Online ISSN : 2249-4626
Print ISSN : 0975-5896
DOI : 10.17406/GJSFR

Global Journal

of Science Frontier Research: C

Biological Science

Botany & Zoology

Remedy of Corona Virus

Study on the Role of Homoeopathy

Highlights

Human Breast Cancer Cell Lines

Development of Hedysarum Alpinum L.

Discovering Thoughts, Inventing Future

VOLUME 20 ISSUE 3 VERSION 1,0

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Volume 20 Issue 3 (Ver. 1.0)

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Incorporation No.: 0423089
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USA Toll Free: +001-888-839-7392
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Global Journal of Science Frontier Research

Contents of the Issue

Global Journal of Science Frontier Research: C
Biological Science
Volume 20 Issue 3 Version 1.0 Year 2020
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Wonder Findings of Number of Cells in a Body Including Sexual Cells, Remedy of Corona Virus, Increase of Memory, And other Cells by Couple System

By Nirmalendu Das

Abstract- It is difficult to calculate fixed data of a number of cells in a body. It is varying on time and age of life. The time increasing that cells are increasing, though we can estimate the number of cells in various nerves in a body, brain, sexual platform, etc through a couple system. The coupling system is a new system of the finding of peculiar series of numbers [1], which is applies to many fields. In the cases of Medical Science, it has been observed by calculation that due to disturbing of the couple caused different difficulties in the body. A smooth Coupling cell may produce a healthy body, and it is possible to increase memory by adding particular cells number in a loss position. The coupling system is interesting that, can explain the real mechanism of every cell. There are many types of cells in a living body. Almost all cells follow a couple of system. The coupling system performs coupling between two (say, A, 1st party & B, 2nd party) with keeping relation as 3rd party, denoted by R (Relative Number) mathematically.

Keywords: number of cells related to couple system, sexual activity, corona virus, cancer cells, neurons of humans & animals, cause of diseases, memory cells.

GJSFR-C Classification: FOR Code: 279999

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Abstract- It is difficult to calculate fixed data of a number of cells in a body. It is varying on time and age of life. The time increasing that cells are increasing, though we can estimate the number of cells in various nerves in a body, brain, sexual platform, etc through a couple system. The coupling system is a new system of the finding of peculiar series of numbers [1], which is applies to many fields. In the cases of Medical Science, it has been observed by calculation that due to disturbing of the couple caused different difficulties in the body. A smooth Coupling cell may produce a healthy body, and it is possible to increase memory by adding particular cells number in a loss position. The coupling system is interesting that, can explain the real mechanism of every cell. There are many types of cells in a living body. Almost all cells follow a couple of system. The coupling system performs coupling between two (say, A, 1st party & B, 2nd party) with keeping relation as 3rd party, denoted by R (Relative Number) mathematically. This system applies to finding the series of Pi [1], ½ values [2], Searching of Properties of Mind, Activity of number of Cells in a brain [3]; Determination of relative numbers by using couple system and its application to the atomic fields and quark coupling strength of the LHCb collaboration [4]. We can determine the atomic number, electron, proton, neutron, splitting of quark from one point to other, etc. So, we can call this system to give birth to all systems. It requires more study and searches in every case, couple system in medical science a touch of light that can bring a revolutionary change to keep fit body from various virus effects.

Keywords: number of cells related to couple system, sexual activity, corona virus, cancer cells, neurons of humans & animals, cause of diseases, memory cells.

I. Introduction

The process of the birth of the body is a natural system. Cell forms accordingly by nature. It has seemed that all processes of birth probably follow one relation to the next relation by coupling each other. Figure 1[5], is indicating that system here.

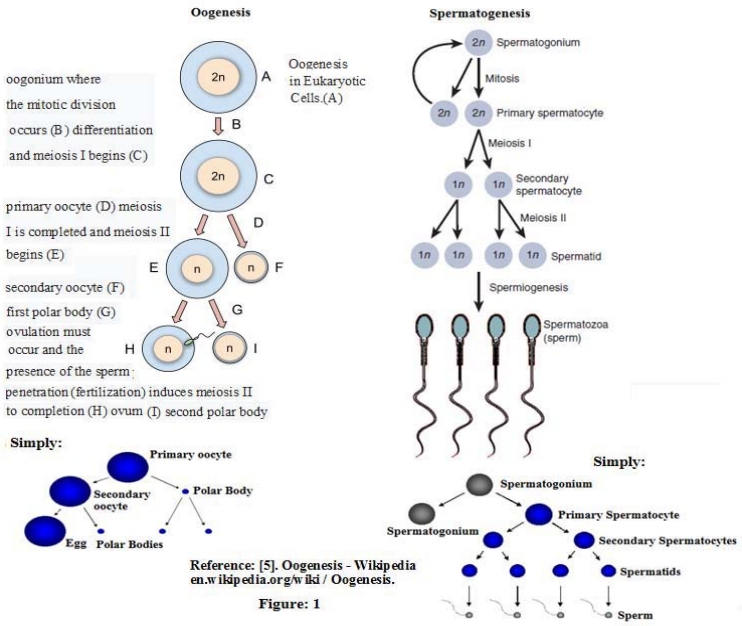

Fig. 1

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The coupling system is related to the above birth mechanism. The obtained different numbers are indicating a new era of cell number of how it forms for every life. Why this number almost fixed to the body? How we can determine these numbers in series, which will become apply to the human body and animals. We can treat the number as day, month, hour, second, mile, km, kg, etc where needed. Again the number is the only number may apply to cells as cell number, two bodies coupling periodically. If a couple destroys somehow by the effect of the virus or by any way, the coupling will disturb. As a result, various disease attacks in a body. If we keep coupling steady in a uniform process by medicine or by such equipment, we can protect ourselves.

Let us go through the Couple System a new process:
Application of "No-1 Formation": [1, 2]

Problem (1):

The well known equation $(r + M)^2 = r^2 + 2rM + M^2$. Similarly, $(r + M)^3 = r^3 + 3r(rM + M^2) + M^3$. Likewise, $(r + M)^4$, $(r + M)^5$, $(r + M)^6$ etc. but new function derived in the form of:
If $(r + M)^3 \rightarrow r^3 + 3r(rM + M^2) + M^3$ and let $M = 1$, then
$(r + 1)^3 \rightarrow r^3 + 3r(r1 + 1^2) + 1^3 \rightarrow r^3 + 3r(r + 1) + 1$ …….. Known fact.

The middle part of the above equation, $3r\ (r + 1)$ means 3 times of the factor $[r(r + 1)]$. Let, $r = A$, $1 = B$ and $R = (r + 1)$. Naturally, R interrelated to A and B shown here in the form of:

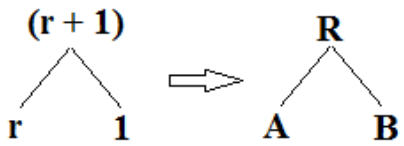


Fig. 2

So, the equation,
$(r + 1)^3 = r^3 + 3rR + 1^3$ is representing the equation of $(r + 1)^3 \rightarrow r^3 + 3r(r1 + 1^2) + 1^3 \rightarrow r^3 + 3r(r + 1) + 1$ (known).
If the figure 1 is extended as:

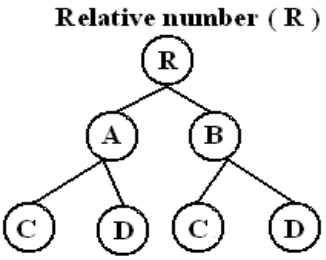


Fig. 2

No. 1, Formation
Reaction of couple:

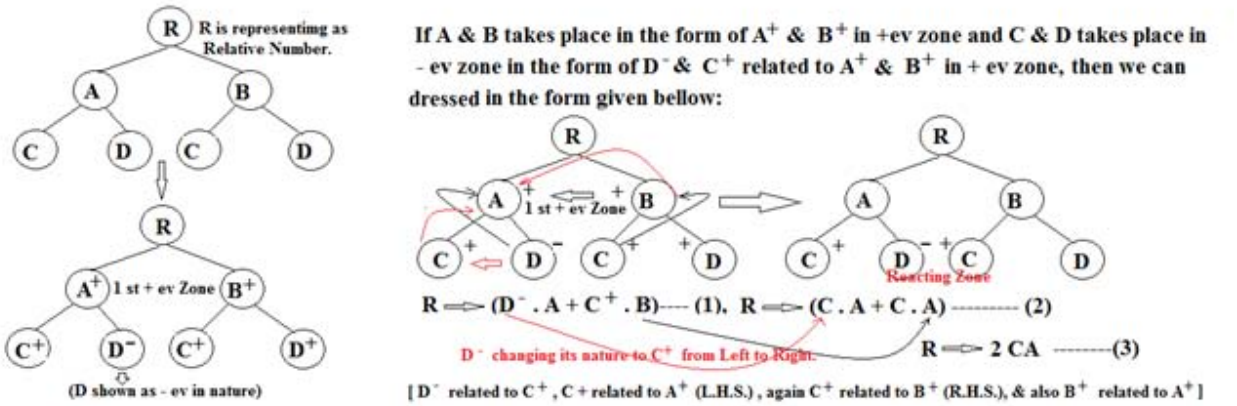


Fig. 3

Description:

1) D is related to A and C is related to B. But A and C are respectively the value of B and D. D^- In L.H.S. means that it is the end of the reaction of problem to form relative number (R) acting with C^+ in R.H.S.
2) The original value of D is C; therefore, D reacts with A and forms CA. Similarly, C is original value of D, but the original value of B is A. So, C reacts with B and forms CA.
3) Total couple reaction with respect to R is 2CA.

No. 2, Formation

When the couple will increase to one step towards E & F (Fig. – 4) from the coupling zone of C & D (Fig- 3) respectively, the positive (+) & negative (-) sign will play as the reverse function of "No. 1, formation" that is, F to F^+ on L.H.S. of the couple & E to E^+ on R.H.S. of the couple. If D^- remains unchanged & C^+ turns to C^- (1st negative row).

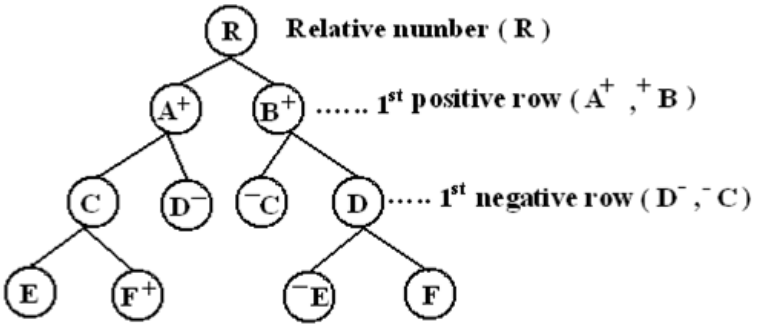


Fig. 4

(No. 2, Formation)

The couple reaction with respect to R as follows:

$$
\begin{aligned}
R \dashrightarrow\ & [F^{+}.C + E.D] + [D^{-}.A + C.B] \\
\dashrightarrow\ & [E.C + (-E.-C)] + [C.A + (-C.A)] \\
\dashrightarrow\ & [E.C + E.C] + [C.A - C.A] \\
\dashrightarrow\ & 2E.C + 0 \\
\text{Hence: } R \dashrightarrow\ & 2E.C
\end{aligned}
$$

When the couple will increase to one more step towards G & H (Fig.- 5) from the couple zone of E & F (Fig.- 4) respectively , then the end of the couple will play as a function of "No. 1 formation `` and if $F^+$ remain unchanged and $E^-$ turns to $E^+$, then another positive row will be formed (Fig.- 5) . If the couple proceeds to another next step, the end of the couple will follow "No. 2, formation `` and again one negative row will be formed (Fig. – 5).

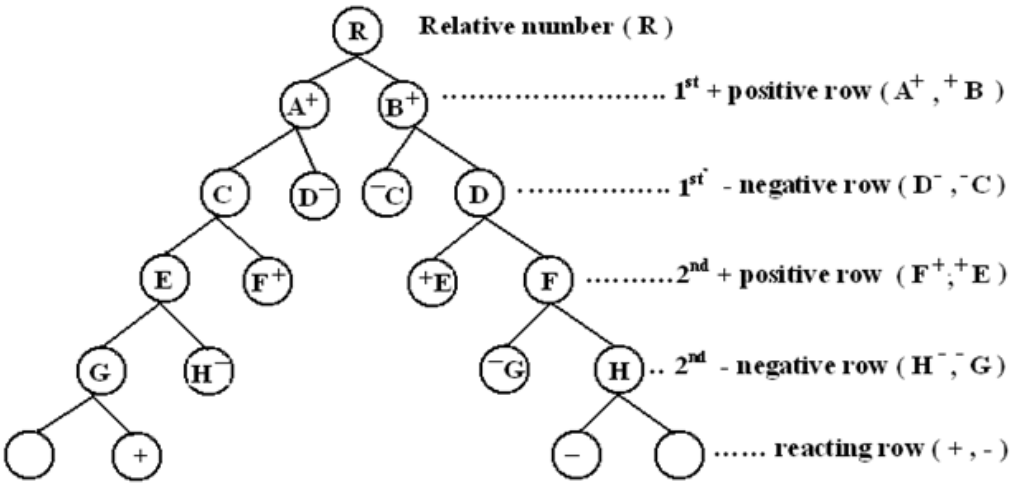


Fig. 5

If the process continues as above alternately as +ve, -ve, +ve, -ve, +ve, -ve,………rows will produced successively and middle part of the couple will be zero after the couple reactions (vide problems). This process is applicable to determination of value of Pi. [1, 2]. Wonder Findings of Number of Cells in a Body Including Sexual Cells, Remedy of Corona Virus, Increase of Memory, and Other Cells by Couple System.

To follow the figure – 3,

Let us an example, $(r + 1)^2 = r^2 + 2r.1 + 1^2$

Middle term is 2r.1 and may represent by R. then according to fig. – 3 we can draw a figure as,

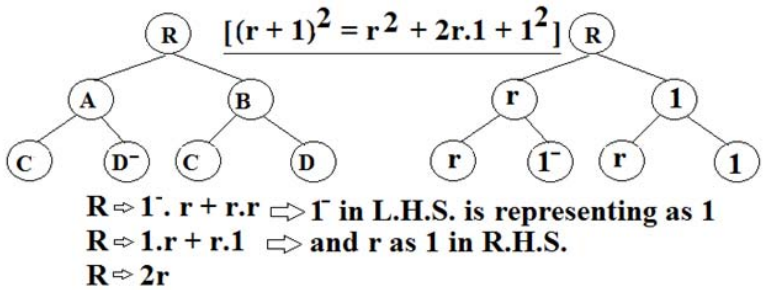


Fig. 6

The equation $(a + b)^2$, $(a + b)^3$, $(a + b)^4$, $(a + b)^5$, etc are very well known simple equation. If we consider middle term of this equation represented by R and put in coupling system to find relative numbers, then we can get a new series of numbers which may apply to some fields. The obtained new equation is:

$$(r \pm M)^N \rightarrow r^N \pm M^{[1 + 2(N - 2)]}.Nr.(N - 1)r \pm M^N$$

And middle part of this equation is $M^{[1 + 2(N - 2)]}.Nr.(N - 1)r$

The process is given here step by step.

We known the equation $(r + 1)^3$, it will turns to $(r + 1)^3 = r^3 + 3r.2r + 1^3$ using couple system.

If M = 2,3,4,5, ….. Then, we can get the following series.

$$(r + 2)^3 \rightarrow r^3 + 2^3.3r.2r + 2^3$$

$$(r + 3)^3 \rightarrow r^3 + 3^3.3r.2r + 3^3$$

$$(r + 4)^3 \rightarrow r^3 + 4^3.3r.2r + 4^3$$

$$(r + 5)^3 \rightarrow r^3 + 5^3.3r.2r + 5^3$$

$$\ldots\ldots\ldots \rightarrow \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

$$(r + M)^3 \rightarrow r^3 + M^3.3r.2r + M^3 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(a)}$$

This equation (a) will satisfy by only 0 & 1.

When, r and M = 0, then result brings 0, but, when r = M = 1, then, on putting this value in L.H.S., $(r + M)^3 = (1 + 1)^3 = 2^3 = 8$ and for R.H.S., $r^3 + M^3.3r.2r + M^3 = 1^3 + 1^3.(3x1).(2x1) + 1^3 = 1 + 6 + 1 = 8$. This equation satisfying Binary Numbers as 0 & 1 only. This equation will not satisfy others numbers like 2,3,4, etc, in this case numbers of L.H.S. and R.H.S. will defer, for example, if r = 1 & M = 2, the we get,

$$(r + M)^3 \rightarrow r^3 + M^3.3r.2r + M^3$$

$$(1 + 2)^3 \rightarrow 1^3 + 2^3.(3x1).(2x1) + 2^3$$

$$27 \rightarrow 1 + 8 \times 3 \times 2 + 8$$

$$27 \rightarrow 57$$

L.H.S is known equation, R.H.S. is unknown.

Here we can say, relative number of 27 is 57. The difference between is 30. We considered numbers as 1 & 2 for r and M. Now 1 + 2 = 3, so, 30 – 3 = 27, this similarity we have from this relation using the number 1 & 2 only. From the above deduction, we can arrange the equation, $(r + 1)^3 = r^3 + 3r (r + 1) + 1$ to $(r + 1)^3 \rightarrow r^3 + 3r.2r + 1^3 \rightarrow (r + 1)^3 \rightarrow r^3 + 1^3.3r.2r + 1^3$. Similarly, on putting next odd number 5, then, we observed that:

Global Journal of Science Frontier Research (C) Volume XX Issue III Version I Year 2020

Problem (2):
When, $(r + M)^5 \rightarrow r^5 + 5r\,(r^3 + 2r^2 + 2r + 1) + 1^5$, then, $R = r^3 + 2r^2 + 2r + 1$, if A, B, C, D represents the corresponding values of r^3, $2r^2$, 2r, 1 (Since, these are the real values of $(r + 1)^5$) respectively. Then, R will relate in Couple Systems as follows:

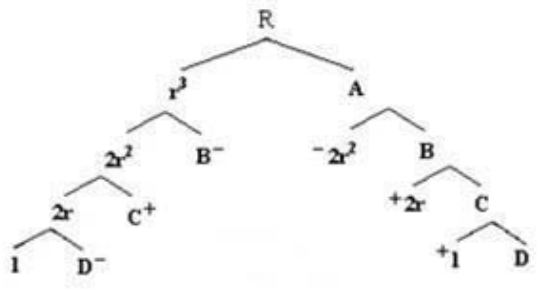


Fig. 7

$$R \rightarrow (D^-.\,2r + 1.C) + (C^+.2r^2 + 2r^+.B) + (B^-.2r^3 + 2r^{-2}.A)$$

$$R \rightarrow (1.\,2r + 1.2r) + (2r.2r^2 + 2r.2r^{-2}) + (2r^2.2r^3 + 2r^{-2}.r^3)$$

$$R \rightarrow 1.(2r + 2r) + (2r.2r^2 - 2r.2r^{-2}) + (2r^2.2r^3 - 2r^{-2}.r^3)$$

$$R \rightarrow 4r + 0 + 0$$

$$R \rightarrow 4r$$

Therefore, $(r + 1)^5 \rightarrow r^5 + 5r.4r + 1^5$ ..... (b)

If M = 2,3,4,5…..[Vide Problem (1)], then,

$$(r + 2)^5 \rightarrow r^5 + 2^7.5r.4r + 1^5$$

$$(r + 3)^5 \rightarrow r^5 + 3^7.5r.4r + 1^5$$

$$(r + 4)^5 \rightarrow r^5 + 4^7.5r.4r + 1^5$$

$$(r + 5)^5 \rightarrow r^5 + 5^7.5r.4r + 1^5$$

.....

$$(r + M)^5 \rightarrow r^5 + M^7.5r.4r + M^5$$

Therefore, the real formation of $(r + 1)^5$ will $r^5 + 1^7.5r.4r + 1^5$

Problem (3):
When, $(r + M)^7 \rightarrow r^7 + 7r\,(r^5 + 3r^4 + 5r^3 + 5r^2 + 3r + 1) + 1^7$, then, R will related to bellow as:

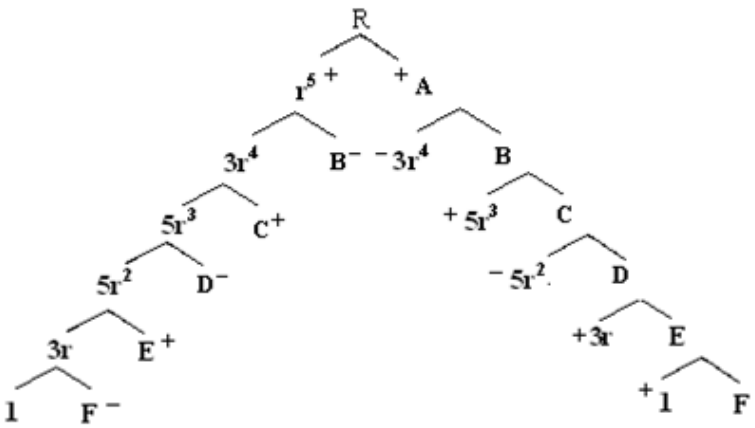


Fig. 8

$R \rightarrow (F^{-}.3r + 1^{+}.E) + (E^{+}.5r^{2} + 3r^{+}.D) + (D^{-}.5r^{3} + 5r^{-2}.C) + (C^{+}.\ 3r^{4} + 5r^{+3}.B) + (B^{-}.r^{5} + 3r^{-}.A)$
$R \rightarrow (1.3r + 1.3r) + (3r.5r^{2} + 3r.5r^{-2}) + (5r^{2}.5r^{3} + 5r^{-2}.5r^{3}) + (5r^{3}.\ 3r^{4} + 5r^{3}.3r^{-4}) + (3r^{4}.r^{5} + 3r^{-4}.r^{5})$
$R \rightarrow (1.3r + 1.3r) + 0 + 0 + 0 + 0 = 1.(3r + 3r) = 1 \text{ x } 6r = 6r.$
$(r + 1)^{7} \rightarrow r^{7} + 1^{7}.7r.6r + 1^{7}$ and if M = 2,3,4,5 ….., then,

$$(r + 2)^{7} \rightarrow r^{7} + 2^{11}.7r.6r + 2^{7}$$
$$(r + 3)^{7} \rightarrow r^{7} + 3^{11}.7r.6r + 3^{7}$$
$$(r + 4)^{7} \rightarrow r^{7} + 4^{11}.7r.6r + 4^{7}$$
$$(r + 5)^{7} \rightarrow r^{7} + 5^{11}.7r.6r + 5^{7}$$
$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$
$$(r + M)^{7} \rightarrow r^{7} + M^{11}.7r.6r + M^{7}$$

"No – 1 formation" is only for the series of *odd numbers* as 1,2,3,,5 …., if the series increases, then the process of couple will increase.

Application of "No – 2 formation" for even number. [1]
Problem (1):
$(r + 1)^{4} \rightarrow r^{4} + 4r.(r^{2} + [3/2] \text{ x } r + 1) + 1^{4} = r^{4} + 4r.\ (R) + 1^{4}$

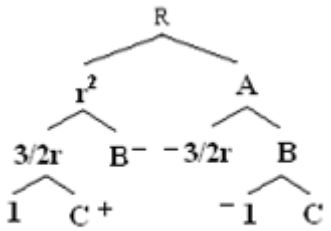


Fig. 9

$R \rightarrow (C.[3/2] \text{ x } r + 1^{-}.B) + (B^{-}.r^{2} + [3/2] \text{ x } r^{-}.A)$

$R \rightarrow (1.[3/2] \text{ x } r + 1^{-}.[3/2] \text{ x } r^{-}) + ([3/2] \text{ x } r.r^{2} + [3/2] \text{ x } r^{-}.r^{2})$

$R \rightarrow 2.[3/2] \text{ x } r + 0 = 3r$

Note:

$(a + b)^{4} = a^{4} + 4a^{3}b + 6a^{2}b^{2} + 4ab^{3} + b^{3} = a^{4} + 2ab\ (2a^{2} + 3ab + 2b^{2}) + b^{4}.$

$= a^{4} + 4ab\ (a^{2} + [3/2] \text{ x } ab + b^{2}) + b^{4}$, when b = 1, then,

$(a + 1)^{4} = a^{4} + 4a.1\ (a^{2} + [3/2] \text{ x } a.1 + 1^{2}) + 1^{4} = a^{4} + 4a\ (a^{2} + [3/2] \text{ x } a + 1^{2}) + 1^{4}$, when, a = r, then, $(r + 1)^{4} = r^{4} + 4r\ (r^{2} + [3/2] \text{ x } r + 1^{2}) + 1^{4}$ = Let, R = $(r^{2} + [3/2] \text{ x } r + 1^{2})$ and so,

$(r + 1)^{4} \rightarrow r^{4} + 4r.(R) + 1^{4} \rightarrow r^{4} + 4r.\ ([3/2] \text{ x } r) + 1^{4}$ and $R \rightarrow [3/2] \text{ x } r = 3.r = 3r$. Now we can get a series in the forms of: $(r + 1)^{4} \rightarrow r^{4} + 1^{5}.4r.3r + 1^{4}$

Similarly,

$$(r + 2)^{4} \rightarrow r^{4} + 2^{5}.4r.3r + 2^{4}$$
$$(r + 3)^{4} \rightarrow r^{4} + 3^{5}.4r.3r + 3^{4}$$
$$(r + 4)^{4} \rightarrow r^{4} + 4^{5}.4r.3r + 4^{4}$$
$$(r + 5)^{4} \rightarrow r^{4} + 5^{5}.4r.3r + 5^{4}$$
$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$
$$(r + M)^{4} \rightarrow r^{4} + M^{5}.4r.3r + M^{4}$$

For power 6, we get,

$$(r + 1)^{6} \rightarrow r^{6} + 1^{9}.6r.5r + 1^{6}$$

$$(r + 2)^6 \rightarrow r^6 + 2^9.6r.5r + 2^6$$

$$(r + 3)^6 \rightarrow r^6 + 3^9.6r.5r + 3^6$$

.....

$$(r + M)^6 \rightarrow r^6 + M^9.6r.5r + M^6$$

"No – 2, formation" is only for the series of powers of (r + M), when integrates acts as a function of, 2,4,6,8 ….. When the series increases, then the process of couple increases.
Hence the series:

$$(r + M)^2 \rightarrow r^2 + M^1.2r.1r + M^2$$

$$(r + M)^3 \rightarrow r^3 + M^3.3r.2r + M^3$$

$$(r + M)^4 \rightarrow r^4 + M^5.4r.3r + M^4$$

$$(r + M)^5 \rightarrow r^5 + M^9.5r.4r + M^5$$

$$(r + M)^6 \rightarrow r^6 + M^9.6r.5r + M^6$$

$$(r + M)^7 \rightarrow r^7 + M^{11}.7r.6r + M^7$$

.....

$$(r + M)^N \rightarrow r^N + M^{[1 + 2(N-2)]}.Nr.(N-1)r + M^N \quad \text{(A)}$$

$$(r + M)^N \rightarrow r^N + M^Z.\ Nr.(N-1)r + M^N \quad \text{(B)}$$

When, $Z = [1 + 2(N – 2)]$ & $N = 2,3.4.....$

In the case of negative functions, this equation will turn to:

$$(r - M)^N \rightarrow r^N - M^{[1 + 2(N-2)]}.Nr.(N-1)r - M^N \quad \text{(C)}$$

$$(r - M)^N \rightarrow r^N - M^Z.Nr.(N-1)r - M^N \quad \text{(D)}$$

Relative Numbers (R):

The middle part of the equation (A) or (B) and (C) or (D) is same. We have the Relative number as [Nr.(N – 1)r] which connected to $M^{[1 + 2(N-2)]}$ or M^Z, when $Z = [1 + 2(N – 2)]$ of $(r + M)^N$ or $(r - M)^N$. so, we may write the general equation in the form of:

$$(r \pm M)^N \rightarrow r^N \pm M^{[1 + 2(N-2)]}.Nr.(N-1)r \pm M^N \quad \text{(E)}$$

And middle part of this equation is $M^{[1 + 2(N-2)]}.Nr.(N-1)r$ (F)

When, N = 1, 2, 3, 4, 5 ….. we get relative numbers 1r, 2r, 3r, 4r, 5r etc both of even and odd numbers. The equation (A) obtained by the couple system and is applicable in forming relative numbers with respect to Z of which numbers become odd in series, when N = 2, 3, 4, 5 ….of the equation, $M^{[1 + 2(N-2)]}.Nr.(N-1)r$. On changing the number of Z as $Z = [2 + 2(N – 2)]$, we get,

$M^{[2 + 2(N-2)]}.Nr.(N-1)r \rightarrow M^2.\ 2r.1r$, when N = 2

$M^{[2 + 2(N-2)]}.Nr.(N-1)r \rightarrow M^4.\ 3r.2r$, when N = 3, where, $(r + M)^3 \rightarrow M^3.3r.2r$, when, N = 3, due to change of Z, power changes as:

When, $Z = [1 + 2(N – 2)]$, Z = - 3, when, N = 0 (not satisfying).

$Z = [1 + 2(N – 2)]$, Z = – 1, when, N = 1 (not satisfying).

$Z = [1 + 2(N – 2)]$, Z = 1, when, N = 2 (satisfying). It shows N > 1

If, $Z = [2 + 2(N – 2)]$, we get,

$Z = [2 + 2(N – 2)]$, Z = - 2, when, N = 0 (not satisfying)

$Z = [2 + 2(N – 2)]$, Z = 0, when, N = 1 (satisfying), because, $M^Z = M^0 = 1$

$Z = [2 + 2(N – 2)]$, Z = 2, when, N = 2 (satisfying), N > 1

At the time of changing of Z, let, Nr.(N – 1)r will change to Nr.(N – 2), then, we get a series as:

$M^{[2 + 2(N - 2)]}.Nr.(N - 2)r \rightarrow M^{0}.\ 0 \text{ x } r.(0 - 2)r = 0$, when N = 0

$M^{[2 + 2(N - 2)]}.Nr.(N - 2)r \rightarrow M^{-1}.\ 1r.(1 - 2)r = M^{-1} \text{ x } 1r \text{ x } - 1 = -\ M^{-1}$ when N = 1

$M^{[2 + 2(N - 2)]}.Nr.(N - 2)r \rightarrow M^{2}.\ 2r.0xr = 0$, when N = 2

$M^{[2 + 2(N - 2)]}.Nr.(N - 2)r \rightarrow M^{4}.\ 3r.1r$, when N = 3 etc., N > 2

When this equation turns to $M^{[2 + 2(N - 2)]}.Nr.Nr$ ……(G), when, (N – 1)r treated as Nr, then we will get even numbers (Z) of M of the series. So,

$M^{[2 + 2(N - 2)]}.Nr.Nr \rightarrow M^{2}.2r.2r$, when, N = 2, N > 1, if, M = r = 1, $M^{2}.2r.2r = 400$ & 400/2 = 200.

$M^{[2 + 2(N - 2)]}.Nr.Nr \rightarrow M^{4}.3r.3r$, when, N = 3

$M^{[2 + 2(N - 2)]}.Nr.Nr \rightarrow M^{6}.4r.4r$, when, N = 4 etc.

Therefore, the deduction (F) and (G) finds,

$M^{[1 + 2(N - 2)]}.Nr.(N - 1)r \rightarrow M^{1}.2r.1r$, when, N = 2, Z = 1 of power of M, odd number.

$M^{[2 + 2(N - 2)]}.Nr.Nr \rightarrow M^{2}.2r.2r$, when, N = 2, Z = 2 of power of M, even number.

$M^{[1 + 2(N - 2)]}.Nr.(N - 1)r \rightarrow M^{3}.3r.2r$, when, N = 3, Z = 3 of power of M, odd number.

$M^{[2 + 2(N - 2)]}.Nr.Nr \rightarrow M^{4}.3r.3r$, when, N = 3, Z = 4 of power of M, even number.

……

From the above deduction, we have the following results as:

i) When, N = 0, the equation (F) yields $M^{-3}.0r.(-1).r$

ii) ″ N = 0, ″ (G) ″ $M^{-2}.0r.0r$

iii) ″ N = 1, ″ (F) ″ $M^{-1}.1r.0r$

iv) ″ N = 1, ″ (G) ″ $M^{0}.1r.1r$

Therefore, when N has tendency to proceed in negative direction, i.e, N = -1, -2, -3, -4 …… then the deduction (F) & (G) will give results, the yielded values are listed here in a table (Zr).

Relative Numbers obtained by Couple System.
Table (Zr):

$$M^{Z} \text{ x } [r_2 \text{ x } r_1] \quad M^{-Z} \quad [\text{x } -r'_2 \text{ x } -r'_1]$$

R.H.S.(Relative No.) (Relative No.) L.H.S.

M^{0} x 1 x 1 = 1 ($M^{0} = 1^{0} = 1$, when, M = 1 and relative number, 1 x 1 = 1

M^{1} x 2 x 1 M^{-1} x 1 x 0 Now, 2/0 = 2

[Relative number = 2 (r_2) x 1(r_1) = 2 x 1 = 2]. On the other hand,

(1^{1} x 2 x 1 = 10 x 2 x 1 = 20 and 20/10 = 2, and 1^{-1} x 1 x 0 = 0.1 x 1 x 0 = 0. so, 2/0 = 2).

M^{2} x 2 x 2 M^{-2} x 0 x 0 Now, 4/0 = 4

M^{3} x 3 x 2 M^{-3} x 0 x -1 Now, 6/0 = 6

M^{4} x 3 x 3 M^{-4} x -1 x -1 Now, 9/1 = 9

M^{5} x 4 x 3 M^{-5} x -1 x -2 Now, 12/2 = 6

M^{6} x 4 x 4 M^{-6} x -2 x -2 Now, 16/4 = 4

M^{7} x 5 x 4 M^{-7} x -2 x -3 Now, 20/6= 3.333

M^{8} x 5 x 5 M^{-8} x -3 x -3 Now, 25/9 = 2.777

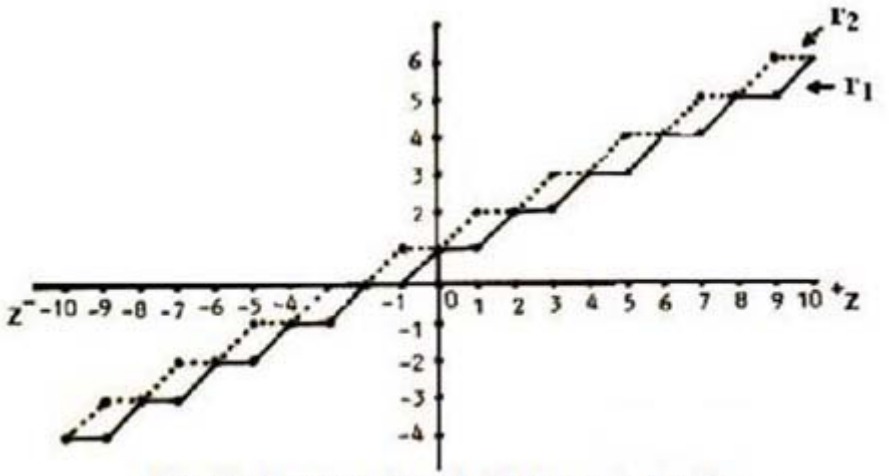
M^{9} x 6 x 5 M^{-9} x -3 x -4 Now, 30/12 = 2.5

M^{10} x 6 x 6 M^{-10} x -4 x -4 Now, 36/16 = 2.25

M^{11} x 7 x 6 M^{-11} x -4 x -5 Now, 42/20= 2.1

M^{12} x 7 x 7 M^{-12} x -5 x -5 Now, 49/25 = 1.96

..... *up to n numbers, but not to infinity.*



Graphical presentation of relative number r_2 & r_1 .

Fig. 10

When the coupling zones presented by +1 in the form of object & image, then it may treat as 1st, 2nd, 3rd, 4th coupling zones are shown in fig-11.

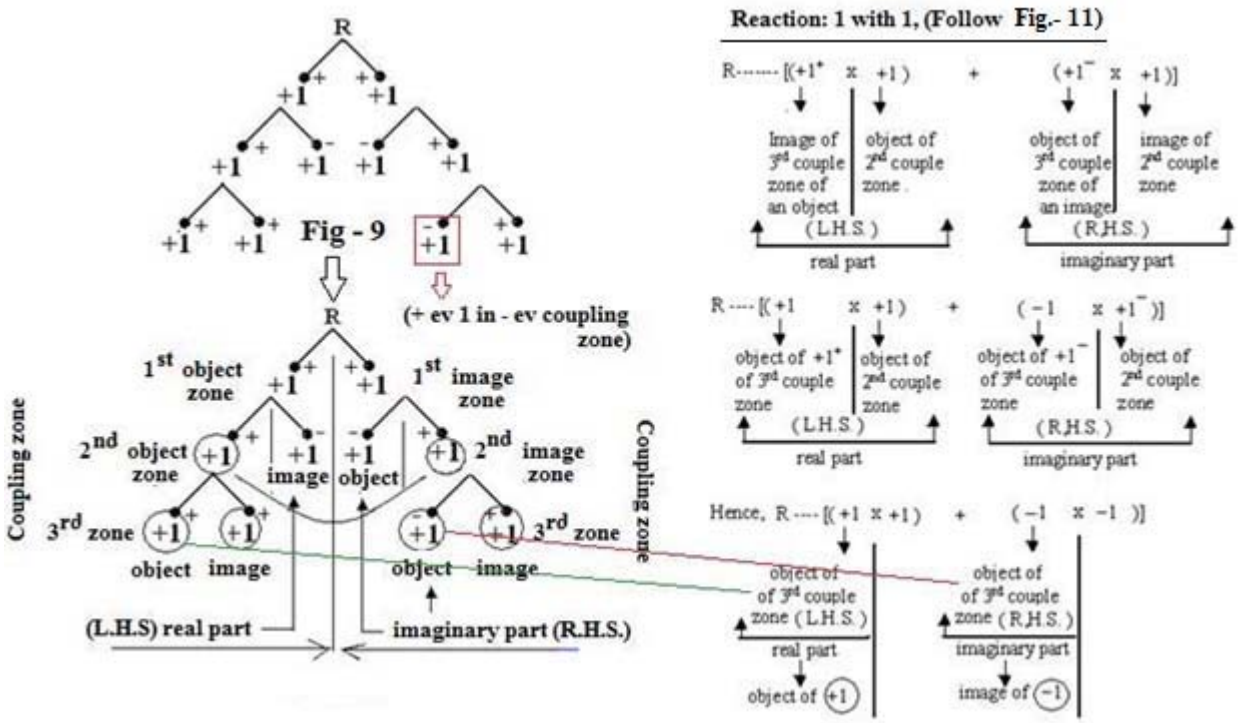


Fig. 11

In this way, if coupling series increases with respect to 1, the reacting results to be 2.
This is also applicable to *No. 1, Formation.* The sum of Fig.-11 is given below for example:

$$R \text{ ------ } [(+1^{+} \times +1) + (+1^{-} \times +1)] + [(+1^{-} \times +1^{+}) + (+1^{-} \times +1^{+})]$$

$$R \text{ ------ } [(+1 \times 1) + (-1 \times +1^{-})] + [(+1 \times +1^{+}) + (-1 \times +1^{+})]$$

R ------ [(1 x 1) + (-1 x -1)] + [(1 x 1) + (-1 x +1)]

R ------ [(1) + (+1)] + [(1) + (-1)]

R ------ [(2)] + [(1 -1)] = 2 + 0 = 2

These formations of relative numbers are most important to find the different types of cells of the brain and other parts of a body. R.H.S. of these formations brings the total number of cells, if we consider M as base1, that is (According to Calculator, 1 ^ EXP – 0 = 1, & same is applies to all M^n, where, n = 0, 1, 2, 3, 4, 5,…..). M^0 x 1 x 1 = 1^0 x 1 x 1 = 1 number (1^0 = 1), here we can assume, 1^0 x X x Y = 1 sex cell for male and for female 1^0 x X x X = 1 sex cell. Ten is representing as the relation between two as 1^0 = 1, which one is commending as love or agrees to meet in sexual functions.

Applications of most basic formation Application:

Sex cells and chromosomes:

M^1 x 2 x 1 = 1^1 x 2 x 1 = 20 numbers (1^1 = 10) and so on. Here we observed that coefficient of M^1 is 2 &1 as relative number and if we add 2 + 1 = 3 & 3 to 20 = 23 numbers. We may treat these 23 numbers as sex cells and 23 + 23 = 46 of human body.

Sex cells and chromosomes [6]

Human body cells each contain 23 pairs of *chromosomes*. Parents pass on their *genes* to their offspring in their sex cells. Female sex cells are called egg cells or ova. Male sex cells are called sperm.

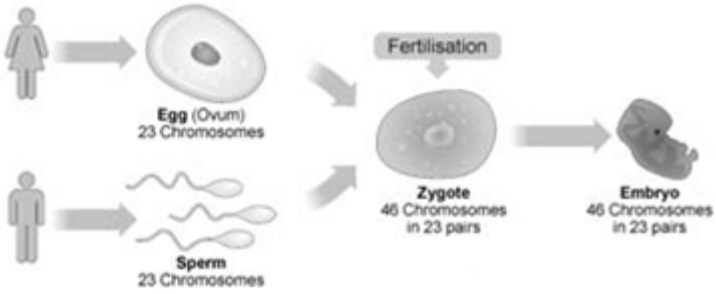


Fig. 12

Process of fertilization:

Sex cells only contain one chromosome from each pair. When an egg cell and sperm cell join together, the fertilized egg cell contains 23 pairs of chromosomes. One chromosome in each pair comes from the mother, the other from the father.

The pair of chromosome is random. Due to this different child in the same family gets a different combination. This is why children in the same family look a little like each other and a little like each parent but are not identical to them.

Again, [M^2 x 2 x 2] = [1^2 x 2 x 2 = 10 x 2 x 2 = 400, when M = 1]. 1/10th of 400 are 40 numbers, If we consider, the coefficient of M^1 is 2 and 2, then, 2 + 2 = 4. Then, 40 + 4 = 44. Then, 44/2 = 22 cells plus with 1 chromosome, then brings 23 cells. But 44 cells + 2 chromosomes = 46 cells of XX and XY sex cells.

$$\text{Again, ratio of } \frac{[M^2 \times 2 \times 2]/10 = [1^2 \times 2 \times 2]/10}{M^1 \times 2 \times 1 = 1^1 \times 2 \times 1} = \frac{40}{20} = 2$$

Here, 2 (two) means for any birth, two couples need to meet together.

Birth of Baby:

When power of M increases 1 to 6, then the equation, M^6 x 4 x 4 = 1^6 x 4 x 4 = 16000000 = 1.6 x 10^7 = 16 million and if we treated as seconds this number, then, 16000000 sec = 185.185 days or 6.17 months. As so, 6 month baby is unmeasured. If we multiply by the factor 3/2, (*j* = 1 + ½ & *j* = 3, in the case of atomic stage, *j* used as angular quantum number) then, 16000000 sec x (3/2), then we see that 24 million seconds = 9.26 months = 9.3 months.

If 2 x [M^1 x 2 x 1] = 2 x [1^1 x 2 x 1] = 40 numbers and if we treated as weeks days, then, "Average human baby pregnancy time vs. other mammals. In general, the larger the animal, the longer the gestation period. In general, the larger the animal, the longer the life.
Human body* 266 days (40 weeks = 280 days)*
*=280 days, from the first day of a woman's last menstrual period.
= 266 days, if measured from the sperm joining the ovum"[7].
40 week days = 9.33 months, this value is tallied to 9.3 months followed by the equation (3/2) x M^6 x 4 x 4 = 1^6 x 4 x 4 = 9.26 months or 277.8 days. Or [M^2 x 2 x 2] / (3/2) = 266.66 days. Therefore, Couple System and obtained relative numbers are most important in the case of birth cells.

We can give other examples from another animal also.

Average Gestation Period Of Different Animals [8]

Animal	Period (months)
African elephant	22 (Again, M^1 x 2 x 2 = 1^2 x 2 x 2 = 40 numbers, its coefficient is 2, then, 2 + 2 = 4, so, 40 + 4 = 44. Then, 44/2 = 22. May use as 22 months.)
Giraffe	15.25 (Now 22/√2 = 15.55 may use as 15.55 months.)
Humpback whale	10 to 12 (22/2 = 11 average period, 11 months) 360 days or 12 months. [M^2 x 2 x 2] / √(√3/2) = 361.44 days = 12.04 months.
Bison (buffalo)	9.5 (22/√5 = 9.83 months)
Human	9 (22/√6 = 8.98 months)
Hippopotamus	8 (22/√7 = 8.31 months)
Grizzly bear	7 (22/√10 = 6.957 months)
Baboon	5 to 6 (22/√14 = 5.88 months or 22/√18 = 5.18 months)
Giant panda	4 to 5 (22//√20 = 4.92 months or 22/√30 = 4.01 months)
Jaguar	3.5 (22/√40 = 3.48 months)
Dog	2 (22/11 = 2 months)
Cat	2 (22/11 = 2 months)
Rabbit	1.33 (22/16 = 1.37 months)
Hamster	0.5 to 1 (22/44 = 0.5 or 22/22 = 1 month)
Lion	108 days (22 days x5 = 110 days = 3.66 months) [7].
Ant 8 to 12 weeks	(22 days x2x√(3/2 = 53.88 = 7.698 weeks & 22x4 = 88 = 12.57 weeks)
Camel = 400 days = [M^2 x 2 x 2] = 400 days or 13.33 months. [7]	
Rhino, Gray = 485 days equivalent to [M^2 x 2 x 2] x √3/2 = 489.89 days = 16.329 months. [7]	
Elephant, Asian = 610 days equivalent to 400 x (3/2) = 600 days = 20 months. [7]	
Likewise, we can estimate the period of other animals.	

Breaking of couple system (for example cancer cell):

What is cancer?

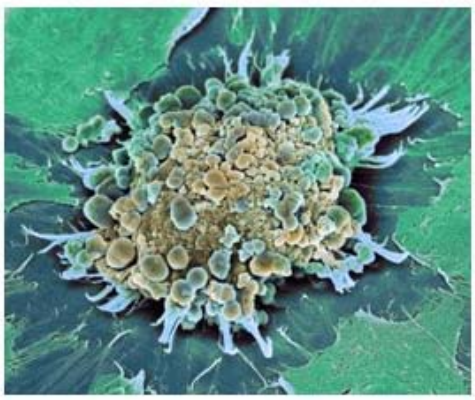
Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is buildup of trillions of cells. Human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. When cancer develops, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide without stopping and may form growths called tumors.

Many cancers form solid tumors, which are masses of tissue of the blood, such as leukemias, generally do not form solid tumors. Cancerous tumors are malignant, which means they can spread into or invade nearby tissues. Also as these tumors grow, some cancer cells can break off and travel to distant places in the body through the blood or the lymph system and form new tumors far from the original tumor. Unlike malignant tumors, benign tumors

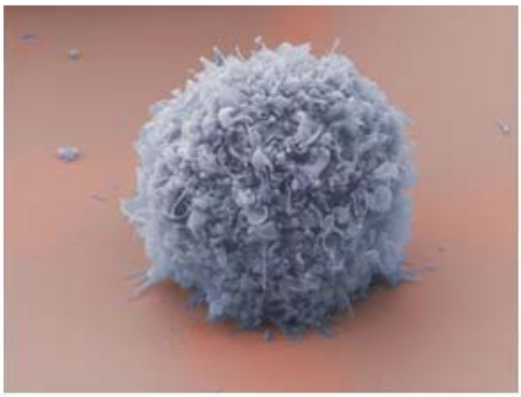
do not spread into or invade nearby tissues. Benign tumors can sometimes be quite large, however. When removed, they usually don't grow back, whereas malignant tumors sometimes do. Unlike most benign tumors elsewhere in the body, benign brain tumors can be life-threatening.

Differences between Cancer Cells and Normal Cells

Cancer cells differ from cells in many ways that allow them to grow out of control and become invasive. The difference is that cancer cells are less specialized than normal cells. That is, whereas cells mature into very distinct cell types with specific functions, cancer cells do not. This is one reason that, cancer cells continue to divide without stopping [9]. So, without the coupling of two cells, it is never possible to give birth to a new generation. When the virus attracts in a body, then the system of a couple feels disturbed, and as a result, it cannot functioned smoothly. The living body feels trouble called diseases. Cancer is one example in this case. These cells do not follow the couple system in the long run; it breaks haphazardly and destroys other connected cells. A figure is given here, for example.



This colored scanning electron micrograph (SEM) Cell shows undergoing programmed **Cell death or apoptosis. [10].**



Human Breast Cancer Cell

Fig. 13

In these figures (13), we see that individual cancer cell have no clear hand to proceed in front, but the cell attack to cell and then progress in couple system by damaging each cell. According to reference [11], a figure is given here that how cancer cells dangerous for the body.

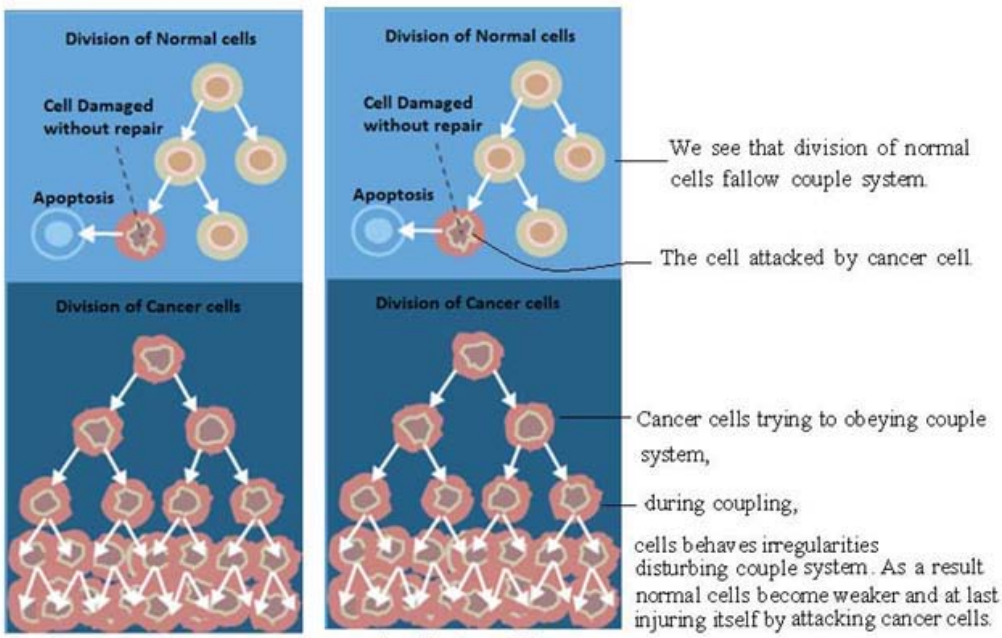


Figure from reference. Classification of figure

Fig. 14

Therefore to fit a body, this is very important that the coupling reaction needs to keep steady by doing exercise. Regular exercise has roll to run coupling cells in every corner of a body and needs balanced food to fit. When the strength of the virus > the strength of coupling, then the virus acts on the body. The same number of cell attacks by the same number of the virus; this is one of the main properties of all cells reaction in the living body. If it is possible to stop growing cancer cells, no entry of cancer cells to the couple system by hook or kook; it is required by adding such medicine or rearranging cells by coupling system in this field to stop the activity of cancer cells. Here is the touch of couple system that how it is important in cell properties.

The probable cause of breaking of cells:

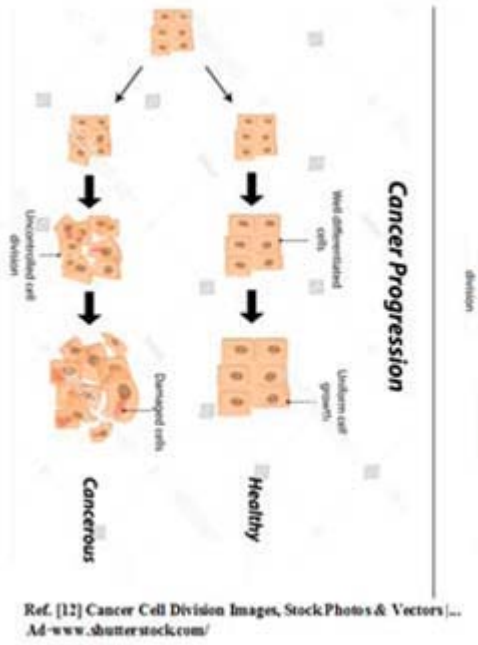


Fig. 15

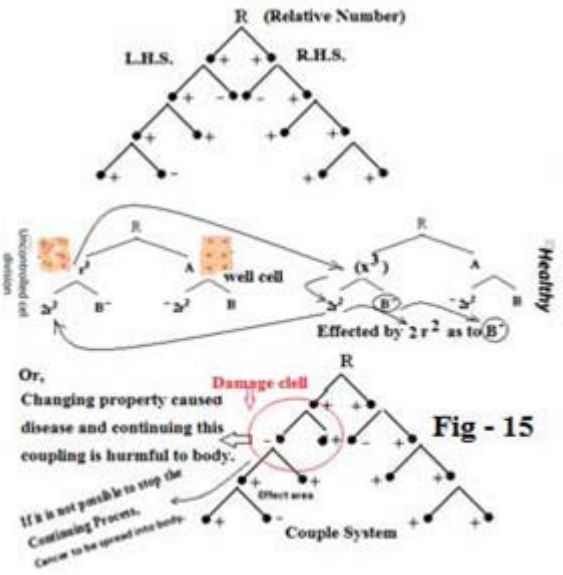


Fig. 16

The life or decay of corona virus [80]

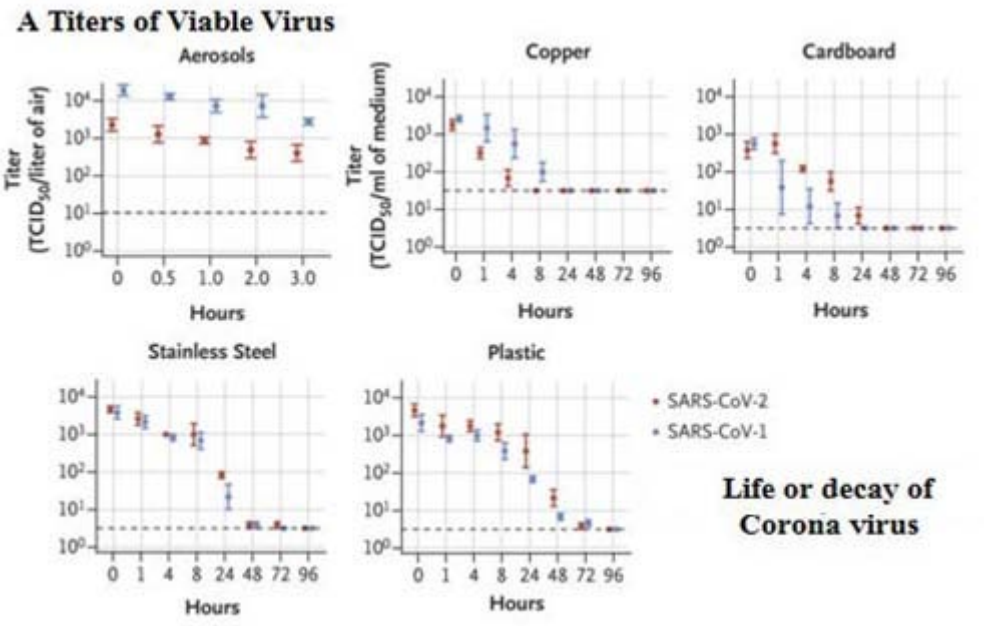


Fig. 17

The coupling system of life of corona virus.
We have the equation,
M^0 x 1 x 1 = 1^0 x 1 x 1 = 1 number ($M^0 = 1^0 = 1$, when M = 1) and 1 number = 1 hour.

For a titers of viable virus (Aerosols)
1) [M^0 x 1 x 1] = 1^0 x 1 x 1 = 1 hour.
2) ½ [M^0 x 1 x 1] = 0.5 hour.
3) 2 [M^0 x 1 x 1] = 2 hours.
4) 3 [M^0 x 1 x 1] = 3 hours
5) 4 [M^0 x 1 x 1] = 4 hours

For Copper, Cardboard, Stainless steel, Plastic: ($M^0 = 10 = 1$, when M = 1)
a) 2 [M^0 x 2 x 1] = 2 [2] = 4 hours on copper.
b) 3 [M^0 x 2 x 1] = 3 [2] = 6 = hours on cardboard.
c) 4 [M^0 x 2 x 1] = 4 [2] = 8 = hours 6 x12 = 72 hours on stainless steel and plastic.
d) 5 [M^0 x 2 x 1] = 5 [2] = 10 = hours
e) 6 [M^0 x 2 x 1] = 6 [2] = 12 = hours, again, (12 x 2) = 24, (24 x 2) = 48, (48 x 2) = 96
f) 6 (6 [M^0 x 2 x 1]) = 6 (6 [2]) = 72 = hours. (72 x 2) = 144 hours.

Virus spread throughout the word. An example is stated here [81]

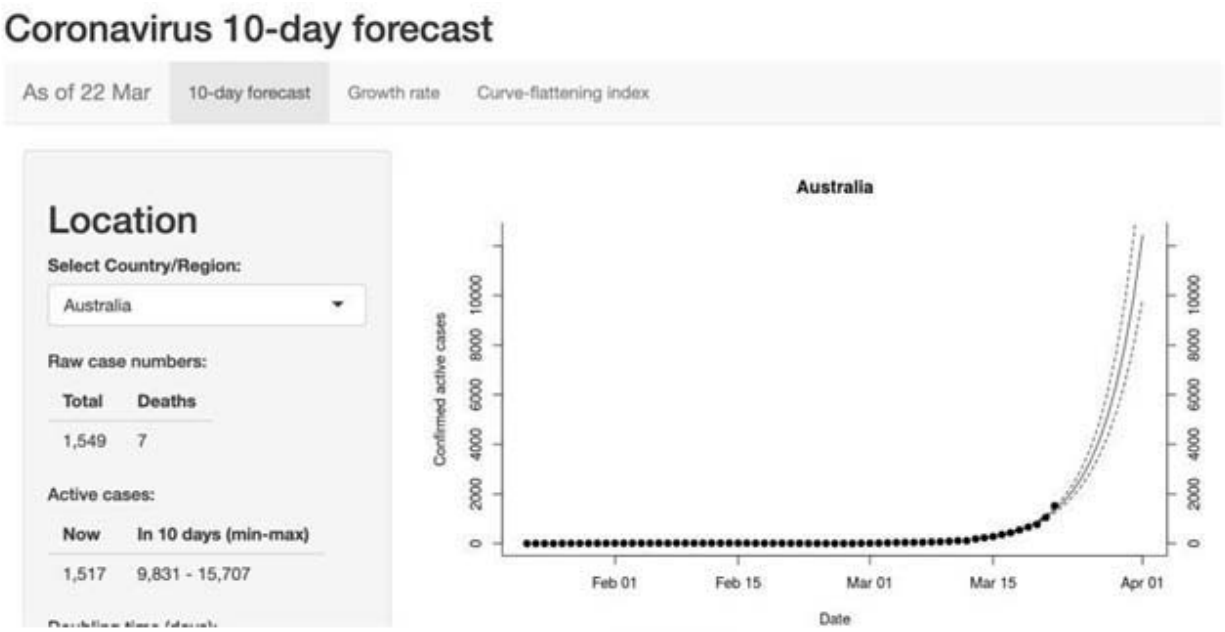


Fig. 18

The app's 10-day forecast for Australia as of 22 March, 2020. Picture: Supplied.

How fast could COVID-19 spread in Australia? And how many people could potentially be infected?

We can't of course know for sure, but we have enough data to make some rough forecasts; and being forewarned is to be forearmed. So we've developed an interactive website that gives a ten-day forecast, by country, on likely numbers of COVID-19 cases. We may compare the above figure of spreading of corona virus in 10 days in Australia with the figure of series of Pi [1, 2] as given here:

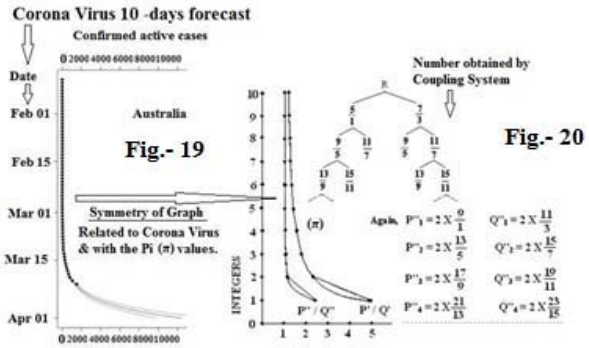


Fig. 19: (Reference - MODELLING THE SPREAD OF COVID-19 Forecasting the spread of COVID-19, including the likely number of undetected cases, is fraught and difficult, but a sense of the numbers is important for appreciating the challenges ahead. *By Associate Professor Ben Phillips, University of Melbourne*).

Fig. 20: (Reference - IJMEIT// Vol.03 Issue 07//July//Page No:1437-1445// ISSN-2348-196x 2015. By Nirmalendu Das).

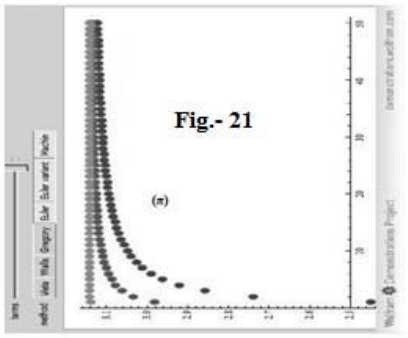


Fig. 21: (Reference - Pi Formulas – from Wolfram Math World)

From the above comparison of the figures - 19, 20, 21 we may get idea that corona virus spread by obeying the couple system. Corona virus behave like host cells and 'spike' protein. Its hand is unstable. Due to this reason cell attacks body seriously. It is possible to destroy a corona virus cells by destroying the couple protein. All cells are interlinked to a couple systems. Couple breaks means cell will weak & cannot mixed with each other, at that time medicine can destroy the virus cells forever. Or we may add another protein cell to corona to change its harmful properties through a coupling process. If it is possible to fit cell by micro-cell-fitting- equipment, then no need medicine. Few medicines has side effect, but to rearranging the cell, body will safe from disease.

II. Brain and other Cells

Neurons are responsible for the transport and uptake of neurotransmitters - chemicals that relay information between brain cells. Depending on its location, a neuron can perform the job of a sensory neuron, a motor neuron, or an interneuron, sending and receiving specific neurotransmitters. In the adult brain, neural circuits are already developed, and neurons must find a way to fit in. As a new neuron settles in, it starts to look like surrounding cells. It develops an axon and dendrites and begins to communicate with its neighbors. The following figure [20] related to the couple system.

a) Death of brain cells

The lives of some neurons can take abnormal turns. Some diseases of the brain are the result of the unnatural deaths of neurons. Due to Parkinson's disease, Huntington's disease, Alzheimer's disease, Blows to the brain, Spinal cord injury and, other cause cells damaged. When virus attacks or for other causes, cells destroyed. Here we see neuron, oligodendrocyte, astrocyte in the form of a couple systems. This system breaks when the attack by diseased. The reaction of coupling disturbs means cells damaged. We can realize these facts to see the following figure [22].

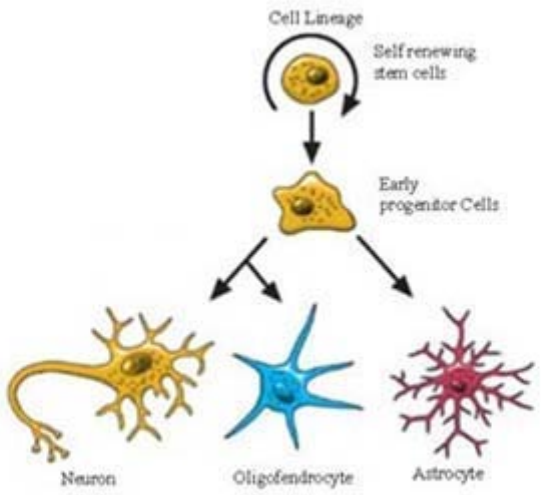


Fig. 22: Stem cells differentiate to produce different types of nerve cells

b) Death of brain cells

The lives of some neurons can take abnormal turns. Some diseases of the brain are the result of the unnatural deaths of neurons. Due to Parkinson's disease, Huntington's disease, Alzheimer's disease, Blows to the brain, Spinal cord injury and, other cause cells damaged. When virus attacks or for other causes, cells destroyed. Here we see neuron, oligodendrocyte, astrocyte in the form of a couple systems. This system breaks when the attack by diseased. The reaction of coupling disturbs means cells damaged. We can realize these facts to see the following figure [23].

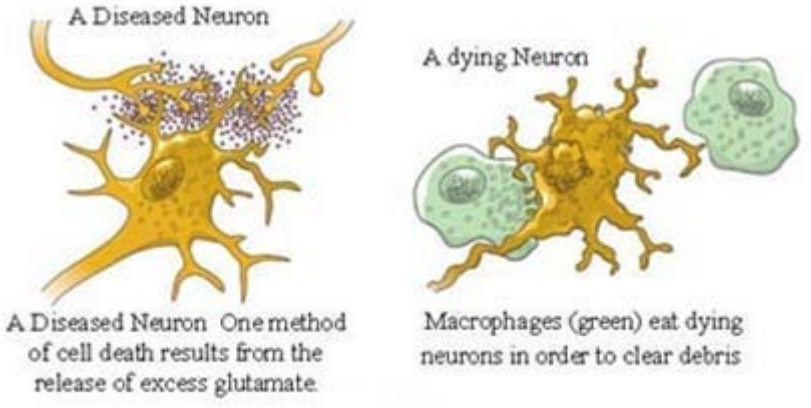


Fig. 23

So, we need to think in what way a couple to be fit to work cells. The proper medicine has the power to clear diseases and can run a couple in proper ways. We need to know the number of cells worked in a body. It is possible to find many numbers from a couple systems. Therefore, the application of finding of a number of cells is vital one. The following figure of Ependymal cells [14] of the brain functioned as a couple system. If one of the cells breaks to complete its function, then it is to be assumed that memory cells will lose their property. As a result, the number of cells differs from the original format.

So, it needs to function in a body in a properly adding medicine or by sending a messenger to the brain to keep fit. The coupling can multiply by numbers as to produce many cells. Many coupling is possible at a time to yielding a cell's birth also.

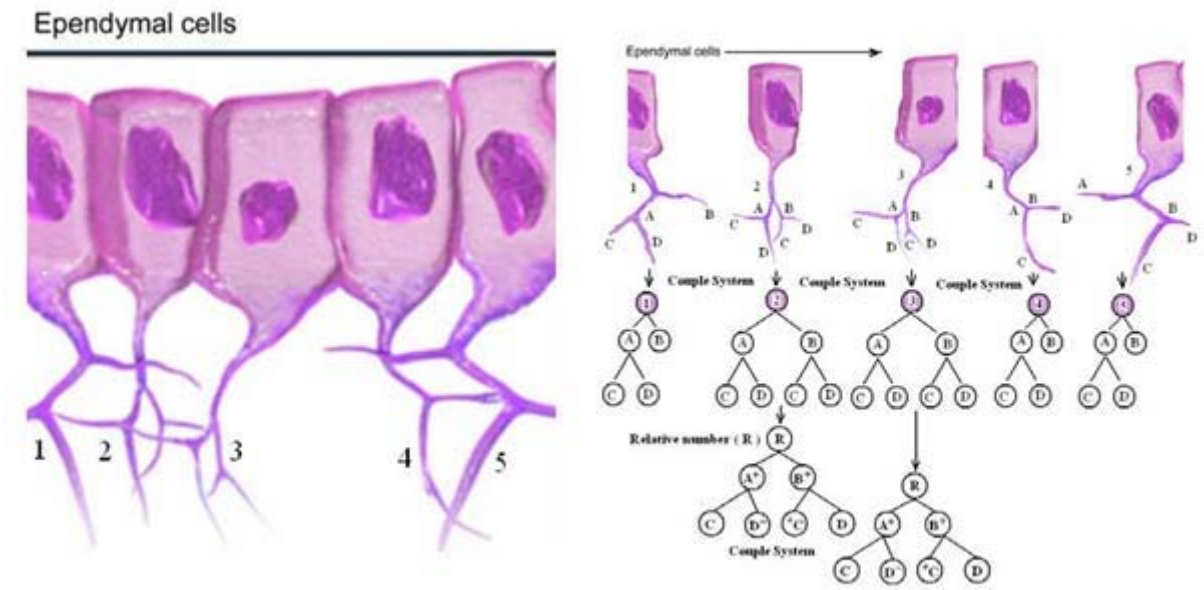


Fig. 24

Functions of Couple System in the Brain Cells, Fig. 24 and 25.

Another example of the brain cells.

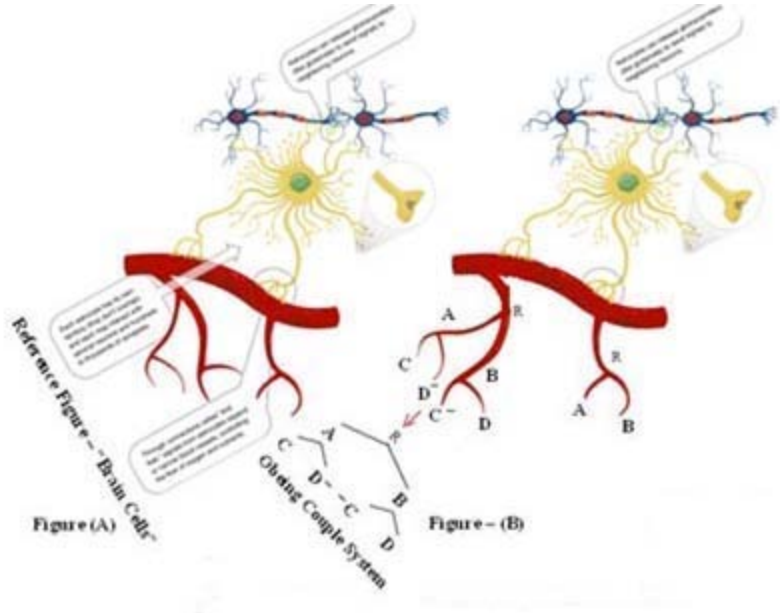


Fig. 25: Brain cells are obeing couple system

Calculation of the number of cells

$M^3 \times 3 \times 2 = 1^3 \times 3 \times 2 = 6000 = 6 \times 10^3 = 6$ thousand x 2 = 12000, (11000 Neurons in Pond Snail brain) [9]. Now ratio of $M^3 \times 3 \times 2 = 1^3 \times 3 \times 2 = 6000$ number and $M^2 \times 2 \times 2 = 1^2 \times 2 \times 2 = 40$ numbers = 6000/40 = 150. But 6000 / (3x2) = 1000 & 1000x100 = 100000, the number of hairs that someone has on their head can vary by individual. However, the average person has about 100,000 hairs on their head at one time [15]. Again, 150 x1000 = 150000 number of blonde hair. [15].

$M^4 \times 3 \times 3 = 1^4 \times 3 \times 3 = 90000 = 9 \times 10^4 = 0.09$ million = 90 thousand of red hair [15].

Ratio of $M^4 \times 3 \times 3 = 1^4 \times 3 \times 3 = 90000$ and $M^3 \times 3 \times 2 = 1^3 \times 3 \times 2 = 6000$ & 90000/6000 = 15.

$M^5 \times 4 \times 3 = 1^5 \times 4 \times 3 = 1200000 = 1.2 \times 10^6 = 1.2$ *million.* Now, 1.2 million / 90000 = 13.33.

Application of $1.2x10^6$ ------

Numbers from Couple System *Cells* *[References]*

1) $1.2x10^6 = 1.2$ million A healthy adult male can release between 40 million and 1.2 billion sperm cells in a single ejaculation. [16]
2) $1.2 \times 10^6 = 1.2$ million The number of fibers in human optic nerve = 1,200,000 [17].
3) $1.2x10^6 / 3 = 400000$ The purple structures inside the ovary are immature egg cells, or oocytes. All of the 400,000 egg cells a woman will ever produce are already present in her ovaries when she is born, although the eggs are in an undeveloped form [18] and 400000 / 10 = 40000, The human gut alone contains on average: 40,000 bacterial species [25d], 40000 / 2 = 20000, According to Asher Mullard, "Between them [the bacteria in our bodies], they harbor millions of genes, compared with the paltry 20,000 estimated in the human genome. [19]
4) 1.2 million / 10 = 120000 Number of fibers in cat optic nerve = 119,000[20].
5) 1.2 million / 16 = 75000 Number of fibers in albino rat optic nerve = 74,800 [20].
6) 75000 / 10 = 7500 Number of neurons in nucleus of the hypoglossal nerve 7500 / (3/2 = 1.5) = 5000… 4,500-7,500[20].
7) 7500 / 2 = 3750 Number of hair cells in cochlea = 3,500 inner hair cells [21].
8) 1.2 million/100 = 12000 Number of hair cells in cochlea 12,000 outer hair cells [21].
9) 1200000 / (3/2)= 800000 Number of retinal ganglion cells = 800 thousand to 1 million.
10) 1200000 /√ (3/2) = 97979.58 ≈ 1 million. Again, 1200000x√(3/2) = 14.6969 ≈ 14.7 million. There are about 0.7 to 1.5 million retinal ganglion cells in the human retina [22].
11) 800000 / 10 = 800000
    800000 / √2 = 565685.42 Number of neurons in the human LGN 565,835[23].

It is not possible to know all cells of all animals in our Earth; these are the examples to support the number yielded from a couple system, and it is related to different cells.

$M^6 \times 4 \times 4 = 1^6 \times 4 \times 4 = 16000000 = 1.6 \times 10^7 = 16$ million. *Ratio of 16 million/1.2 million = 13.33:1,* for example, Currently the largest artificial neural networks, built on supercomputers, have the size of a frog brain (about 16 million neurons) [20].

$M^7 \times 5 \times 4 = 1^7 \times 5 \times 4 = 200000000 = 2 \times 10^8 = 0.2$ billion = 200 million / 10 = 20 million, (Neurons in the brain of rat, 15000000 - 21000000) [24]. *Ratio = 200 million/16 million = 12.5:1*

$M^8 \times 5 \times 5 = 1^8 \times 5 \times 5 = 25000000000 = 2.5 \times 10^9 = 2.5$ billion /10 = 250 million (Number of fibers in corpus callosum = 250,000,000) [25]. *Ratio = 2.5 billion/0.2 billion = 12.5:1*

$M^9 \times 6 \times 5 = 1^9 \times 6 \times 5 = 30000000000 = 3 \times 10^{10} = 30$ billion. *Ratio = 30 billion/2.5 billion =12:1.*

$M^{10} \times 6 \times 6 = 1^{10} \times 6 \times 6 = 360000000000 = 36 \times 10^{10} = 360$ billion, $1/10^{th}$ of this is number 36 billion. Average number of neocortical glial cells (older adults) =36 billion [26].

Ratio = 360 billion/30 billion = 12:1.

Here we see, when $M^1 \times 2 \times 1$ then, negative portions is $M^{-1} \times 1 \times 0$, we can arrange this figure as:

1) $(M^1 \times 2 \times 1) \times (M^{-1} \times 1 \times 0) = M^1 \times M^{-1} \times 2 \times 1 \times 1 \times 0 = 0$ Now, $2/0 = 2$
2) $(M^1 \times 2 \times 1) / (M^{-1} \times 1 \times 0) = 10 \times 2 / 0 = 20$ Now, $2/0 = 2$
3) $(M^1 \times 2 \times 1) + (M^{-1} \times 1 \times 0) = 10 \times 2 + 0 = 20$ Now, $2/0 = 2$
4) $(M^1 \times 2 \times 1) - (M^{-1} \times 1 \times 0) = 10 \times 2 - 0 = 20$ Now, $2/0 = 2$
5) $M^2 \times 2 \times 2 \times M^{-2} \times 0 \times 0 = 0$ Now, $4/0 = 4$
6) $(M^2 \times 2 \times 2) / (M^{-2} \times 0 \times 0) = 100 \times 4 / 0 = 400$ Now, $4/0 = 4$
7) $(M^2 \times 2 \times 2) + (M^{-2} \times 0 \times 0) = 100 \times 4 + 0 = 400$ Now, $4/0 = 4$

$(M^2 \times 2 \times 2) - (M^{-2} \times 0 \times 0) = 100 \times 4 - 0 = 400 \times \sqrt{2} = 5656.8$, (Neurons in the brain of Jellyfish is 5600) [27]. Again, 400 x 10 = 4000, The ANC is found by multiplying the WBC count by the percent of neutrophils in the blood. For instance, if the WBC count is 8,000 and 50% of the WBCs are neutrophils, the ANC is 4,000 (8,000 × 0.50 = 4,000). The most important infection-fighting WBC is the neutrophil (NEW-truh-fil). The number doctors look at is called your absolute neutrophil count (ANC). A healthy person has an ANC between 2,500 and 6,000 [28].

Again, $(M^2 \times 2 \times 2) - (M^{-2} \times 0 \times 0) = 100 \times 4 - 0 = 400 \times 4 = 1600$ is for acting as "Within germinal centers, B cells proliferate and mutate the genetic region coding for their surface antibody (also known as immunoglobulin). The process is called somatic hypermutation and is responsible for introducing spontaneous mutations with a frequency of about 1 in every 1600 cell division (a relatively high frequency considering the low mutation frequency of other cells of the body is 1 in 10^6 cell divisions)…." [29].

In this case, for 1600 cell division, when acts with 10^6 cells, then we get 1.6×10^9 cells, and it is equal to $(M^6 \times 4 \times 4) = 1.6 \times 10^6$, and 1000 times of this value is $= 1.6 \times 10^9$ or 1.6 billion. Again, $(M^6 \times 4 \times 4) = 1.6 \times 10^6 / (3/2) = 1.066 \times 10^6$ or about 1 million. The *total number of Sodium pumps for a small neuron = 1 million* [30]. Again, $\sqrt{1.066 \times 10^6}$ number = 1032.47number and $800 \times \sqrt{1.5} = 979.79$ number. The scientists estimated that "Researchers have learned a lot about this worm — enough for several Nobel Prizes — and they know that there are exactly 1,031 cells in the adult male and 959 in the adult hermaphrodite (there is no female C. Elegans)" [31].

$$M^3 \times 3 \times 2 \times M^{-3} \times 0 \times -1 = M^3 \times M^{-3} \times 3 \times 2 \times 0 \times -1 = 0$$

8) $(M^3 \times 3 \times 2) / (M^{-3} \times 0 \times -1) = 1000 \times 6 / 0 = 6000$
9) $(M^3 \times 3 \times 2) + (M^{-3} \times 0 \times -1) = 1000 \times 6 + 0 = 6000$
10) $(M^3 \times 3 \times 2) - (M^{-1} \times 0 \times -1) = 1000 \times 6 - 0 = 6000$
11) $(M^4 \times 3 \times 3) \times (M^{-4} \times -1 \times -1) = 90000 \times 0.0001 = 9$, but 90000 The nervous system: more than 90,000 miles of sensations [32] and according to University of Rochester Medical Center (UMRC), these are the normal range of WBCs per microliter of blood (mcL) [33]

Age range	*WBC count per mcL of blood.*
Newborns	9000 to 30000, let $9000 \times (3/2)^2 \times 3/2 = 3.375 = 30375 \approx 30000$
Children under 2	6200 to 17000, let $9000 / \sqrt{2} = 6363.9 \approx 6200$ & $9000 \times 2 = 18000 \approx 17000$
Children over 2 and adults	5000 to 10000, let $18000 / 4 = 4500 \approx 5000$ & $9000 \times \sqrt{3}/2 = 11022.7 \approx 10000$. Or 9000 is near value of 10000.

12) $(M^4 \times 3 \times 3) / (M^{-4} \times -1 \times -1) = 90000 / 0.0001 = 9 \times 10^8 = 0.9$ billion x 100 = 90 billion, application of 90 billion:

a) $9x10^8$ x10 = $9x10^9$ x3 = $2.7x10^{10}$ = (difference of $5.00x10^{10}$ & $3.20x10^{10}$ is $2.7x10^{10}$ cell of mean cell number of Adipocytes and standard deviation of it, An average human adult has 30 billion fat cells with a weight of 30 lbs or 13.5 kg[34].
b) $9x10^8$ /6 = $1.5x10^8$ ≈ Femoral cartilage cells ($1.49x10^8$ cells) [35].
c) $9x10^8$ /7 = $1.5x10^8$ = $1.285x10^8$ ≈ $1.23x10^8$ = (Humeral head cartilage cells) [35].
d) $9x10^8$ /11 = $8.18x10^7$ ≈ $8.06x10^7$ = (Talus cartilage cells) [35].
e) $4x10^9$ cells / 2 = $2x10^9$ = $2x10^9$ cells (Heart muscle cells) [36].

12) (M^4 x 3 x 3) + (M^{-4} x -1 x -1) = 90000 + 0.0001 = 90000.0001
13) (M^4 x 3 x 3) – (M^{-4} x -1 x -1) = 90000 – 0.0001 = 89999.9999 = 90000 x10 = 9 million,
14) (M^5 x 4 x 3) x (M^{-5} x -1 x -2) = 1200000 x 0.00002 = 24
15) (M^5 x 4 x 3) / (M^{-5} x -1 x -2) = 1200000 / 0.00002 = 6 x 10^9 = 6 billion x 10 = 60 billion ≈ 60.84 billion glia cells in brain [37].

Discussion: Coefficient of M^5 & M^{-5} is (4 x 3) / (-1 x -2) = 12/2 = 6, Number of cortical layer = 6. The percentage of oxygen consumption by white matter = 6 and by gray matter = 94 % [38].

In general, neuroglial cells are smaller than neurons; there are about 86 billion neurons and 85 billion "nonneuronal" (glial) cells in the human male brain. While that of the cerebellum is only 0.23 (16.04 billion glia; 69.03 billion neurons). The ratio in the cerebral cortex gray matter is 1.48, and the combined gray and white matter is 3.76.[33] The ratio of the basal ganglia, diencephalon, and brainstem combined is 11.35 [39].

16) We see that, 60 billion x√2 = 84.852 billion ≈ 85 billion glial cells. Therefore, the equation (M^5 x 4 x 3) / (M^{-5} x -1 x -2) is tallied to glia cells in human brain. Again, in the case of cerebral cortex gray matter is 16.04 billion glia and 69.03 billion neurons. Again, 84.852/(3/2) billion = 69.28 billion ≈ 69.03 billion neurons. When th is equation takes place in the form of (M^5 x 4 x 3) x (M^{-5} x -1 x -2), then we get = 1200000 x 0.00002 = 24 number / (3/2) = 16. So, when 69 billion neuron cells will present in brain, then, rest of the glia will 16 billion. Now, 69 billion / 16 billion = 0.23 (present cerebellum in brain). If glia cell decreased as 16 billion cells /√(3/2), we get 13.06 billion and then, 13.06 billon / 69 billion = 0.189 or 0.19, this factor save from cerebellum attack.

If we arrange, (4 x 3) & (-1 x -2) as (4 + 3) = 7 and 1/ (-1 x -2) = ½ = 0.5, now, 7 + 0.5 = 7.5/2 = 3.75 this number brings the ratio of combined gray and white matter in the brain (3.76). Again, (4 – 3) = 1 and 1/ (-1 x -2) = ½ = 0.5, now, 1 + 0.5 = 1.5 which is almost same value of 1.48, the ratio of cerebral cortex gray matter. These facts prove that the cells obey the couple system in all respect.

17) (M^5 x 4 x 3) + (M^{-5} x -1 x -2) = 1200000 + 0.00002 = 1200000 = 1.2 million x 2 = 2.4 million. There are about 2.4 million to 3 million *ganglion cells* in the human visual system.
18) (M^5 x 4 x 3) – (M^{-5} x -1 x -2) = 1200000 – 0.00002 = 1200000 = 1.2 million / (3/2) = 800000, (Number of retinal ganglion cells = 800 thousand to 1 million) [40]
19) (M^6 x 4 x 4) x (M^{-6} x -2 x -2) = 16000000 x 0.000004 = 64
20) (M^6 x 4 x 4) / (M^{-6} x -2 x -2) = 16000000 / 0.000004 = 400 x 10^9 = 400 billion x√2 = 565.68 (In fact, the average male will produce roughly 525 billion sperm cells over a lifetime and shed at least one billion of them per month) [41]. So, 565.68 will be the maximum sperm cells over a life time.
21) (M^6 x 4 x 4) + (M^{-6} x -2 x -2) = 16000000 + 0.000004 = 16000000 = 16 million / 4 = 4000000, (Neurons in the brain of Mouse, 4000000) [9]. But 4000000/40 = 100000 or 10^5, the average loss of neocortical neurons = 100000 or 10^5 per day [42]. Again, 100000/100 = 1000 and 100000/10 = 10000 number, number of synapses for a "typical" neuron = 1000 to 10000 [43].
22) (M^6 x 4 x 4) – (M^{-6} x -2 x -2) = 16000000 – 0.000004 = 16000000 = 16 million, (Neurons in the brain of Frog, 16000000) [44].
23) (M^7 x 5 x 4) x (M^{-7} x -2 x -3) = 200000000 x 0.0000006 = 120
24) (M^7 x 5 x 4) / (M^{-7} x -2 x -3) = 3.333333333 x 10^{14} = 333 x 10^{12} = 333 trillion, 3.33 x 10^{14} / 9 = 3.737 x 10^{13} or 37.04 trillion, Dr. Bianconi and her colleagues concluded that there were 3.72 x 10^{13} cells in each of us. That is, 37.2 trillion [45].
25) (M^7 x 5 x 4) + (M^{-7} x -2 x -3) = 200000000 + 0.0000006 = $2x10^8$ = 200 million, now ½ of this 100 million Number of neuron of Cockroch cells [46]. Again, 100 million x 5 = 500 million --- An octopus brain is formed by 500 million large neurons (while the human brain is made of roughly 100 billion smaller neurons), but the intelligence of this aquatic creature is comparable with that of the apes. [47].
26) (M^7 x 5 x 4) – (M^{-7} x -2 x -3) = 200000000 - 0.0000006 = $2x10^8$ = 200 million, Brown rat contain 200 million cells [48].

On the other hand, we can arrange the series as:

A) $M^1 \times 2 \times 1 - M^0 \times 1 \times 1 = 20 - 1 = 19$ (Ratio = (2x1)/(1x1) = 2:1)

$M^2 \times 2 \times 2 - M^1 \times 2 \times 1 = 400 - 20 = 380$, (Neurons in the brain of Roundworm, 302) [49]. *Caenorhabditis elegans* (roundworm)

(Ratio = (2x2)/(2x1) = 2:1)

$M^3 \times 3 \times 2 - M^2 \times 2 \times 2 = 6000 - 400 = 5600$ (Ratio = (3x2)/(2x2) = 3/2 = 1.5:1)

B) $M^4 \times 3 \times 3 - M^3 \times 3 \times 2 = 90000 - 6000 = 84000 = 8.4 \times 10^4 \times 3 = 252000$ = Brain cells of ant is about 250000 [50] (Ratio = (3x3)/(3x2) = 3/2 = 1.5:1)

$M^4 \times 3 \times 3 - M^3 \times 3 \times 2 = 90000 - 6000 = 84000 = 8.4 \times 10^4 \times 3 = 252000$ = Brain cells of ant is about 250000 [50] (Ratio = (3x3)/(3x2) = 3/2 = 1.5:1)

Again, a) $8.4 \times 10^4 \times 3/2 = 1.26 \times 10^5$, (Cell-associated viral loads for sorted memory CD4+ T cells) and it losses day after day from the memory, range from $1x10^5$ to $2x10^5$ cells) [51]. Therefore, we can increase the memory by adding at least $1.26x10^5$ cells to memory through proper medicine. In almost primary stage of couple system this $M^4 \times 3 \times 3 - M^3 \times 3 \times 2$ factors is responsible to loss of memory. b) $8.4 \times 10^4 \times \sqrt{3}/2 = 1.0287x10^5$, the average loss of neocortical neurons = 100000 or 10^5 per day [52]. Both the results are almost same. More investigation is required in this field. 3/2 is the spin of the cells when to be act in time in the reaction.

The decrease in PV-specific CD8 T cells was also even more apparent when the percentage was translated into absolute numbers per spleen, as there was an overall reduction in the size of the spleens of the virus-infected mice at this point (NP38: PV-immune, $5.8 \pm 2.5 \times 10^5$; PV + LCMV Armstrong, $2.4 \pm 1.3 \times 10^5$; PV + LCMV-clone 13, $1.5 \pm 0.4 \times 10^5$). We assume that these observed reductions in virus-specific T cells as monitored by intracellular IFN-γ production indicate a loss in T cell number instead of just function, because there is a loss in the total number of CD44highCD8+ cells [53].

Now value of

i) For $5.8 \pm 2.5 \times 10^5$ cells,
$5.8 + 2.5 \times 10^5 = 8.3 x10^5$ cells supporting the couple number, $8.4 \times 10^4 \times 10 = 8.4 x10^5$
$5.8 - 2.5 \times 10^5 = 3.3x10^5$ cells supporting the couple number, $8.4x10^5/2\sqrt{(3/2)} = 3.429 x10^5$

ii) For $2.4 \pm 1.3 \times 10^5$ cells,
$2.4 + 1.3 \times 10^5 = 3.7x10^5$ cells supporting the couple number, $8.4 \times 10^4 \times 4 = 3.36 x10^5$
$2.4 - 1.3 \times 10^5 = 1.1x10^5$ cells supporting the couple number, $3.36 x10^5 / 3 = 1.12 x10^5$

iii) For $1.5 \pm 0.4 \times 10^5$ cells,
$1.5 + 0.4 \times 10^5 = 1.9x10^5$ cells supporting the couple number, $8.4 \times 10^4 \times 2 = 1.68 x10^5$
$1.5 - 0.4 \times 10^5 = 1.1x10^5$ cells supporting the couple number, $3.36 x10^5 / 3 = 1.12 x10^5$

To calculate these values, we can assume that minimum of $1.26x10^5$ cells be active in loss of memory. So, it requires filling the cell in a proper way by adding medicine, which can play in the coupled system to increase memory.

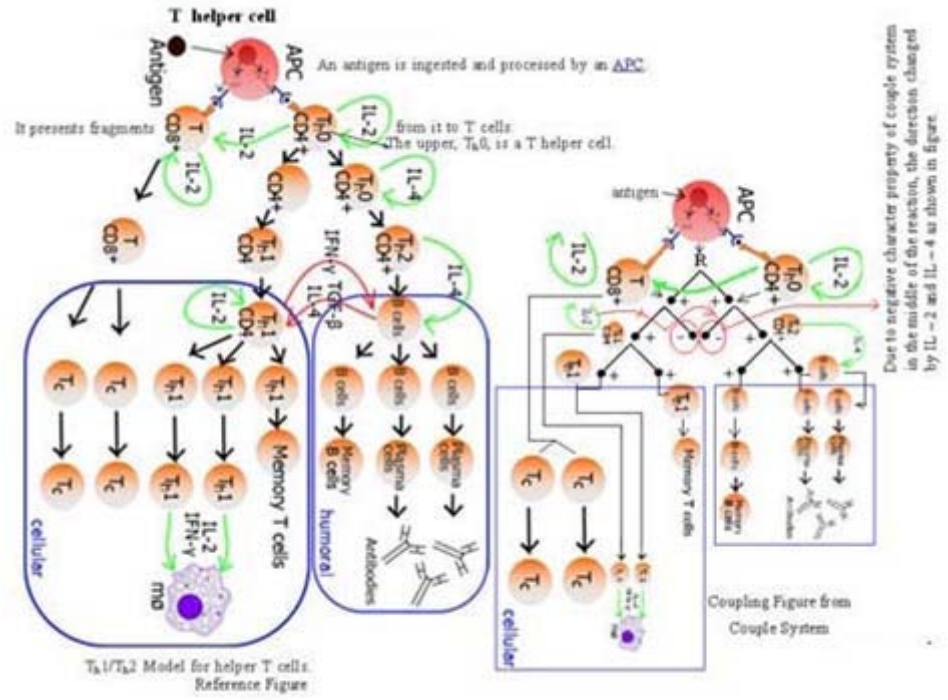


Fig. 26

Cell-associated viral loads for sorted memory CD4+ T cells

C) $M^5 \times 4 \times 3 - M^4 \times 3 \times 3 = 1200000 - 90000 = 1110000 = 1.1$ million,(Neurons in the brain of Cockroach, 1 million) [54]. (Ratio = (4x3)/(3x3) = 1.3333)

D) $M^6 \times 4 \times 4 - M^5 \times 4 \times 3 = 16000000 - 1200000 = 14.8 \times 10^6 = 14.8$ million (Ratio = (4x4)/4x3) = 1.7777. but $14.8 \times 10^6 / \sqrt{(3/2)} = 12.084 \times 10^6$. We see that ½ of 12 million or 6 million cone cells and 10 times of 12 million or 120 million rod cells are in human retina [55]. $M^7 \times 5 \times 4 - M^6 \times 4 \times 4 = 200000000 - 16000000 = 1.84 \times 10^8 = 184$ million $\times \sqrt{2} = 260.2$ million, (Neurons in the brain of Common treeshrew is 261×10^6 cells [56].

E) $M^8 \times 5 \times 5 - M^7 \times 5 \times 4 = 2500000000 - 200000000 = 2.3 \times 10^9 = 2.3$ billion $\times 10 = 23$ billion and 23 billion $/\sqrt{(3/2)} = 18.7794$ billion ≈ 19 billion. [57]. The average number of neocortical neurons was 19 billion in female brains and 23 billion in male brains. in terms of cells for male brain, 23 billion/2 = 11.5 billion, African Elephant, 11000000000 [58]. (Ratio = (5x5)/(5x4) = 1.25

F) $M^9 \times 6 \times 5 - M^9 \times 5 \times 5 = 30000000000 - 2500000000 = 27.5 \times 10^9 = 27.5$ billion. (Ratio = (6x5)/(5x5) = 1.2. now 27.5 x109 / $\sqrt{(3/2)} = 22.45 \times 109$ cells in human body. "The average number of neocortical neurons was 19 billion in female brains and 23 billion in male brains."[59]

G) $M^{10} \times 6 \times 6 - M^{10} \times 6 \times 5 = 360000000000 - 30000000000 = 330 \times 10^9 = 330$ billion /√3/2 = 269.44 billion, (Neurons in the brain of Elephant is 267 billion) [60]. (Ratio = (6x6)/(6x5) = 1.2

H) $M^{11} \times 7 \times 6 - M^{11} \times 6 \times 6 = 4200000000000 - 360000000000 = 3840 \times 10^9 = 3840$ billion

Couple shifting type formation: (Ratio = (7x6)/(6x6) = 1.166

a) $M^2 \times 2 \times 2 - M^0 \times 1 \times 1 = 400 - 1 = 399$

b) $M^3 \times 3 \times 2 - M^1 \times 2 \times 1 = 6000 - 20 = 5980$

c) $M^4 \times 3 \times 3 - M^2 \times 2 \times 2 = 90000 - 400 = 89600$

d) $M^5 \times 4 \times 3 - M^3 \times 3 \times 2 = 1200000 - 6000 = 1194000 = 1.194$ million

e) $M^6 \times 4 \times 4 - M^4 \times 3 \times 3 = 16000000 - 90000 = 15910000 = 15.91$ million

f) $M^7 \times 5 \times 4 - M^5 \times 4 \times 3 = 200000000 - 1200000 = 198800000 = 198.8$ million $\times 2 = 3.976 \times 10^8$, (Neuron of Octopus - 300,000,000/500,000,000) [61].

$M^8 \times 5 \times 5 - M^6 \times 4 \times 4 = 2500000000 - 16000000 = 2484000000 = 2.484$ billion $\times 4 = 9.9 \times 109 \approx 10$ billion, Neuron of *False killer whale*, 10500000000 and 2.484 billion x 2 = 4.968 billion, Neuron cells of Greater Kudu [62].

$M^8 \times 5 \times 5 - M^6 \times 4 \times 4 = 2500000000 - 16000000 = 2484000000 = 2.484$ billion. Now,

2.484 billion x 3 = 7.452 billion and 2.484 billion x 4 /√1.5 = 8.1127 billion

It was estimated that long-finned pilot whales have an average of 2.3×10^9 neurons and 8.3×10^9 glial cells in the auditory cortex, and 2.3×10^9 neurons and 7.6×10^9 glial cells in the visual cortex. [63].

g) M^9 x 6 x 5 – M^7 x 5 x 4 = 30000000000 - 200000000 = 29.8 billion x √1.5 = 36.13 billion, Neurons of *Long-finned pilot whale,* 37200000000 [64].

h) M^{10} x 6 x 6 – M^8 x 5 x 5 = 360000000000 - 2500000000 = 357.5 billion. Here we see that 40 times of $2.5x10^9$ is 10^{11} or 100 billion, average number of neurons in the brain is 100 billion [65].

i) M^{11} x 7 x 6 – M^9 x 6 x 5 = 4200000000000 - 30000000000 = $4.17x10^{12}$ = 4170 billion

M^{12} x 7 x 7 – M^{10} x 6x 6 = 49000000000000 - 360000000000 = $4.864\ x10^{13}$ = 48.64 trillion, 48.64 trillion x 2 = 97.28 trillion ≈ about 100 trillion atom ≈ cells for human body [66] (Another way of looking at it is that this is 100,000,000,000,000 or 100 trillion atoms. Interestingly, the number of cells in the human body is estimated to be about the same as the number of atoms in a human cell). We can determine more accurate value as $49x10^{12}$ x 2 = 98 trillion ≈ 100 trillion.

48.64 trillion ∜(3/2) = 39.7144 trillion & 48.64/√2 trillion = 34.3496 trillion, now, average is (39.7144 + 34.3496) trillion /2 = 37.032 trillion or $3.7032x10^{13}$ that we can consider as cell in human body. [As per reference, probably the best we have, falls closer to the low end: Dr. Bianconi and her colleagues concluded that there were 3.72 x 10^{13} cells in each of us. That is, 37.2 trillion [67].

Odd - odd function:
M^{11} x 7 x 6 - M^9 x 6 x 5 = 4,2 x 10^{12} – 3 x 10^{10} = $4.17x10^{12}$ & ratio = (7 x 6) / (6 x 5) = 1.4

Even – even function:
M^{12} x7 x7 – M^{10} x6 x6 = 4.9 $x10^{13}$ – 3.6 x 10^{11} = $4.864\ x10^{13}$ & ratio = (7 x 7) / (6 x 6) = 1.36

Even – odd function:
M^{12} x7x7 – M^{11} x7x6 = 4.9 x 10^{13} - 4.2 x 10^{12} = 4.48 x 10^{13} & ratio = (7x7) / (7x6) = 1.166
Now, √4.48 x 10^{13} = 6693280 and 6693280 x (3/2) = $10.04x10^6$ this value is equivalent to animal adult zebrafish ≈ 10000000 neuron in the brain. [68]

Odd - odd function:
M^{13} x8 x7 – M^{11}x7x 6 = 5.6 x 10^{14} – 4.2 x 10^{12} = 5.558 x 10^{14} & ratio = (8x7) / (7x6) = 1.33
Now, √5.558 x 10^{14} = 23575410.9, this value is equivalent to 23000000 of Mechow's mole rat or 24000000 cells of Hedgehog [69]

Even – odd function:
M^{14} x 8x8 - M^{13} x8 x7 = 6.4 x 10^{15} - 5.6 x 10^{14} = 5.84 x 10^{15} & ratio = (8x8) / (8x7) = 1.14
Now, √5.84 x 10^{15} = 76419892, this value equivalent to 762570000 cells of Gearter kudu [70].

Odd - odd function:
M^{15} x 9x8 - M^{13} x8 x7 = $7.2x10^{16}$ - M^{13} x 8 x 7 = 7.144 x 10^{16} & ratio = (9x8) / (8x7) = 1.28
Now, √7.144 x 10^{16} = 267282622 this value equivalent to 258000000 cells of Cockatiel [71].

For higher derivatives do not apply to living bodies, cells probably will not be permitted to build their bodies. The individual body has its limited power to form cells. Again, from g): M^9 x 6 x 5 – M^7 x 5 x 4 = 30000000000 - 200000000 = 29.8 billion≈ 30 billion x √1.5 = 36.74 billion, Neurons of *Long-finned pilot whale*, 37200000000. Again, 30 billion x 3 = 90 billion, Number of neurons in the Human Nervous System – ~90 billion Number of glial cells [72].

For a long time, scientific estimates of the number of cells in the human body ranged between 10^{12} and 10^{16} [73]
M^{16}x10x10 - M^{15} x 9x8 = 10^{18} – $7.2\ x10^{16}$ = $9.28x10^{17}$, but $9.28x10^{17}/\sqrt{(3/2)}$ = $7.577x10^{17}$ it is 75.77 times larger value than 10^{16}. Maximum limitation of cell number.

How couple equation acts on the body, few examples are there: [74]

Name	Neurons in the brain/whole nervous system	Synapses	Details
Sponge	0	$M^{[2 + 2(N-2)]}.Nr.(N-2)r \rightarrow M^{0}$. 0 x r.(0 – 2)r = 0, when N = 0	
Trichoplax	0	$M^{[2 + 2(N-2)]}.Nr.(N-2)r \rightarrow M^{0}$. 0 x r.(0 – 2)r = 0, when N = 0	Despite no nerve system, it exhibite a coordinated feeding and behaviors.[4]
Asplanchna brightwellii (rotifer)	about 200	$M^{[2 + 2(N-2)]}.Nr.Nr \rightarrow M^{2}.2r.2r$, when, N = 2, N > 1, if, M = r = 1, M2.2r.2r = 400 & 400/2 = 200.	Brain only
Ciona intestinalis larva (sea squirt)	231	8,617 (central nervous system only) $M^{[2 + 2(N-2)]}.Nr.Nr \rightarrow M^{4}.3r.3r$, when, N = 3. If M = r = 1, then, $M^{4}.3r.3r$ = 9000 near value of 8617.	
Caenorhabditis elegans (roundworm)	302	$M^{[1+2(N-2)]}.Nr.(N-1)r \rightarrow M^{3}.3r.2r$, when, N = 3, and $M^{[2+2(N-2)]}.Nr.Nr \rightarrow M^{4}.3r.3r$, when, N = 3. if M = r = 1, then average number= ($M^{3}.3r.2r$+$M^{4}.3r.3r$) /2 = (6000 + 9000) /2 = 7500. ~7,500	is the only organism to have its whole connectome (neuronal "wiring diagram") completed.[9][10][11]
Jellyfish	5,600	$(M^{2}$ x 2 x 2) – (M^{-2} x 0 x 0) = 100 x 4 – 0 = 400 x √2 = 5656.8	*Hydra vulgaris* (*H. attenuate*)
Megaphragma mymaripenne	7,400	$M^{4}.3r.3r$ = 9000 √1.5 = 7348.5 ≈ 7350. Whern M = r = 1	
Box jellyfish	8,700–17,500	$M^{4}.3r.3r$ = 9000 and $2(M^{4}.3r.3r)$ = 18000 ≈ 17,500.	adult *Tripedalia cystophora* (8 mm diameter) – does not include 1000 neurons in each of the four rhopalia
Medicinal leech	10,000	$M^{[1+2(N-2)]}.Nr.(N-1)r \rightarrow M^{3}.3r.2r$, when, N = 3,if M = r = 1, then $M^{3}.3r.2r$ = 6000, but coefficient of M^{3} is 3r.2r. If $M^{3}.3r.2r$ / 3r.2r = 1000.	

Name	Neurons in the brain/whole nervous system	Synapses	Details
Pond snail	11,000	M^3 x 3 x 2 = 13 x 3 x 2= 6000 = 6 x 10^3 = 6000 x 2 = 12000 ≈ 11000.	
Sea slug	18,000	M^4 .3r.3r = 90000 and	
		2x(M^4 .3r.3r) = 18000	
Amphioxus	20,000	$M^{[1+2(N-2)]}$ .Nr.(N – 1)r→ M^3 .3r.2r, when, N = 3,if M = r = 1, then M^3 .3r.2r = 6000, but coefficient of M^3 is 3r.2r. If M^3 .3r.2r / 3r.2r = 1000. If we multiply 1000 x 20 = 20000.	central nervous system only
Larval zebrafish	100,000	$M^{[1+2(N-2)]}$ .Nr.(N – 1)r→ M^3 .3r.2r, when, N = 3,if M = r = 1, then M^3 .3r.2r = 6000, but coefficient of M^3 is 3r.2r. If M^3 .3r.2r / 3r.2r = 1000. If we multiply 1000 x 100 = 100000	
Lobster	100,000	$M^{[1+2(N-2)]}$ .Nr.(N – 1)r→ M^3 .3r.2r, when, N = 3,if M = r = 1, then M^3 .3r.2r = 6000, but coefficient of M^3 is 3r.2r. If M^3 .3r.2r / 3r.2r = 1000. If we multiply 1000 x 100 = 100000	
Fruit fly	250,000	M^4 x 3 x 3 – M^3 x 3 x 2 = 90000 – 6000 = 84000 = 8.4 x 10^4 x 3 = 252000 ≈ $< 10{,}000{,}000$ 250,000	In 2020 a research group announced the most sophisticated connectome [23]
Ant	250,000	M^4 x 3 x 3 – M^3 x 3 x 2 = 90000 – 6000 = 84000 = 8.4 x 10^4 x 3 = 252000 ≈ 250,000	Varies per species
Honey bee	960,000	$\sim 1 \times 10^9$ M^4 .3r.3r = 9000 x 100 = 900000	
Cockroach	1,000,000	$M^{[1+2(N-2)]}$ .Nr.(N – 1)r→ M^3 .3r.2r, when, N = 3,if M = r = 1, then M^3 .3r.2r = 6000, but coefficient of M^3 is 3r.2r. If M^3 .3r.2r / 3r.2r = 1000. If we multiply 1000 x 1000 = 1000000	
Guppy	4,300,000	(M^5 x 4 x 3 = 1^5 x 4 x 3) x 4 = = 4800000	
Adult zebrafish	~10,000,000	[(M^5 x 4 x 3 = 1^5 x 4 x 3) / (4x3)] x 10 =	cells (neurons + other)

Name	Neurons in the brain/whole nervous system	Synapses	Details
		1000000	
Frog	16,000,000	M^{6} x 4 x 4 = 1^{6} x4x4 = 16000000	
Naked mole-rat	26,880,000	⌈(M^{6} x 4 x 4 = 1^{6} x4x4)⌉ x 2 = 32000000 /√1.5 = 26127890. 59	
Smoky shrew	36,000,000	⌈M^{4} x 3 x 3⌉ x4 = ⌈1^{4} x 3 x 3⌉ x 4 = ⌈90000⌉ x 4 = 360000 and ⌈360000⌉ x 100 = 36000000	
Short-tailed shrew	52,000,000	⌈(M^{6} x 4 x 4 = 1^{6} x4x4)⌉ x 4 = 64000000 /√1.5 = 52.2255781.18	
Hottentot golden mole	65,000,000	⌈(M^{6} x 4 x 4 = 1^{6} x4x4)⌉ x 4 = 64000000	
House mouse	71,000,000	$\sim 1\times10^{12}$ ⌈(M^{6} x 4 x 4 = 1^{6} x4x4)⌉ x 4 = ⌈64000000 x √1.2 + 64000000 x√1.3⌉ /2 = 71539857.28	
Nile crocodile	80,500,000	⌈(M^{6} x 4 x 4 = 1^{6} x4x4)⌉ x 4 = 64000000 x 5 = 80000000 Or. {⌈M^{7} x 5 x 4 = 1^{7} x 5 x 4⌉ x⌈5 x 4⌉ } / 5 = 40000000 x 2 = 80000000	
Golden hamster	90,000,000	M^{4} .3r.3r = 9000 x 10000 = 90000000	
Ansell's mole-rat	103,000,000		
Mashona mole-rat	113,000,000	M^{6} x 4 x 4 = 1^{6} x4x4)⌉ x 10 = 160000000 /√2 = 113137085	
Hairy-tailed mole	124,000,000	M^{7} x 5 x 4 − M^{5} x 4 x 3 = 200000000 − 1200000 = 198800000 = 198.8 million =	

Name	Neurons in the brain/whole nervous system	Synapses	Details
		$0.1988 \times 10^{9} / {}^{16} = 12425000 \times 10 = 124250000$	
Eastern rock elephant shrew	129,000,000	$100 \times (M^{6} \times 4 \times 4) = 1.6 \times 10^{6} / \sqrt{(3/2)} = 1.306 \times 10^{8} \approx 129,000,000$	
Star-nosed mole	131,000,000	$M^{7} \times 5 \times 4 - M^{5} \times 4 \times 3 = 200000000 - 1200000 = 198800000 = 198.8$ million $= 0.1988 \times 10^{9} / 1.5 = 132533333$	
Zebra finch	131,000,000	Do	Brain only
Silvery mole-rat	148,000,000	$[(M^{6} \times 4 \times 4 = 1^{6} \times 4 \times 4)] \times 10 = 160000000 \times 3 = 148000000$	
Four-toed elephant shrew	157,000,000	$[(M^{6} \times 4 \times 4 = 1^{6} \times 4 \times 4)] \times 10 = 160000000 \times 3 = 148000000 \times \sqrt{1.1} = 155223709$	
Eurasian blackcap	157,000,000	Do	
Goldcrest	164,000,000	$[(M^{6} \times 4 \times 4 = 1^{6} \times 4 \times 4)] \times 10 = 160000000 \times 3 = 148000000 \times 1.1 = 162800000$	

III. Regarding Brain Cells

Herculano-Houzel and her colleagues used this technique to analyze the brains of four deceased men and published their results in 2009: they consistently found whole human brain glia to neuron ratio of almost 1:1. Specifically, they found that the human brain contains about 170.68 billion cells, 86.1 billion of which are neurons, and 84.6 billion of which are glial cells. Their study also suggests that the ratio of glia to neurons differs dramatically from one general brain region to the next. 60.84 billion cells in the cerebral cortex are glia, while only 16.34 billion cells are neurons, giving this region glia to neuron ratio of about 3.76 to 1. It's the inverse in the cerebellum, an evolutionarily ancient part of the brain that sits astride the brain stem.

According to Herculano-Houzel's study, the cerebellum contains 69.03 billion neurons and only 16.04 glial cells, which means there are about 4.3 neurons for every glia in this region.[75]. If we apply the relative number of couple system & classify, we can get different numbers of the brain and shown in Fig 27- Brain here.

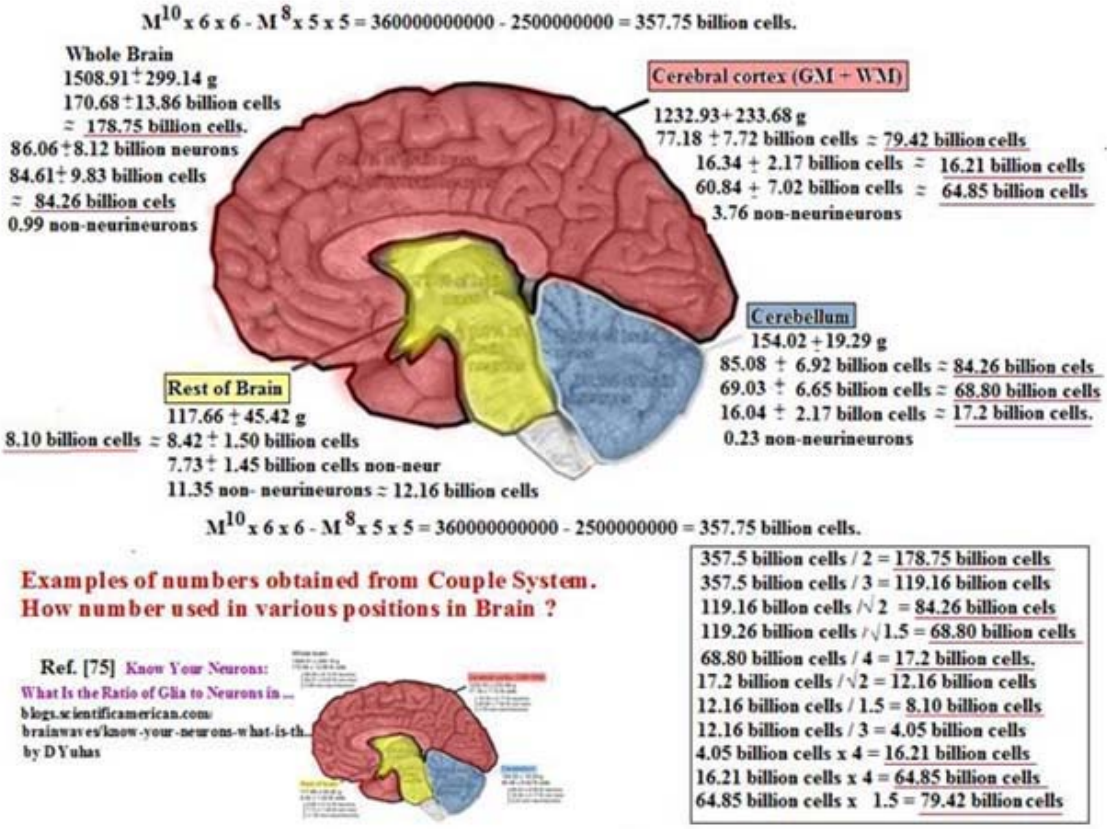


Fig. 27: Coupling mechanism of Brain Cells

Adapted from "Equal Numbers of Neuronal and Nonneuronal Cells Make the Human Brain an Isometrically Scaled-Up Primate Brain"

a) Memory (Mind & its activity)

What is gray matter in the brain?

According to reference [76], the grey matter is mainly composed of neuronal cell bodies and unmyelinated axons. Axons are carrying signals between those bodies. The grey matter serves to process information in the brain. Structures within the grey matter process signals generated in the sensory organs or other areas of the grey matter. These signals reach the grey matter through myelinated axons that make up the bulk of the white matter in the cerebrum, cerebellum, and spine. Also found in the grey matter are the glial cells (astroglia and oligodendrocytes) and capillaries. The glial cells transport nutrients and energy to the neurons and may even influence how well the neurons function and communicate.

What is the white matter in the brain?

White matter, on the other hand, is mainly composed of long-range myelinated axons (that transmit signals to the grey matter) and very few neuronal cell bodies. Myelin forms a protective coating around these axons, insulating them and improving their transmission of neuronal signals. White matter is found buried in the inner layer of the brain's cortex, while the grey matter is mainly located on the surface of the brain. The spinal cord is arranged in the oppositely way, with grey matter found deep inside its core and the insulating white matter wrapped around the outside. Some grey matter is also found deep inside the cerebellum in the basal ganglia, thalamus, and hypothalamus and white matter is also found in the optic nerves and the brainstem. How Gray and White Matter functioned through a couple system, that is given here. Glia and neuron cells are present in the gray matter. Brain and mind are related, and this relationship works to do think which stored in memory. It transmits timely from a store and hits mind that what happened in the past. In the present situation, the mind has determinable property. But for the future, which we think for tomorrow, all-time it may not be applicable due to variation of the present situation. A couple system is a system of dual property between two objects (A) & (B) related to the third party (R). This third party ordered to do function. Suppose, he loves a girl, love comes out from the mind; here mind is a third property which calls each other to meet in the form of the direction of love. A figure is given here, for an example:

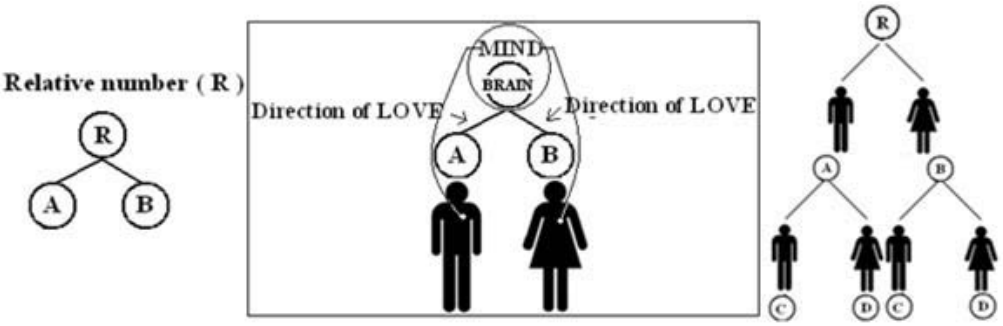


Fig. 28

Couple system is related to Mind and Brain

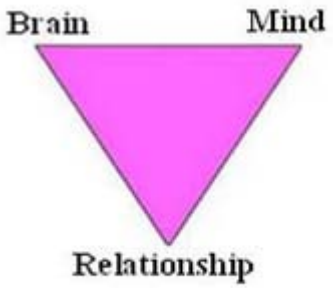


Fig. 29

Fig. 30

[Reference – 77] [From the view of Couple System]

A taken from Reference and Figure – 30 is the reverse of Figure – 29 and obeying the figure of Couple System In figure – 28, we see that A & B is related to R. This R may term as RELATIVE NUMBER between A & B. This relation will continue when the series extend from A & B to C & D to keep relation R. Let, A & B placed in A zone and B zone respectively, and C & D will produce another zone as C zone and D zone. If C & D another two number makes relation to A & B together, then the zone may be represented by a round symbol as , and we can write it in the form of:

No. 1, Formation:

The Reaction of a couple:

If A & B takes place in the form of A+ & B+ in the positive zone and C & D takes place in the negative zone in the form of D- & C+ related to A+ & B+ in the positive zone, then if dressed it in the form of:

$R \rightarrow (D^- . A + C^+ B^+)$, we can write, $D^- = -D$ & $C^+ = +C$ ----------(1)

$R \rightarrow (C . A + C . A)$ ---------- (2)

$R \rightarrow 2\,CA$ ---------- (3)

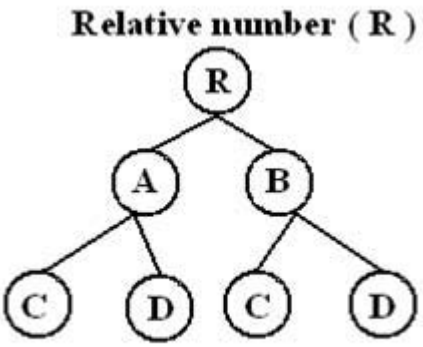


Fig. 31

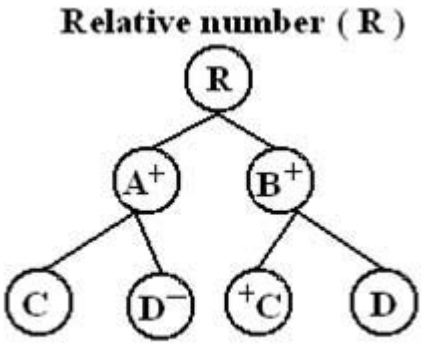


Fig. 32

The Reaction of a couple:

If A & B takes place in the form of A^+ & B^+ in the positive zone and C & D takes place in the negative zone in the form of D^- & C^+ related to A+ & B+ in the positive zone, then if dressed it in the form of:

$R \rightarrow (D^- . A + C^+ . B^+)$, we can write, $D^- = - D$ & $C^+ = + C$ ---------- (1)

$R \rightarrow (C . A + C . A)$ ---------- (2)

$R \rightarrow 2\ CA$ ---------- (3)

Description:

1) D is related to A (Fig-31, L.H.S.), and C to B (Fig -32, R.H.S.). But the value of A and C will be same as the value of B and D. D- in L.H.S. means that it is the end of the reaction of the problem to form relative number (R) acting with C+ in R.H.S.
2) The original value of D is C; therefore, D reacts with A and forms CA. Similarly, C is the value of D, but the real value of B is A. So, C reacts with B and forms CA.
3) The total couple reaction with respect to R is 2CA.

When the value of A, B, C, D…., individual, then the result of 2 CA will bring another number [1].

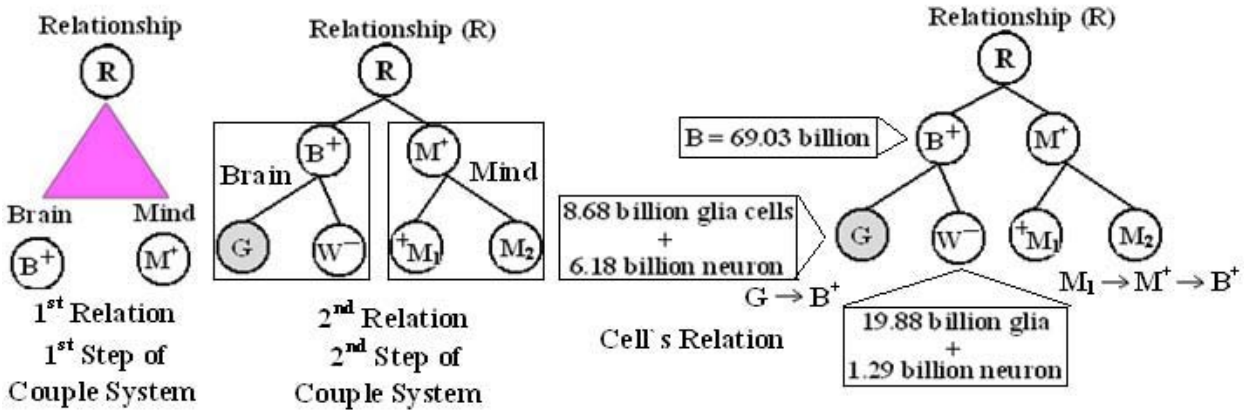


Fig. 33

Here in this figure- 33, suppose, R = Relation between Brain and Mind = 1 Brain + 1 Mind = 2
Total neuron in the brain, B = 69.03 billion, M^+ = Conscious mind
G = Gray Matter (8.68 billion glia cells + 6.18 billion neuron cells),
M_1 = Same to Conscious mind, M_2 = Sub Conscious mind

Effect of Gray Matter by Couple System: (Figure – 33)

$R \rightarrow$ 8.68 billion glia cells x 69.03 billion neuron cells in brain + M_1 x 69.03 billion neuron cells
Or, $R \rightarrow$ 599.1803 billion neuron cells^2 + M_1 x 69.03 billion neuron cells.
Or, M_1 x 69.03 billion neuron cells + 599.1803 billion neuron cells^2 = 2 billion cells^2 need for couple (Assuming that at least 2 billion cells^2 need for function where, R = 1 brain + 1 mind = 2)
Or, M_1 x 69.03 billion neuron cells = 2 billion neuron cells^2 - 599.1803 billion neuron cells^2 = - 597.1803 billion^2.
Or, M_1 = - 599.1803 billion neuron cells^2 / 69.03 billion neuron cells = - 8.65102 billion neuron cells will function mind.

This value 8.65 billion is almost $1/10^{th}$ of 86.1 billion neuron cells out of total of 170.68 billion cells in the brain [75]. - negative sign indicating that mind on functioned to do give the order to work, which is mind-minus. When any view/ think/ …, enter into brain, then we may call it mind-plus. These two compartments (+Memory & - Memory) are very active for the living body. We keep it in mind and stored in memory.

Effect of white matter from the view of a couple systems. (Figure – 33)

$R \rightarrow$ 19.88 billion glia cells x 69.03 billion neuron cells in brain + M_1x69.03 billion neuron cells.
Or, $R \rightarrow$ 1371.779 billion neuron cells^2 + M_1 x 69.03 billion neuron cells
Or, M_1 x 69.03 billion neuron cells + 1371.779 billion neuron cells^2 = 2 billion cells^2 need for couple
Or, M_1 x 69.03 billion neuron cells = 2 billion neuron cells^2 – 1371.779 billion neuron cells^2 = - 1369.779 billion^2.
Or, M_1 = - 1369.779 billion neuron cells^2 / 69.03 billion neuron cells = - 198.432 billion neuron cells will function mind.

For the white region, (Figure – 34, R.H.S.)

Effect of mind = 198.432 billion neuron cells x 1.29 billion cells / (8.68 billion glia + 6.18 billion neuron) = - 17.225 billion cells which is almost 1/10th of 170.68 billion cells of human brain.

This value 8.61 billion is almost 1/10th of 86.1 billion neuron cells out of total of 170.68 billion cells in the brain [74]. The negative sign indicating that mind on functioned to do give the order to work.

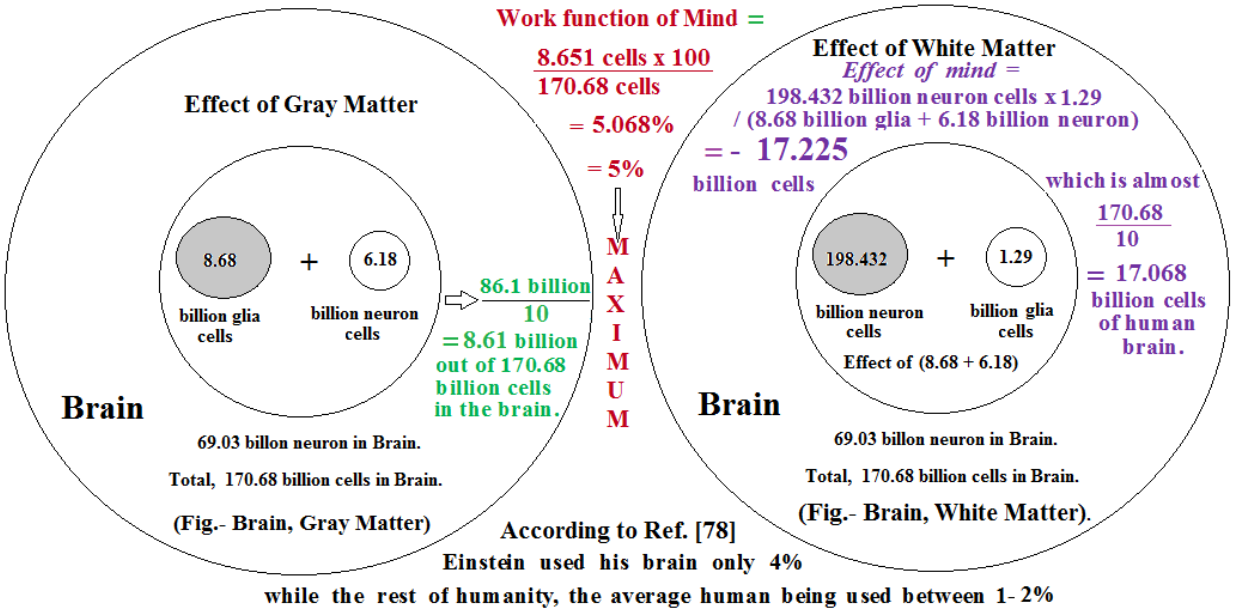


Fig. 34

How many percentage cells work for mind?

For figure – Brain, Gray Matter: (Fig.- 34, L.H.S.)

Work function of the mind = 8.65102 billion cells x 100 / 170.68 billion cells = 5.068% = 5%. Only 5% of cells function for mind in the brain. According to reference [78], Vijay Kumar, who Realized God In 1993 - the connecting link between human form - cosmic mind. He said, "Albert Einstein used his brain only 4% while the rest of humanity, the average human being used between 1-2%, the balance portion always lying dormant. The human brain primarily acts as a receiving and transmitting station. No human being ever had an independent mind. The mind of the entire cosmos is one; it had only two compartments... reservoir of mind plus and reservoir of mind-minus".

For Figure – Brain, White Matter: (Fig.- 34, R.H.S.)

In the white region, this effect will, 17.225 billion cells x 100 / 170.68 billion cells = 10.09 % = 10%. According to reference [79], nearly 90 percent of the brain is composed of glial cells, not neurons. Andrew Koob argues that these overlooked cells just might be the source of the imagination. Astrocytes are also the adult stem cell in the brain and control blood flow to regions of brain activity. Because of all these important properties, and since the cortex is believed responsible for higher thought, scientists have started to realize that astrocytes must contribute to thought. Calcium waves in the cortex are leading scientists to infer that this style of communication may be conducive to the processing of certain thoughts. This idea stems from dreams, sensory deprivation. Without input from our senses through neurons, how is it that we have such vivid thoughts? How is it that when we are deep in thought, we seemingly shut off everything in the environment around us? In this theory, neurons are tied to our muscular action and external senses. We know astrocytes monitor neurons for this information. Similarly, they can induce neurons to fire. Therefore, astrocytes modulate neuron behavior. This could mean that calcium waves in astrocytes are our thinking mind. Neuronal activity without astrocyte processing is a simple reflex; anything more complicated might require astrocyte processing.

From the above writings, we can assume that, for ordinary people used less than 4% comparing Einstein`s brain. Though that will vary from time to time depending on age, brain weight, power of thinking, keeping capacity of the memory of the brain. Altogether, in all cases, mind will touch the field of the body. The brain has capacity 100% to do work, anyone can cover this field. But this is very tub to do. Because we are losing memory, we do not keep the mind in attention in the same direction concerning time. Due to these reasons, a lot of things went out of the

memory which plays with the mind. The function of memory comes from the brain and remarkable memory stored in it; when the mind wishes to search and order to the brain, then it comes out, and we describe on that past facts. Again, we lost many memories every day. Suppose the most common matter, the sun is rising, we are observing this, but we do not keep in mind. That is the uses of everyday things sometimes ignore the mind. But in the case of the sexual field, most of the persons (Men & Women) want to meet together to get their mental and sexual satisfactions. These two opposite nature of the body always wants to attract each other. Due to our social construction, we can't meet an unknown men or women to do sex work. For example, when a beautiful lady passing you, your mind attracts her by eyes and action to be start in your mind that, Oh! Shall I marry her? May I use it? So-so thinks stored in your brain as memory. It is very difficult to explain perfectly about the mind and its behavior connected to the brain. Again, the brain is connected to every parts of the body; mind also obeys this path with brain activity. If I feel pain in my body, immediately brain will attain in that place and my mind does not think other on that moment, I shall not think on universe, planets, other picture, wife, person etc. how I get pain or how I can relieve from it, that will be main feature at that time. Therefore, our mind will work maximum 5 billion cells with effect of 10 billion cells of brain as shown in the *Figure*: Brain, Gray Matter and Brain, White Matter.

IV. Conclusion

Lot of examples is there, the relative number obtained from couple system naturally important. We can apply it in other fields. Body cell is most important, body fit means mentally fit. So need such discover equipment by which cell in a body will stay in normal position. Math is manmade properties; its application to nature helps us to increase the knowledge many ways.

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Global Journal of Science Frontier Research: C
Biological Science
Volume 20 Issue 3 Version 1.0 Year 2020
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Ontogenetic Development of *Hedysarum Alpinum* L. in Prebaikalia

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Abstract- *Hedysarum alpinum L.* is an important medicinal plant, which also has ornamental value and is used as a non-conventional feed resource. The medicinal value of this plant species is attributable to the content of the glycoside mangiferin in the aerial parts of a plant, which demonstrates immunostimulating properties. Harvesting of *H. alpinum* as a herbal raw material leads to a fast depletion of natural coenopopulation areas. When cultivated in Prebaikalia, the plant passes through all ontogenetic stages, entering the reproductive phase at year 2-3 of growth. The mid-reproductive stage, which is the most productive for feeding purposes, lasts 6 to 10 years. For introduced species, the life span of a plant growing in the same place ranges between 12 and 20 years. *H. alpinum* is a promising medicinal and fodder plant, which tolerates dry summer periods, is winter-hardy, and has high shoot biomass production.

Keywords: *hedysarum alpinum, fodder plant, medicinal, ornamental, ontogenesis, introduction.*

GJSFR-C Classification: *FOR Code: 060499*

Strictly as per the compliance and regulations of:

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Elena G. Khudonogova [α], Alena A. Mikhlyaeva [σ] & Svetlana V. Polovinkina [ρ]

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I. Introduction

Fodder production in an especially relevant issue in Prebaikalia with its developed livestock farming. In recent years, along with the bringing of wild plants into cultivation and development of new varieties of fodder crops, special attention has been paid to non-conventional plants, in particular *Hedysarum alpinum L.* (common name: alpine sweetvetch), which is a valuable fodder, medicinal and ornamental plant. In the study territory *H. alpinum* is has not been used as a fodder or ornamental plant, both due to conventional reasons and insufficient knowledge of this species.

H. alpinum is a perennial herbaceous plant of the family *Fabaceae*, growing up to 40-120 cm in height. Stem: upright, bare. Rhizome: thick, long, branched. Leaves: unpaired, 5–9 (10) pairs, oblong-ovate or elongated-elliptical, appressed-pilose on the top. Inflorescence: long thick multi-flowered raceme with 20-30 flowers. Flowers: up to 15 mm long, papilionaceous, on short pedicels with linear bracts, corollas pink, lilac or purple, rarely white, turn purple when dried. Bracts are usually shorter than pedicels. Calyx: about 4 mm long, bell-shaped, irregular, wide-triangular, the smallest calyces are 2-4 times shorter than the calyx tube. Corollas: 10-14 mm long, the keel is slightly longer than wings and the banner, or is nearly the size of the latter. Fruit: bean (8-10 mm long), bean segments: 1-4 (2-5), round-oval, with narrow margins, easily breaking off. Each segment contains one seed enclosed in a hard shell. Bloom period: June-July, ripening: from August to early September [1].

Herbal raw materials are harvested for medicinal use in the blooming period (herb) and during withering of the aerial parts of plants, usually in September (roots). *H. alpinum* herb contains mangiferin, which demon states antiviral activity against herpes simplex, chickenpox, and cytomegaloviruses. Mangiferin is also known as an immunostimulant due to its stimulating action on cellular and humoral immunity and stimulation of the production of gamma-interferon in blood cells; it is used as an active ingredient in the newly developed "Alpizarin" drug. The aerial part contains quercetin, quercetrin, avicularin. Leaves contain flavonoids: hyperoside, mangiferin, isomangiferin, polystachoside and hedizaride, traces of alkaloids, ascorbic acid (up to 137.5 mg%); herb contains triterpene saponins. The underground parts of a plant contain 30-40% of polysaccharides, which hydrolyze down to galactose, xylose, galacturonic acid, and low amounts of rhamnose. The sweetvetch root decoction is used in traditional medicine as an expectorant for treatment of coughing, bronchitis and pulmonary tuberculosis, as well as a sedative for treatment of nervous disorders, insomnia, epilepsy, heartache, and atherosclerosis [2,3,4,5,6].

In the territory of the Russian Federation *H. alpinum* its habitat area spreads from the south of the Kola Peninsula to the Urals and Siberia. It grows in the forest and forest-steppe zone along river and stream valleys. It grows riotously in shrub-and-grass meadows on well-drained, elevated sections of the central parts of floodplains [1].

It grows in the southern regions of Irkutsk Oblast, in the Sayan Mountains, in the Lake Baikal area, in Buryatia, in the Yablonovy Mountains and in Dauria, where is occupies moist forest meadows, shrubs, and river valleys.

No large beds of *H. alpinum* were found in Prebaikalia. Harvesting of *H. alpinum* as a herbal raw material leads to a fast depletion of natural coenopopulation areas.

To preserve this species, introduction studies must be carried out based on the investigation of

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ontogenesis of a species under conditions of the study area.

The aim of the present study was to investigate the ontogenetic development of *H. alpinum* introduced in the forest-steppe zone of the southwestern Prebaikalia. Research objectives: based on morphological characteristics, identify ontogenetic states of the species, trace the progress of *H. alpinum* ontogenesis in the Western Cisbaikalia.

II. Materials and Methods

Study object: H. alpinum. Investigation of the ontogenetic state of the species was carried out in the training and experimental field of Irkutsk State Agrarian University. Scarified *H. alpinum* seeds were planted in May and September.

Planting method: wide-row planting. The soil in the *Experimental field:* grey wood soil.

The age-state structure of coenopopulations of useful plants was determined using a standard method for studying the age-state structure of populations making up the plant association, and in compliance with principles and methods for studying the age structure of coenopopulations developed by Yu.A. Zlobin [7] and Ye.L. Nukhimovskiy [8]. Description of ontogenesis *H. alpinum* growing in Prebaikalia also took into account the data on species biology obtained in the studies by L.I. Fomina carried out in Chita Oblast [9], by N.S. Zinner in the forest-steppe zone of Siberia [10], by N. Portnyagina et al. in the Komi Republic [11,12,13], and by N.A. Karnaukhova, D.V. Sandanov, I.Yu.Selyutina in Buryatia [14].

III. Results and Discussion

Cisbaikalia (or Pribaikalia) is a vast region in the south of Eastern Siberia located in the center of Asia with an area of over 800 thousand sq.km. Cisbaikalia is considered to include the entire area adjacent to Lake Baikal, while the western territories, in a similar way to Transbaikalia (meaning "beyond" (trans-) Lake Baikal), is called Prebaikalia (i.e. "before" Lake Baikal). In the system of physical and geographical zoning of Siberia, most of the Cisbaikalia territory is occupied by two physical and geographical areas: Central Siberia and the mountains of Southern Siberia. According to the landscape zoning of the south of Eastern Siberia, the Cisbaikalia territories occupied by taiga belong to three physical and geographical regions of North Asia: Central Siberian, South Siberian, and Baikal-Dzhugdzhur regions.

The climate of Prebaikalia is determined by the geographical latitude and the position of Lake Baikal almost in the center of Asia, which result in a harsh continental climate. The average January temperature in Cisbaikalia can go down to - 20°C. The average air temperature in July reaches + 15°C in the mountainous regions along the shores of Lake Baikal, and + 18 °C in the central regions of Cisbaikalia. Climate of Prebaikalia is determined by the geographical latitude and the position of Lake Baikal almost in the center of Asia, which result in a harsh continental climate. The average January temperature in Cisbaikalia can go down to - 20 °C. The average air temperature in July reaches + 15 °C in the mountainous regions along the shores of Lake Baikal, and + 18 °C in the central regions of Cisbaikalia. The sum of air temperatures above 10°C is 1550-1670 °C, and the frost-free period lasts about 100 days. Maximum air fluctuations are observed in early spring and late autumn. Annual precipitation ranges 380-480 mm [15].

In the entire Prebaikalia, the most prevalent type of soils is grey wood soils [16], while in island steppes chestnut soils, and less often chernozem and meadow chernozem can be found. There are also saline-alkali soils, and black alkali soils.

Our study results show that *H. alpinum* introduced in the region goes through the following ontogenetic stages (Fig. 1):

1. *Seedling stage:* a 1-1.5 high shoot is formed, with 1-3 simple broadly ovate or rarely round leaves and a 1-3 cm long taproot, with clearly visible round-shaped, yellowish cotyledons [17].
2. *Juvenile stage:* monopodial shoot growth continues, cotyledons whither. At this stage, two subgroups can be distinguished: subgroup 1 of juvenile plants (shoot height increases to 1.6-2 cm, the number of simple leaves increases to 3-6, the primary root begins to branch off to secondary roots), and subgroup 2 of juvenile plants (shoot height of 1.7-2.3 cm, with 1-3 ternate leaves appearing on a shoot in addition to simple leaves). In the basal portion of a shoot, 1-3 cataphyll leaves are formed, the 3-5 cm-long primary root begins to branch off to secondary roots, the root is thin, with a diameter of 1.0 to 1.6 mm. In the region of cotyledonary nodes, two primary buds are formed (1 mm wide, 2-2.5 mm high), in the axils of which an additional small third bud is formed, and in turn, in the axils of the third bud is the fourth bud. At this, all buds are formed in a ladder-like manner: with the base of each bud slightly above the other. All buds are different in terms of the stage of formation, they are mostly poorly formed, but in the next year of life they give fast-growing shoots with sympodial branching.

   The juvenile stage duration varies from 1-3 months to a year. Some plants enter the immature stage already in the first year of growth, while others enter the immature stage only the next year.
3. Immature stage. Individuals that entered the immature stage during the first vegetative phase remain single-shooted, the shoot (2.5-3 cm high) continues to grow monopodially, sometimes with

weak branching, in addition to 3-6 simple leaves, 1-3 small ternate leaves (0.1-0.5 cm long) are formed on a shoot, the primary root thickens and begins to deepen into soil.

In plants that entered the immature stage in the following year, the major shoot withers away, and a new major shoot grows from innovation buds located in the cotyledonary node regions. Shoots of immature plants branch in a sympodial manner. The first imparipinnate leaves, in the number of 2-5 pairs or more, appear on shoots. The primary root (5-7 cm long) deepens 10-20 cm into soil and thickens (root diameter 1.7 - 2.1 mm), root buds can be seen, giving growth to multiple secondary and tertiary roots.

4. Virginile stage. *H. alpinum* enters the virginile stage during the second year of growth. The innovation starts in the buds located in axils of cataphyll leaves. Multiple imparipinnate leaves (2 - 6 pairs) unfold along the shoot axis. Plants begin to form caudex from the remaining last year's shoots (root length - 15-20 cm, root diameter - 2-2.5 cm). New shoots emerge from latent buds. The root has auxiliary buds, which can also give rise to new shoots. The duration of the virgin state is about 1 year.
5. Plants usually enter the reproductive stage at the third year of growth. In the case of underwinter sowing, single plants may bloom in the second year.

   Plants at the early-reproductive stage form 1-2 (2-5) reproductive shoots with 4-7 pairs of imparipinnate leaves. Caudex becomes two- or many-headed, taproot develops multiple thickened lateral roots. Cultivated plants remain in this state for about 1-2 years.

   Plants at the mid-reproductive stage form multiple reproductive shoots (15-20 or more) bearing 7-9 or rarely up to 10-14 pairs of imparipinnate leaves. At the tops of shoots an inflorescence is formed as a dense raceme of flowers. During this period, shoots rapidly grow and thicken (shoot height 1.0-1.25 м or more), the partial caudex and the primary root become significantly thicker (primary root diameter: 2.8 cm or more). This stage lasts 6-10 years.

   Plants enter the late-reproductive stage at the age of 9-14 years, and at this time the number of reproductive shoots of a plant decreases down to 2-5, their seed productivity is significantly reduced. The caudex particulation begins in the central part. This stage lasts about 2-3 years or more.
6. Subsenile plants bear vegetative shoots growing from latent buds located in the caudex, at the same time the number of leaf pairs decreases (to 6-8). Plants stop flowering. The subsenile stage is characterized by incomplete caudex particulation. This stage lasts about 2 years.
7. Senile stage: at this stage individuals are in a depressed state, the number of vegetative of shoots is 1-2, necrotic caudex particulation is clearly visible, with the caudex completely destroyed in the center. Plant individuals remain at this stage for 1-2 years.

In Prebaikalia *H. alpinum* goes through all age periods of development: from immature and virginile to reproductive and senile.

The lifespan of *H. alpinum* introduced in the southwest Prebaikalia is from 12 to 20 years, at this, seedlings germinate in 10-15 days, the juvenile stage lasts from 1-3 months to 1 year. Some individuals enter the immature stage already at their first year of life, while others enter the immature stage only next year. The virginile stage lasts for about one year. Plants usually enter the reproductive stage in the third year of their growth; and winter-sown individuals may flower during the second year, but only very few of them. The duration of the reproductive stage is from 9 to 14 years. The subsenile stage is characterized by an incomplete caudex particulation and lasts for 2 years. A plant quickly enters a senile stage, which is characterized by a complete caudex particulation and the subsequent death after overwintering.

Alpine sweetvetch goes through a full development cycle: it grows, blooms, bears fruit, forms viable seeds (3.02 ± 0.1 mm long and 1.97 ± 0.09 mm wide). The weight of 1000 pieces of seeds ranges from 4.24 to 6.17 g. Seed germination rate is 40–68%.

Mechanical scarification, light, and removal of seed pericarp improve germination rates and increase the germination energy to 58–88%.

In the first year after sowing, the plant develops slowly, forming one brittle shoot, so in this period it is nearly unable to compete with weeds. Like many other legumes, the sweetvetch forms a symbiotic relationship with nitrogen-fixing bacteria. Many researchers consider the slow growth of *H. alpinum* in the first year of life to be due to an insufficient number of nitrogen-fixing bacteria; therefore, when seeds are planted in the soil where this species did not grow before, it is recommended to sprinkle the seeds with soil taken from under adult individuals of the species. In the next years, *H. alpinum* perfectly adapts to the climatic conditions of Prebaikalia, being a frost- and drought-tolerant species, competitively resistant to weeds. Plants have a relatively long life span and can grow in the same place for 10 years or longer.

The 7-year study of biomorphology (Table 1), demonstrated that the productivity was the highest in middle-age reproductive stage plants, which had the highest shoot growth gain (up to 16.5 ± 1.2), shoot height (up to 130.2 ± 4.0 m), number of leaves per shoot (up to 16.0), plant mass per plant (up to 120.2 ±

1.9 g). The productivity of plants at the mid-reproductive stage was affected by the plant age, geographical location of the region, climatic conditions of the study area, environmental [18,19] and other factors. Thus, for example, several authors observed the highest biomass in 4-year-old reproductive plants in the Chita region (up to 13.7 g) [9], and in 8-year-old plants cultivated in the middle-taiga subzone of the Komi Republic (from 91 to 114 g) [11,12,13] and in Tomsk Oblast (up to 174.2 g) [20]. n Prebaikalia, the most productive are 7-year-old plants (up to 120 g).

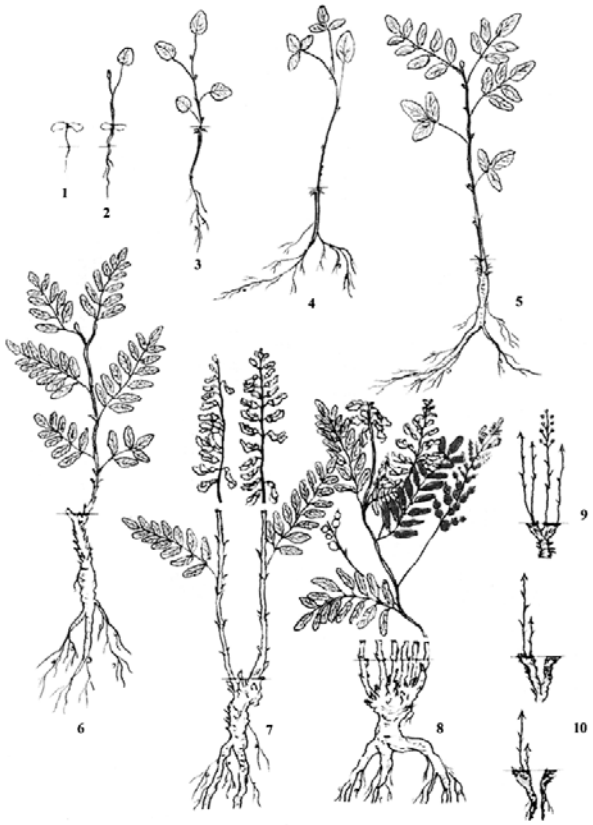

Fig. 1: Ontogenetic development of *Hedysarum alpinum L.* growing in Prebaikalia: 1. Seedling; 2. Juvenile plant of subgroup 1; 3. Juvenile plant of subgroup 2; 4. Immature plant, 5. Virginile plant; 6. Early-reproductive stage plant; 7. Middle-reproductive stage plant; 8. Late- reproductive stage plant; 9. Subsenile plant; 10. Senile plant

Table 1: Biomorphology of *Hedysarum alpinum* L. growing in the conditions of Prebaikalia

Year of life	Reproductive shoots			Weight of seeds per one plant (g)	Phytomass per plant (g) (air-dry)
	Number of shoots	Shoot height (cm)	Number of leaves per shoot		
3	3.1±0.,5	69,4±4,8	12.0±1.1	-	30.2±2.7
4	4.5±0.8	88.6±6,5	13.5±0.7	9.0±0.6	42.5±1.9
5	8.3±0.,3	112.5±5.5	13.6±1.0	17.2±1.0	86,5±3.6
6	13.7±0.9	120.0±3.6	16.0±1.5	25.8±2.9	117.0±1.5
7	16.5±1.2	130.2±4.0	15.3±1.9	38.5±3.0	120.2±1.9

IV. Conclusion

When cultivated in Prebaikalia, the plant passes through all ontogenetic stages, entering the reproductive phase at year 2-3 of growth. The duration of the most productive for feeding purposes mid-reproductive stage is 6-10 years. For introduced species, the life span of a plant growing in the same place ranges between 12 and 20 years. *H. alpinum* is a promising medicinal and fodder plant, which tolerates dry summer periods, is winter-hardy, and has high shoot biomass production (up to 120.2 g per plant on year 7 of growth).

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Global Journal of Science Frontier Research: C
Biological Science
Volume 20 Issue 3 Version 1.0 Year 2020
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

"A Clinical Study on the Role of Homoeopathy in Managing Anxiety Disorders in School Going Children of Kanniyakumari District"

By Dr. Vineetha Sreekumar & Dr. Vasanth C Kurup
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Abstract- *Childhood anxiety disorder* is one of the emotional disorders which are not due to any abnormalities in brain development or function but improper thoughts and emotions. It has become very common in this world of competition. But these are usually not identified and treated in children. The prime aim of the study is to assess the effective use of individualized Homoeopathic medicines in treating anxiety disorders in school going children of Kanniyakumari district of Tamil Nadu. This study also aims to determine the probable causes and also the type of anxiety disorder prevalent in school-going children. A sample of 30 cases presenting with anxiety disorders was selected using purposive sampling technique from school health programs conducted at Sarada Krishna Homoeopathic Medical College, Kanniyakumari district. Every case is subjected to screening using a basic diagnostic tool Screen for child anxiety related emotional disorders (SCARED), and those cases identified with anxiety disorders will be sent for detailed case taking. Medicine was prescribed according to the individualization and totality of symptoms. The improvement was monitored after 3 to 6 months of prescription by recording the variations in the scoring criteria of the SCARED tool.

Keywords: *childhood anxiety disorder, homoeopathic medicine, individualization.*

GJSFR-C Classification: *FOR Code: 279999*

Strictly as per the compliance and regulations of:

A Clinical Study on the Role of Homoeopathy in Managing Anxiety Disorders in School Going Children of Kanniyakumari District

Dr. Vineetha Sreekumar [α] & Dr. Vasanth C Kurup [σ]

Abstract- *Childhood anxiety disorder* is one of the emotional disorders which are not due to any abnormalities in brain development or function but improper thoughts and emotions. It has become very common in this world of competition. But these are usually not identified and treated in children. The prime aim of the study is to assess the effective use of individualized Homoeopathic medicines in treating anxiety disorders in school going children of Kanniyakumari district of Tamil Nadu. This study also aims to determine the probable causes and also the type of anxiety disorder prevalent in school-going children. A sample of 30 cases presenting with anxiety disorders was selected using purposive sampling technique from school health programs conducted at Sarada Krishna Homoeopathic Medical College, Kanniyakumari district. Every case is subjected to screening using a basic diagnostic tool Screen for child anxiety related emotional disorders (SCARED), and those cases identified with anxiety disorders will be sent for detailed case taking. Medicine was prescribed according to the individualization and totality of symptoms. The improvement was monitored after 3 to 6 months of prescription by recording the variations in the scoring criteria of the SCARED tool.

According to the study result, twelve patients (40%) have antenatal risk factors, 18 patients (60%) have natal risk factors, six patients (20%) have environmental risk factors, seven patients (23.33%) have developmental delay. Out of the twelve patients, ten of them had antenatal maternal emotional stress as a risk factor. The most commonly identified type of anxiety disorder was social anxiety and separation anxiety disorder. The common co-morbid conditions were learning disability, attention deficit disorder, and nocturnal enuresis. In this study, nine patients (30%) had marked improvement, ten patients (33.33%) had moderate and eleven patients (36.66%) had mild betterness with individualized Homoeopathic medicines. Therefore this medicine can be effectively used as psychologic medicine, which can give emotional healing apart from other systems of medicines as Homoeopathic treatment is based on individualization, i.e treating the person as a whole.

Keywords: *childhood anxiety disorder, homoeopathic medicine, individualization.*

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I. Introduction

Anxiety is a feeling of threat experienced in anticipation of an undesirable event that may be unknown or specified and is a normal phenomenon (Kleigman, 2015). But when it becomes disabling, causing distress and impairs overall functioning, it is considered as an anxiety disorder (Robin M Murray, 2008). Anxiety disorders can significantly interfere with academic, social, and emotional, and family functioning. The onset of any anxiety disorder was seen in childhood. In different studies of impairing childhood anxiety disorder, 5% to 13% of children younger than 18yrs have an anxiety disorder.

a) *Need for the study*

- Childhood anxiety disorders are the most common type of psychiatric problem in children, which can cause excessive distress, are usually not assessed, diagnosed, and treated properly and if left untreated, can follow a chronic and fluctuating course into adulthood.
- Children experience anxiety just as the adults who can begin at a very early age and is said to be mainly due to the pressures that come from outside sources such as family, friends, or school (Nidhi Luthra, 2007).
- In this competitive world, fear of examination in students is highly increasing. The thought and the pressure to perform well in exams have heightened fear and anxiety and even panic attacks (Rajive Sood, 2004).
- Status of anxiety disorder research from India about management is lacking, and research areas like family studies are not touched.

b) *Role of Homoeopathy*

The experience of anxiety is unique for each person, and the Homoeopathic approach is personalized, which is a 'holistic' method of diagnosis and of prescription which is called *individualization*.

Individualized Homoeopathic medicine (IHM) was found effective in reducing the symptoms of anxiety in patients. The current study established the fact that Homoeopathic medicines were very useful in

psychological medicine (Lakshmipathy R Prabhu, 2012). The anxiolytic effect of the Homoeopathic preparation of Pulsatilla nigricans in Swiss albino mice is proved (S Keerti Jogdand, 2015).

II. Materials and Methods

a) Study Setting

A sample of 30 cases presenting with an anxiety disorder was obtained from the school health program conducted by Sarada Krishna Homoeopathic Medical College in Kanyakumari district and also from its out-patient department, and rural centers.

b) Selection of Samples

A sample of 30 cases presenting with anxiety disorders was selected using a purposive sampling technique. For that, every child will be screened for anxiety disorders using SCARED (screening for childhood anxiety related emotional disorders) diagnostic tool.

c) Inclusion Criteria

- Patients of pediatric age group between 8yrs to 18yrs
- Children of both sex
- School going children

d) Exclusion Criteria

- Patients taking medicines for any other chronic illness

e) Study Design

Interventional before and after treatment without control

f) Brief of Procedures

Every case is subject to screening using a diagnostic tool, Screen for child anxiety related emotional disorders (SCARED), and those cases identified with anxiety disorders will be sent for detailed case taking. Data was obtained from the patients, bystanders, and physician's observation and the physical examination. Medicine was prescribed according to the individualization and totality of symptoms. The improvement was monitored after 3 to 6 months of administration of medicine by recording the variations in the scoring criteria of the SCARED tool. Pre and post-treatment analysis is done using Screen for child anxiety related emotional disorders (SCARED). Observations are noted in tables and charts. Statistical analysis is done, and results are presented.

g) Data Collection

Data were obtained from the patient & bystanders through the interview technique and also from the physician's observation and physical examination. The diagnosis was made through proper screening and assessment of the patient using proper diagnostic tools (SCARED).

h) Statistical Techniques

- Paired 't' test

i) Statistical Analysis

Table 1: Mean and mean difference of before and after treatment score

SL. No.	X	Y	d = x-y	$d - \underline{d}$	$(d - \underline{d})^2$
1	22	12	10	1.7	2.87
2	16	9	7	-1.3	1.69
3	28	18	10	1.7	2.87
4	20	7	13	4.7	22.09
5	41	22	19	10.7	114.49
6	15	9	6	-2.3	5.29
7	13	9	4	-4.3	18.49
8	21	13	8	-0.3	0.09
9	16	13	3	-5.3	28.09
10	20	14	6	-2.3	5.29
11	28	13	15	6.7	44.89
12	27	18	9	0.7	0.49
13	31	24	7	-1.3	1.69
14	32	18	14	5.7	32.49
15	18	12	6	-2.3	5.29
16	20	11	9	0.7	0.49
17	29	20	9	0.7	0.49
18	19	8	11	2.7	7.29
19	32	23	9	0.7	0.49
20	14	11	3	-5.3	28.09
21	27	21	6	-2.3	5.29
22	24	15	9	0.7	0.49

23	29	16	13	4.7	22.09
24	29	17	12	3.7	13.69
25	30	16	14	5.7	32.49
26	23	20	3	-5.3	28.09
27	30	26	4	-4.3	18.49
28	19	16	3	-5.3	28.09
29	38	33	5	-3.3	10.89
30	33	29	4	-4.3	18.49

X = Score before treatment, Y= Score after treatment, D = Mean difference

Null Hypothesis:

$\sum d = 251$

$\bar{d} = 251/30 = 8.366$

$\sum d-\bar{d} = 101$

$\sum (d-\bar{d})^2 = 501.06$

Standard error of the mean differences:

The mean of the differences, $\bar{d} = \Sigma d/n = 251/30 = 8.36$

The estimate of population standard deviation is given by,

$$SD = \sqrt{\Sigma(d1 - \bar{d}1)^2}/n - 1$$

$$= \sqrt{501.06/29} =$$

$$= \sqrt{17.22} = 4.149$$

The estimate of standard error of mean, Standard error (S.E)

$$= S.D/\sqrt{n} = 4.149/\sqrt{30} = 0.757531495$$

The test statistics is Paired t:

$$\text{Critical ratio, } t = \frac{\bar{d}}{S.D/\sqrt{n}} = 8.366/\ 0.757531495 = 11.043$$

Table 2: t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	24.8	16.43333
Variance	51.68276	40.59885
Observations	30	30
Pearson Correlation	0.818731	
Hypothesized Mean Difference	0	
df	29	
t Stat	11.02574	
P(T<=t) one-tail	3.44E-12	
t Critical one-tail	1.699127	
P(T<=t) two-tail	6.88E-12	
t Critical two-tail	2.04523	

Interpretation of results:

- *Comparison with the tabled value:*

On comparing the score before and after treatment, the means were 24.8 and 16.43333 and the variances were 51.68276, and 40.59885 respectively (*Table 1, Table 2*). The data showed a positive correlation of 0. Since the calculated value is greater than the tabled value at 5% and 1%, the null hypothesis was rejected at 95% significance and the hypothesis that Homoeopathy is effective in treating anxiety disorders in children is accepted.

j) Data Analysis

Data presentation including charts and tables

Table 3: Distribution of cases according to age

Age	Number of Patients	Percentage
6-8	12	40
9-11	9	30
12-14	6	20
15-17	3	10

Table 4: Distribution of cases according to probable risk factors

Probable Risk Factors	Antenatal Causes	Natal Causes	Environmental (At Home & School)	Developmental Delay	Unknown Causes
Number Of Patients	12	18	6	7	6
Percentage	40	60	20	23.33	20

Table 5: Distribution of cases according to screening with the SCARED tool

Score	10-15	16-20	21-25	26-30	31-35	36-40	41-45
No. Of Patients	3	8	4	9	4	1	1
Percentage	10	26.66	13.33	30	13.33	3.33	3.33

Table 6: Distribution of cases according to the type of anxiety disorder

Type of anxiety disorder	Number of patients	Percentage
Generalized Anxiety Disorder (GD)	3	10
Separation Anxiety Disorder (SP)	23	76.66
Social Anxiety Disorder (SC)	25	83.33
Panic Disorder (PD)	5	16.66
Significant School Avoidance (SH)	5	16.66

Table 7: Distribution of cases according to co-morbidity of anxiety disorders in children

Co-morbidity	Number of Patients	Percentage
ADHD	3	10
Epilepsy	2	6.66
Intellectual Disability	4	13.33
Learning Disability	7	23.33
Attention Deficit Disorder	5	16.66
Oppositional Defiant Disorder	1	3.33
Nocturnal Enuresis	5	16.66
Cerebral Palsy	2	6.66

Table 8: Distribution of cases according to the remedy prescribed

Remedy prescribed	Number of patients	Percentage
Lycopodium clavatum	2	6.66
Carcinosinum	2	6.66
Calcarea carbonica	4	13.33
Cuprum metalicum	1	3.33
Tuberculinum	1	3.33
Silicea terra	3	10
Calcarea phoshorica	4	13.33
Natrum muriaticum	3	10

Phosphorous	4	13.33
Tarantula	1	3.33
Arsenicum album	2	6.66
Opium	1	3.33
Baryta carbonica	1	3.33
Natrum sulphuricum	1	3.33

Table 9: Distribution of cases according to the improvement in the anxiety disorder

	Mild Improvement	Moderate Improvement	Marked Improvement
Number of patients	11	10	9
Percentage	30	33.33	36.66

III. Results

a) *Distribution of cases according to age*

Out of thirty cases, twelve patients (40%) were of age 6 to 8 years old, nine patients (30%) were of age 9 to 11 years old, six patients (20%) were of age 12 to 14 years old, and three patients (10%) were of age 15 to 17 years old.

b) *Distribution of cases according to probable risk factors*

Out of 30 cases, twelve patients (40%) have antenatal risk factors, eighteen patients (60%) have natal risk factors, six patients (20%) have environmental risk factors, seven patients (23.33%) have developmental delay and six patients (20%) have unknown probable risk factors.

c) *Distribution of cases according to antenatal risk factors*

Out of 30 cases, twelve patients (40%) have antenatal risk factors. Out of the twelve patients, ten of them had antenatal maternal emotional stress as a risk factor.

d) *Distribution of cases according to screening with the children with anxiety-related emotional disorders (SCARED) tool*

Out of 30 patients, three patients (10%) had the score range from 10 -15, eight patients (26.66%) had the score between 16 -20, four patients (13.33%) had the score range from 21 – 25, nine patients (30%) had score 26 – 30, four patients (13.33%) had score 31 -35, one patient (3.33%) had score between 36 – 40, and one patient (3.33%) had score between 41 – 45.

e) *Distribution of cases according to the type of anxiety disorder*

Out of 30 cases, twenty five patients (83.33%) had social anxiety, twenty three patients (76.66%) had separation anxiety, five patients (16.66%) had panic disorder, five patients (16.66%) presented with significant school avoidance, and three patients (10%) had a generalized anxiety disorder.

f) *Distribution of cases according to co-morbidity of anxiety disorders in children*

Out of 30 patients, three patients (10%) had attention deficit hyperactivity disorder (ADHD), two patients (6.66%) had epilepsy, four patients (13.33%) had intellectual disability, seven patients (23.33%) had learning disability, five patients (16.66%) had attention deficit disorder, one patient (3.33%) had oppositional defiant disorder, five patients (16.66%) had nocturnal enuresis, and two patients (6.66%) had cerebral palsy.

g) *Distribution of cases according to the remedy prescribed*

Out of 30 cases, two patients were given Lycopodium clavatum, two patients with Carcinosinum, four patients were given Calcarea Carbonica, one patient with Cuprum metallicum, one patient with Tuberculinum, three patients with Silicea terra, four patients with Calcarea phosphorica, three patients with Natrum muriaticum, four patients with Phosphorous, one patient with Tarentula, two patients with Arsenicum album, one patient with Opium, one patient with Baryta carbonica, and one patient were given Natrum sulphuricum.

h) *Distribution of cases according to the improvement in the anxiety disorder*

Out of 30 cases, 9 patients (30%) had marked improvement, 10 patients (33.33%) had moderate improvement and 11 patients (36.66%) had mild improvement.

IV. Discussion

In the study, out of 30 cases, twelve patients (40%) were of age 6 to 8 years old, nine patients (30%) were of age 9 to 11 years old, six patients (20%) were of age 12 to 14 years old, and three patients (10%) were of age 15 to 17 years old. Therefore commonly affected age group was 6 to 8 years *(Table 3, Figure 1).*

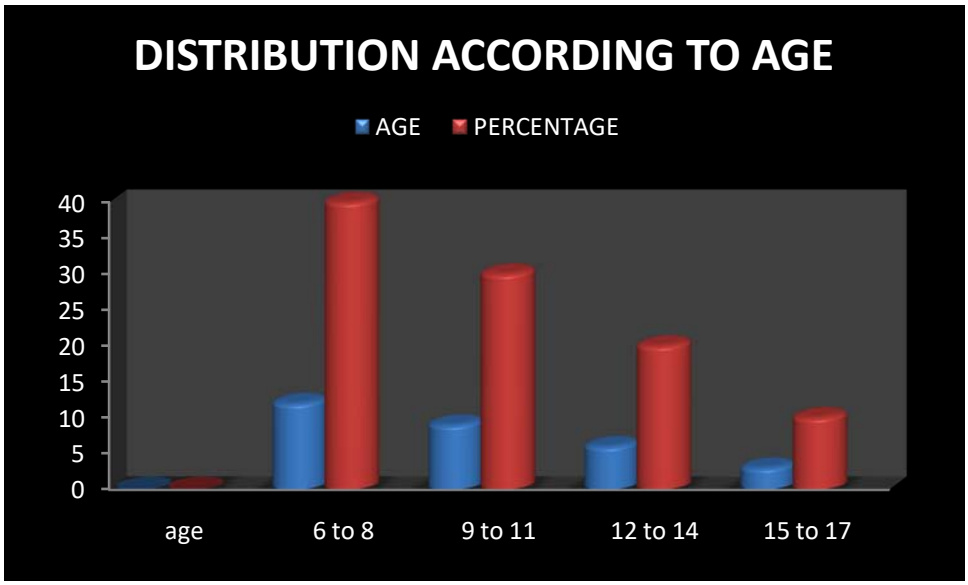

Figure 1: Distribution of cases according to age

There was no gender difference, according to this study. Among the 30 cases, fifteen patients (50%) were females and the fifteen patients (50%) were males.

Out of 30 cases, twelve patients (40%) have antenatal risk factors, eighteen patients (60%) have natal risk factors, six patients (20%) have environmental risk factors, seven patients (23.33%) have developmental delay and, six patients (20%) have unknown probable risk factors. Out of the twelve patients with antenatal risk factors, ten of them had antenatal maternal emotional stress as a risk factor (Table 4, Figure 2).

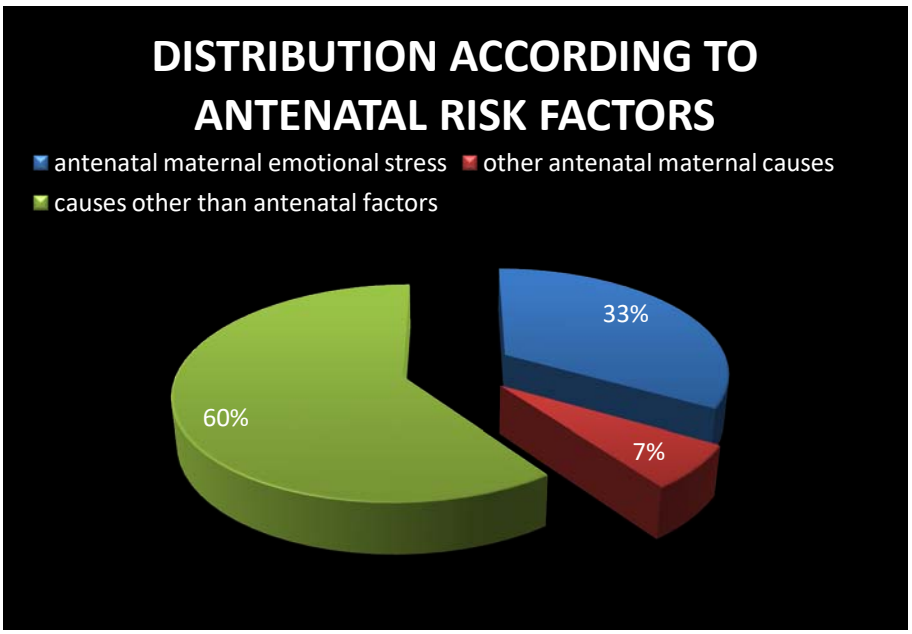

Figure 2: Distribution according to antenatal risk factors

Environmental risk factors like parenting style, childhood adversities, relationship stress, etc. were found to be associated with anxiety disorder. Nearly 20% of women suffer from mental health disorders during gestation. 5.12% had anxiety and 6.69% were having anxiety, and depression *(De Mond M Grant, 2013)*. In the study, 60% of cases have natal risk factors, and 40% have antenatal risk factors, and 20% have developmental delay as risk factor than environmental causes, which is 23.33%. Also, antenatal maternal stress is found to be another important antenatal risk factor where further research is required.

Out of 30 patients, three patients (10%) had the score range from 10 -15, eight patients (26.66%) had the score between 16 -20, four patients (13.33%) had the score range from 21 – 25, nine patients (30%) had score 26 – 30, four patients (13.33%) had score 31 -35, one patient (3.33%) had score between 36 – 40, and one

patient (3.33%) had score between 41 – 45 *(Table 5)*. The tool consists of 41 items and five factors that parallel the DSM –IV classification of anxiety disorders. In this study, many patients had specific social phobias. But these couldn't be assessed using the SCARED tool, which were generally considered as social phobia.

Out of 30 cases, twenty five patients (83.33%) had social anxiety, twenty three patients (76.66%) had separation anxiety, five patients (16.66%) had panic disorder, five patients (16.66%) presented with significant school avoidance, and three patients (10%) had a generalized anxiety disorder *(Table 6, Figure 3)*.

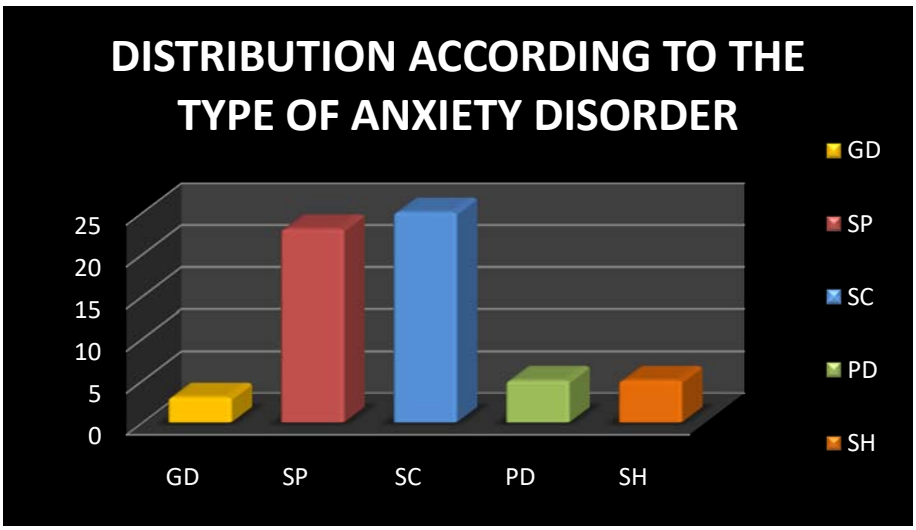

Figure 3: Distribution according to the type of anxiety disorder

The most commonly identified type of anxiety disorder was social anxiety, which includes specific social phobias, and separation anxiety disorder. An inter anxiety co-morbidity was seen in most of the cases.

The majority of the cases have a learning disabilities, attention deficit disorder, and nocturnal enuresis. Other co-existing conditions were attention deficit hyperactivity disorder, epilepsy, intellectual disability, oppositional defiant disorder, and cerebral palsy *(Table 7, Figure 4)*. In most of the cases, these co-morbid conditions can be a causative or risk factor of anxiety disorder. Therefore managing anxiety disorders can also improve the co-morbid conditions simultaneously.

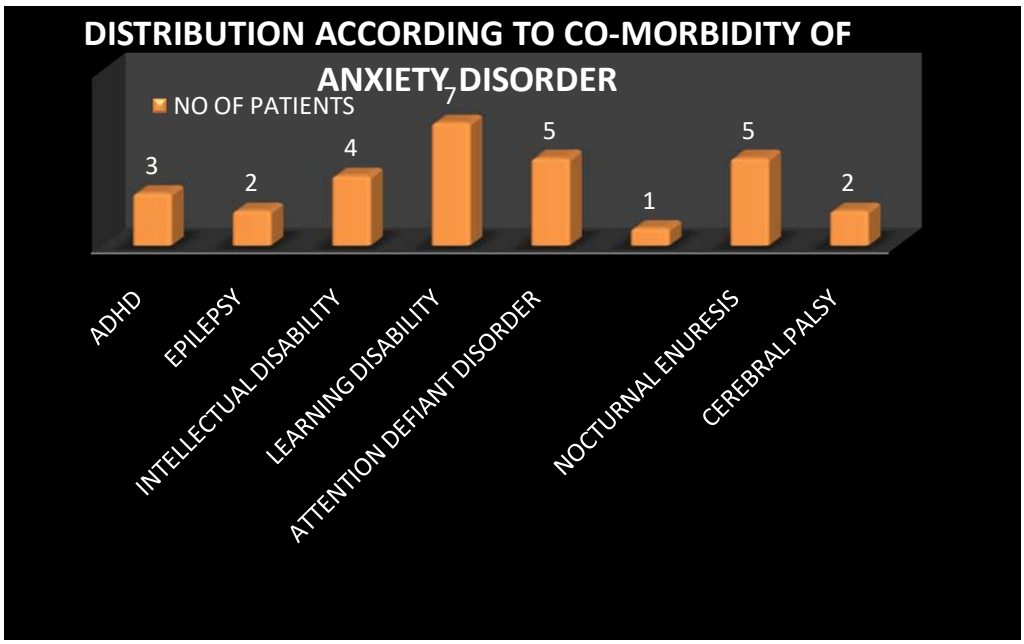

Figure 4: Distribution according to co-morbidity of anxiety disorder

Out of 30 cases, two patients were given Lycopodium clavatum, two patients with Carcinosinum, four patients were given Calcarea Carbonica, one patient with Cuprum metallicum, one patient with Tuberculinum, three patients with Silicea terra, four patients with Calcarea phosphorica, three patients with

Natrum muriaticum, four patients with Phosphorous, one patient with Tarentula, two patients with Arsenicum album, one patient with Opium, one patients with Baryta carbonica, and one patient was given Natrum sulphuricum. According to the remedy analysis, the individualized Homoeopathic medicines are effective in managing anxiety disorders in children. Among these medicines, *Calcarea Carbonica, Calcarea phosphorica,* and *Phosphorus* are the mostly used remedies *(Table 8).* The improvement is assessed after a duration of 3 - 6months after Homoeopathic treatment using individualized Homoeopathic medicines.

Out of 30 cases, nine patients (30%) had marked improvement, ten patients (33.33%) had moderate improvement, and eleven patients (36.66%) had mild improvement *(Table 9, Figure 5).*

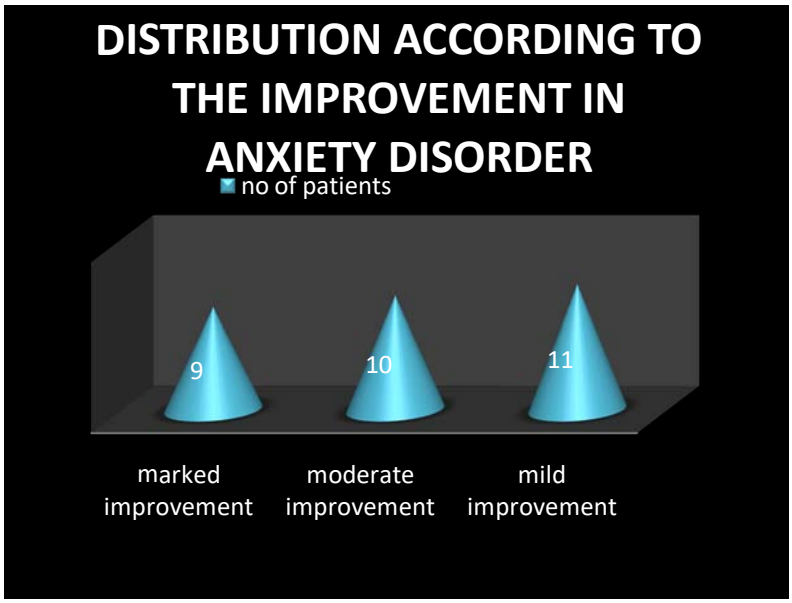

Figure 5: Distribution according to the improvement in anxiety disorder

V. Conclusion

The following conclusions are drawn from the study:

- The majority of patients belong to age groups 6 – 8 years (40%) and 9 – 11 years (30%).
- There is no sex difference according to the study's prevalence of anxiety disorders in children.
- Out of 30 patients, the number of patients from average socio-economic status was fourteen (46.66%), from below-average socio-economic status were three (10%), and above-average socio-economic status were thirteen (43.33%).
- In the study the probable risk factors identified were antenatal, natal, environmental and developmental delay as risk factors. Twelve patients (40%) have antenatal risk factors, eighteen patients (60%) have natal risk factors, six patients (20%) have environmental risk factors, seven patients (23.33%) have developmental delay and, six patients (20%) have unknown probable risk factors. Out of 30 cases, twelve patients (40%) have antenatal risk factors. Out of the twelve patients, ten of them had antenatal maternal emotional stress as a risk factor.
- The majority of patients had a score 26 – 30 and 16 -20 with a percentage 30% and 26.66%, respectively.
- The type of anxiety disorder more prevalent in the study was social anxiety and separation anxiety disorder, with a percentage of 83.33% and 76.66%, respectively.
- The most common co-morbidity identified in the study was learning disability (23.33), attention deficit disorder (16.66%), and nocturnal enuresis (16.66%).
- *The Individualized Homoeopathic medicine* was given, and the mostly used remedies were Calcarea carbonica, Calcarea phosphorica, and Phosphorus.
- The concept of disease in Homoeopathy is of prime importance in the Homoeopathic system. It stresses the importance of individuality of a person as a whole.
- Results of the study suggest that the *individualistic approach of Homoeopathy* was found effective in the management of anxiety disorders in school going children. Also, studies have proven that Homoeopathic medicines are effective as Psychological medicine.

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Appendix

Screen For Child Anxiety Related Emotional Disorders (Scared)

	0 Not true or hardly ever true	1 Somewhat true or sometimes true	2 Very true or often true
1. When I feel frightened, it is hard to breath			
2. I get headache when I am at school			
3. I don't like to be with people I don't know well			
4. I get scared if I stay away from home			
5. I worry about other people liking me			
6. When I get frightened, I feel like passing out			
7. I am nervous			
8. I follow my mother or father wherever I go			
9. People tell me that I am nervous			
10. I feel nervous with people I don't know			
11. I get stomachaches at school			
12. When I get frightened I feel like I am going crazy			
13. I worry about sleeping alone			
14. I worry about being as good as other kids			
15. When I get frightened, I feel like things are not real			
16. I have nightmares about something bad happening to parents			
17. I worry about going to school			
18. When I get frightened, my heart beats fast			
19. I get shaky			
20. I have nightmares of something bad happening to me			
21. I worry about things working about me			
22. When I get frightened, I sweat a lot			
23. I am a worrier			
24. I really get frightened for no reason at all			

25. I am afraid to be alone at home			
26. It is hard for me to talk with people I don't know well			
27. When I get frightened I feel like choking			
28. People tell me that I worry too much			
29. I don't like to be away from family			
30. I am afraid of having anxiety (or panic) attacks			
31. I worry that something bad might happen to my parents			
32. I feel shy with people I don't know well			
33. I worry about what is going to happen in future			
34. When I get frightened I feel like throwing up			
35. I worry about how well I do things			
36. I am scared to go to school			
37. I worry about things that have already happened			
38. When I get frightened, I feel dizzy			
39. I feel nervous when I am with other children or adults and I have to do something while they watch me(for example: read aloud, speak, 40. play a game, play a sport)			
41. I feel nervous when I go to parties, dances, or any place where there will be people that I don't know well			
42. I am shy			

Scoring:

A total score of≥ 25 may indicate the presence of an Anxiety Disorder. Scores higher than 30 are more specific. TOTAL =

A score of 7 for items 1, 6, 9, 12, 15, 18, 19, 22, 24, 27, 30, 34, 38 may indicate Panic Disorder or Significant Somatic Symptoms. PN =

A score of 9 for items 5, 7, 14, 21, 23, 28, 33, 35, 37 may indicate Generalized Anxiety Disorder. GD =

A score of 5 for items 4, 8, 13, 16, 20, 25, 29, 31 may indicate Separation Anxiety SOC. SP =

A score of 8 for items 3, 10, 26, 32, 39, 40, 41 may indicate Social Anxiety Disorder. SC =

A score of 3 for items 2, 11, 17, 36 may indicate Significant School Avoidance. SH =

Global Journal of Science Frontier Research: C
Biological Science
Volume 20 Issue 3 Version 1.0 Year 2020
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Active Fractions of Methanol Crude Obtained from *Acacia Seyal Gum* and their Anti-Proliferative Effects against Human Breast Cancer Cell Lines

By Ahmed. A. M. Elnour, Mohamed E. S. Mirghani, N. A. Kabbashi, Djabir Daddiouaissa, Khalid Hamid Musa, Md Z. Alam & Nour Hamid Abdurahman

International Islamic University

Abstract- *Background:* This study is on Acacia seyal gum, which is an exudate from Talha (Acacia seyal) tree. It provides a rich source of prebiotic that is used traditionally in folk medicine.

Aims: The anti-proliferative effect (APE) of Acacia seyal gum (ASG) and Prebio-T-commercial (PTC) samples on human breast cancer (MCF-7) cell lines, and their antioxidant activities (AA) were investigated. Methods: The methanol crude extracts of both Acacia seyal gum and Prebio-T-commercial were fractioned into acetone and methanol, respectively. The anti-proliferative effect on human breast cancer cell lines for each fraction was examined using sulphorhodamine assay (SRB assay). Methanol crude extracts and their active compositions were analysed carefully using Gas chromatography-mass spectrometry technique.

Keywords: *cytotoxicity activity; acacia seyal gum; breast cancer(MCF-7); methanol extract/fraction, and GC-MS/MS.*

GJSFR-C Classification: *FOR Code: 069999*

Strictly as per the compliance and regulations of:

Active Fractions of Methanol Crude Obtained from *Acacia Seyal Gum* and their Anti-Proliferative Effects against Human Breast Cancer Cell Lines

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Abstract- Background: This study is on *Acacia seyal* gum, which is an exudate from Talha (*Acacia seyal*) tree. It provides a rich source of prebiotic that is used traditionally in folk medicine.

Aims: The anti-proliferative effect (APE) of *Acacia seyal* gum (ASG) and Prebio-T-commercial (PTC) samples on human breast cancer (MCF-7) cell lines, and their antioxidant activities (AA) were investigated. Methods: The methanol crude extracts of both *Acacia seyal* gum and Prebio-T-commercial were fractioned into acetone and methanol, respectively. The anti-proliferative effect on human breast cancer cell lines for each fraction was examined using sulphorhodamine assay (SRB assay). Methanol crude extracts and their active compositions were analysed carefully using Gas chromatography-mass spectrometry technique.

Results: The most anti-proliferative effect was detected in the sample collected from Prebio-T-commercial (IC50=8.97µg/mL) as compared to *Acacia seyal* gum (IC50=9.56µg/mL). Regarding total phenolic content (TPC), the methanol crude extracts values are 694±2.58mg, GAE/100g for Prebio-T-commercial as compared to 155.78±2.58, GAE/100g for *Acacia seyal* gum. However, both acetone and methanol fractions of *Acacia seyal* gum and Prebio-T-commercial were found to be highly anti-proliferative to human breast cancer. For bioactive compounds determinations, the methanol crude extract from *Acacia seyal* gum is mainly dominated by Isovitamin C (42.37%), Crypton (5.86%), and Hydroquinone (4.86%) as major components. Conclusion: Finally, the antioxidant and anti-proliferative properties of the active fraction have shown some evidence regarding its use in traditional medicine as well as the prevention of cancer cell growth. This suggests the potential use of their bioactive compounds as natural anticancer agents.

Keywords: *cytotoxicity activity; acacia seyal gum; breast cancer(MCF-7); methanol extract/fraction, and GC-MS/MS.*

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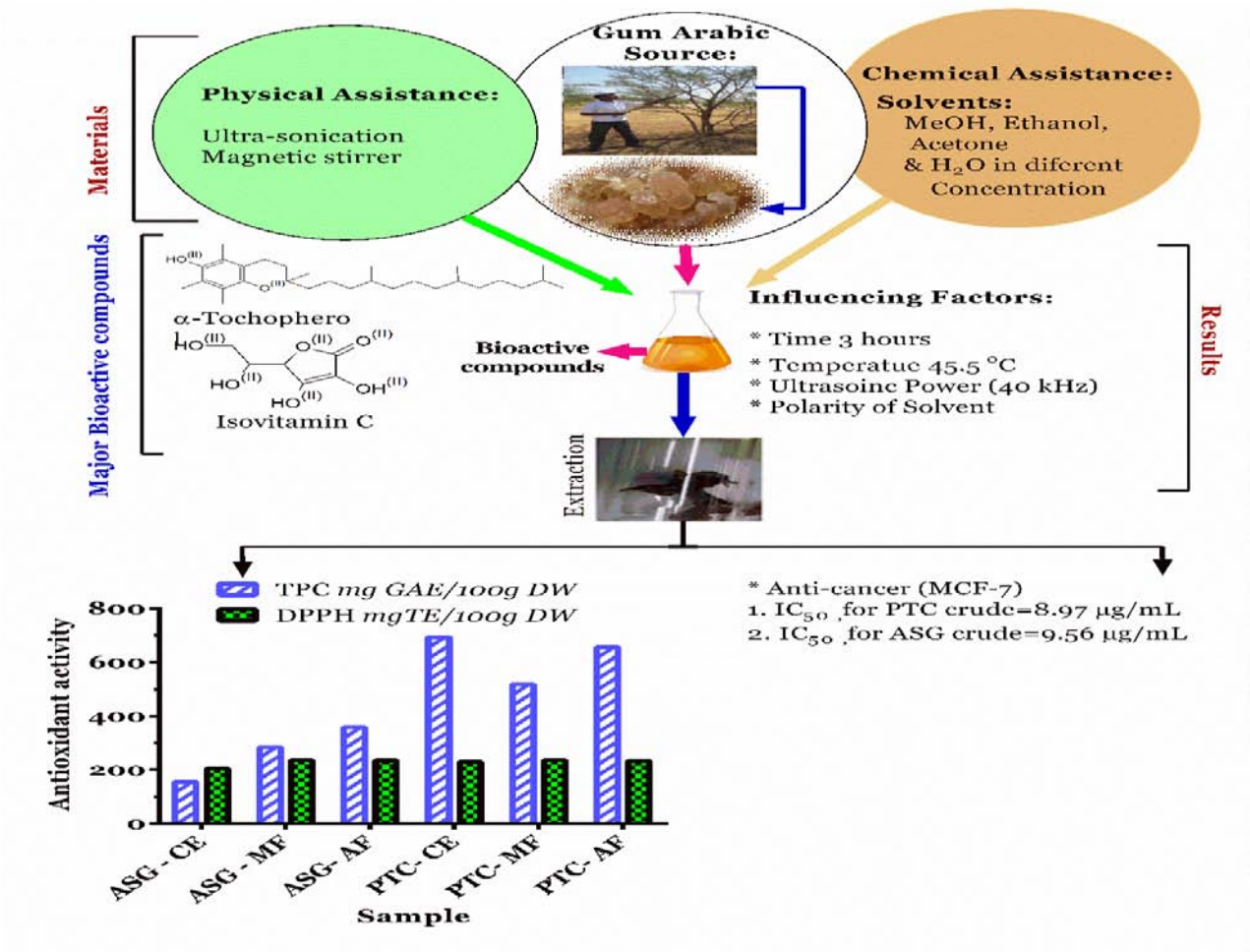
Graphical Abstract



I. Introduction

Breast adenocarcinoma (MCF-7) cell line is one of the most frequently diagnosed cancer in women globally. American Cancer Society (ACS) reported about 268,600 new cancer cases corresponding to a 30 % increase in 2019,whereas 41,670 is the estimated deaths in the USA only[1]. The World Health Organization (WHO) estimated that 84 million people would die from cancer between 2005 and 2015[2]. Thus, it constitutes a public severe health problem in both developed and developing countries.

Current protocols of treatment include radiation therapy, surgical intervention, and chemotherapy, which induce numerous side effects such as nausea, fatigue, vomiting, weak of the immune system and hair loss. Nowadays, treatment for breast cancer (BC) involveshormonal therapy, chemotherapy, surgical intervention, and radiotherapy. Exhaustive treatment with chemotherapy or radiotherapy is frequently related to few side effects ranging from the failure of bone marrow, fatigue, hair loss, vomiting, nausea, and weakness of the immune system[3]. Hence, clinical treatment remained a challenge, and new natural bioactive compounds are urgently needed. Furthermore, cancer cells are regularly not responding to chemotherapy [4]. Consequently, polyphenols from *Acacia seyal* gum (ASG) might be a potential anticancer agent in the future.

Some studies have confirmed that polyphenolics isolated from ASG components are potent biological activities with anti-inflammatory capability[5],aimed at kidney failure treatment[6], focused on a cure for cardiovascular disease[7] and also relieving gastrointestinal diseases [8] have also reported. The most abundant bioactive compounds of ASG are phenolic acids, flavonoids, terpenoids, lignans, tannins, quinones, coumarins, and alkaloids [9].Even though the anti-proliferative effect of ASG compounds was studied on several biological activities, it has not been reported in any cancer cell lines, including breast cancer (MCF-7). In this study, we focused on the cytotoxic effect of MCE and the effect of its active fraction on breast cancer cell lines. Finally, the GC-MS/MS analysis was employed for the quantification of bioactive compounds, which were thought to be responsible for the cancer treatment.

II. Materials and Methods

a) Chemicals and reagents

Folin-Ciocalteu (FC) phenol reagent and Sodium carbonate were obtained from Merck (Darmstadt, Germany) and RDH (Germany), respectively. Moreover, Trolox, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid, were from Sigma-Aldrich (St. Louis, MO, USA). Chloroform, n-Hexane, Acetone, and Methanol were obtained through the fractionation process. All chemicals and reagents used in the study were of analytical grade.

b) Extract preparation and solvent-guided of methanol crude extract

The primary raw material was *Acacia seyal* gum (ASG), it was obtained from Blue Nile state (Sudan), in the year 2015whereas Prebio-T commercial (PTC) was obtained from Perfect Life Food company locatedin Dubai-U.A.E. Spectrophotometer (Spectro-Star Nano) was used for recording the samples'absorbance readings. 500grams of the mechanical ASG powdered was extracted with methanol by using optimized ultrasonic parameters for 3 hrs at a power of 40 kHz, and 42.5^{0}C. Chloroform, n-Hexane, Acetone, and Methanol were used in the fractionation process according to the method reported by Elnour *et al.*[5], as presented in Figure 1. The extract was concentrated and dried under nitrogen gas supply at room temperature. Finally, the extract and its active fractions were stored at 4^{0}C.

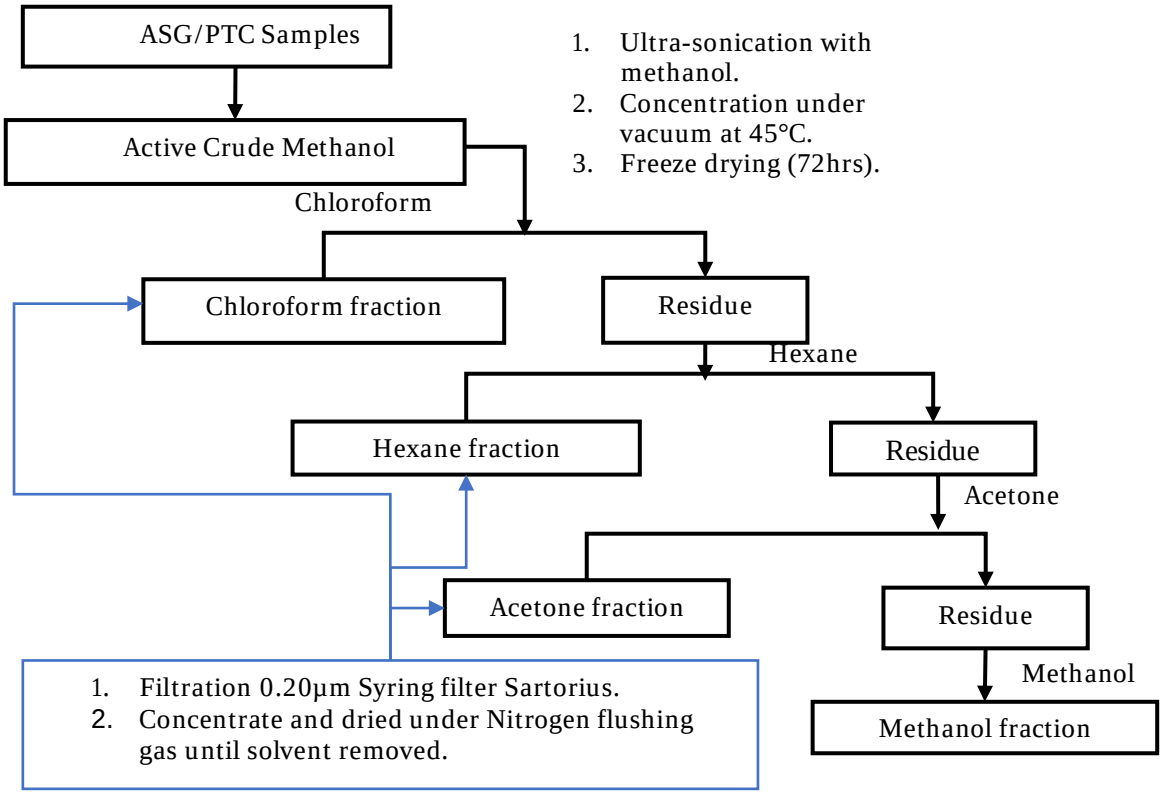


Figure 1: Schematic flow representation of the Kupchan solvent-solvent partitioning of a methanolic crude extract of *Acacia seyal* gum (ASG) and PTC crude methanol and its fractions

c) Cell lines and Culture Conditions

In this research, breast cancer MCF-7 cell lines were used as *in-vitro* experimental cancer cells (ATCC N: HTB-22™). These cells were purchased from American type Collection Culture (ATCC). Frozen MCF-7 cells were thawed and inoculated into 5 mL of RPMI 1640 medium, enhanced with 10% fetal bovine serum (FBS) and supplemented with 100 µg/ml streptomycin and 100 U/ml penicillin. The MCF-7 cells were cultured in T-25 flasks and incubated at 37°C in 95% humidified incubator with 5% CO_2. The cells were used for further experiments after reaching 70 % confluency.

i. *Anti-proliferative effect of methanol extract of Acacia seyal gum*

Were determined in the *in-vitro* cytotoxic activity of the methanol crude extract (MCE) and its active fractions against MCF-7 cell lines were determined using sulphorhodamine assay (SRB assay) as previously described by Samarakoon et al[10];however, a slight modification was done in the procedure. Briefly, cells were trypsinised and inoculated (5x10^3 cells/well) into 96-multiwell plates then incubated for 24 hours. After that, different concentrations of the compound under test (0, 1, 2.5, 5, and 10 µg/mL) were

administered to the cell wells and incubated for 48 hours. Moreover, Taxol was used as a positive control as well as DMSO at (01% v/v) served as a negative control. The cells were placed on the ice-cube with 50% trichloroacetic acid solution and incubated for 60min at 4°C, then washed five times with tap water before marking them with sulforhodamine-B stain. After that, cells were stained with 100 µL of 0.4% SRB solution/well for 15 min at room temperature. The acetic acid solution (1%) was used to remove the unbound dye, and then unbuffered. On the other hand, a Tris-based solution was utilized to solubilise the bound SRB dye. In the end, the plates were shaken for 1 hour at room temperature then the readings were recorded by a microplate reader at a wavelength of 540 nm. The obtained results were used to calculate the percentage of cell viability, and fifty percent of inhibitory concentration (IC_{50}) of each extract was determined, using equation 1[11] as follows:

$$\%\, cellviability = \frac{mean\ OD\ of\ extract\ wells}{mean\ OD\ of\ control\ wells} \times 100$$

d) Antioxidant activities of methanol extract of Acacia seyal gum

i. *Total phenolic content (TPC)*

The procedure adopted follows the method described by Musa et al. [12], whereby approximately 0.5 mL diluted Folin-Ciocalteu (FC) reagent was added to 100 µL of ASG sample. The extraction procedure was conducted using 1.0 g sample of ASG, and 10 mL of solvent for 5 min beforeadding1 mL (7.5%) of sodium carbonate (w/v). The absorption spectrophotometer of wavelength 765 nm was used after 2 hours for analysis. Gallic acid was presumed as the standard, and the results were reported as mg gallic acid equivalents to mg GAE/100g of sample dry weight (DW).

ii. *DPPH free radical scavenging assay*

Following Musa et al., [12] discussions, DPPH was freshly prepared by dissolving 40 mg DPPH in 1000 mL methanol to obtain the absorbance of 1.00±0.01 at 517 nm wavelength using a spectrophotometer, however, with slight modification. Also, 100 µL of the sample was mix with 1 mL DPPH solution and kept closed in the dark for 2 hours. On the other hand, Trolox was taken as the baseline, and the results were reported as mg Trolox equivalent (TE)per 100 g of dry sample (mgTE/100g of DW).

e) Phytochemical analysis using gas chromatography-mass spectrometry (GC-MS/MS)

Methanol crude extract (MCE), methanol fraction (MF), acetone fraction (AF), and active fractions were analysed using GC-MS/MS technique, according to Stankov et al.[13]. The GC-MS/MS was Agilent 7890A, GC/7000 MSD-Triple Quad (Agilent Technologies, Palo Alto, CA, USA), electron impact (EI) ionisationmode (70 eV, acquisition mass range of 50-600) and HP-5MS (integrated with cross-link 5%-phenyl methyl polysiloxane, 30mm×0.25mm, coating thickness 0.25µm) capillary column. Injector and detector temperature were set at 200^{0}C. The temperature of the oven was held at 50^{0}C for 30 min, then speed up to 250^{0}C at therate of 3^{0}C. Helium (99.99%) was used as the carrier gas witha flow rate of 1 ml/min. Diluted sample (1/100 in hexane, w/v) of 1.0 µl were injected. The identification of bioactive compounds depended on varies comparison. Their mass spectra (MS) is compared with those of Wiley 7N (392,086 bioactive compounds spectra), NIST 2011(contains 300,234 compounds spectra), EPA/NIH mass spectral libraries and retention times (RT).

f) Statistical analysis

The cell viability calculation was performed in triplicates. Moreover, each resulting point indicates the overall average of at least three independent trials. The results were examined and expressed in terms of the mean of the samples as well as the standard deviation. Graph Pad Prism Version 7.00 (Inc., La Jolla, CA, USA) and Minitab Software version 17® were used to calculate the statistical parameters. Finally, one-way ANOVA and Dunnett's t-test were used to identify any significant differences between the means of several independent samples.

III. Results and Discussion

a) Anti-proliferative effect of methanol extract of Acacia seyal gum

Table 1 and Figure 2-3 present the results of the anti-proliferative effect (APE) for the methanol crude extract (MCE) *Acacia seyal* gum (ASG), and PTC samples and their active fractions against the human breast tumor MCF-7 cell lines. To the best of the Author's knowledge, this is the first time for the APE of *Acacia seyal* gum investigated using MCE on *in vitro* cell lines based. In this experimental study, the human breast adenocarcinoma (MCF-7) cell lines were studied comprehensively, and therefore, the results in Figure 2 shows active crudes of methanol and acetone extraction, as well as their active fractions from both ASG and PTC.

Also, to the American National Cancer Institute (NCI) guidelines, which have also been mentioned by Fouché et al. [14], SRB assay was also used in this research. NCIC technique defines the mean value of the logarithm growth inhibition at 50% cell lines (GI_{50}) for MCF-7 tumor cell lines. Based on the NCI procedure, the methanol crude extract (MCE) obtained from ASG, and PTC showed an average means of log GI_{50}=0.980 and 0.944, respectively, for the MCF-7 cell lines. The NCI criteria show individual growth inhibition (GI_{50}) values indicate potent activity when log GI_{50}<0, similar to ' 'Taxol's values as elaborated by Table 1. Therefore,

Taxol is one of the most effective drugs used to inhibit cancer cell lines, thus termed as a positive control.

Despite excellent growth inhibition (GI) of cancer cells, Taxol also affects the growth of tumor cells, as illustrated by Table 1 and Figures 2. Unfortunately, non-tumor cells, for instance, the ones from the VERO cell line, were not included in this study for better comparison. In this regard, all assayed of methanolic extracts seem to be like Taxol, since toxicity to tumor cells had reached high concentrations only. For example, the methanolic crude extract (MCE) of both samples (PTC and ASG) was the most active reagent against MCF-7 cell lines, reaching IC_{50} value of as low as8.79±0.046 µg/mL and9.56±0.047 respectively. On the other hand, the mean value of IC_{10} was 1.51±0.02 and µg/mL1.81±0.012, whereas an average value of $IC_{90}$was recorded (51.08±9.02 µg/mLand50.39±6.01) for the MCF-7cell line respectively. On the other hand, gum arabic is more than emulsifier of food additive (E414), as many people thought.

However, methanol fractions (MF) of both ASG and PTC, had the lowest inhibition potential of log GI_{50}=1.315 for ASG and log IG_{50}=1.391 for PTC regarding MCF-7. In contrast, methanol crude extract (MCE) of both samples(ASG and PTC) presented moderate activity with mean of log GI_{50} = 0.980 and 0.944 respectively. The above trend is well elaborated in Table 1 and Figure 2. Therefore, the highest growth inhibition of MCF-7 cell lines was illustrated by MeOH crude rather than the active fractions.

Among the fractions, ASG methanol fraction displayed various means; IC_{50}=20.66±0.01, IC_{10}=10.87±0.13 and IC_{90}=39.27±4.13µg/mL, thus showing a slight selectivity with MCF-7 cell line. On the other hand, the PTC acetone fraction (AF) manifested moderate selectivity with MCF-7 cell lines as shown by the means; IC_{50}=18.58±0.03, IC_{10}=6.62±0.11, and IC_{90}=52.13±4.23µg/mL. Finally, by looking for all fractions, the acetone fraction (AF) for both ASG and PTC was found to be more selective with MCF-7 cell lines, indicating a mean of IC_{50}=12.17±0.08 and 18.58±0.03 µg/mL, respectively. Regrettably, no previous study shaded lights on similar results regarding gum arabic.

However, Manthey et al[15] reported that the activity of extracts with IC_{50} values lower than 10µg/mL should be recommended as healthy. Considering this new perspective, only four fractions, including methanol extract of PTC and ASG, as well as the acetone fraction of PTC and ASG, have shown significant results for the cell strains analysis as elaborated by Table 1. Therefore, inhibition growth IG_{50} is a superior measurement technique recommended by the American National Institute of Cancer. Nevertheless, some researchers have used different parameters. For example, previously, Boyd [16] claim that medicinal plant extract is usually valued as significant for in vitro cytotoxic activity when the IC_{50} value is less than 100 µg/mL. Moreover, another study by Kuete *et al.*[17], optimized limit of the activity for crude plant extracts at 50% inhibition (IC_{50}) of proliferation is less than 30µg/mL after exposure for 72 hours. For this reason, a comprehensive study has to be conducted for optimising the optimum level of the IC_{50} values for plants extracts.

In this regard, the findings of the study indicated that methanol crude extracts (MCE) showed the best cytotoxic activity against the selected cell lines as shown in Table 1, compared to other six solvent extracts. Also, the anti-proliferative activity can significantly be affected by gum processing and type of solvent partitioning.

Interestingly, both samples (ASG-AF and PTC-AF) have revealed results ($p \leq 0.5$) very significantly with MCF-7 human cell lines. For instance, PTC-AF found to be nearly doubled the mean(IC_{50}=18.58±0.03 µg/mL) of MCF-7 cell lines. In contrast, the mean value (IC_{50}=12.17±0.08µg/mL) of ASG–AF for the same cell lines is elaborated well by Figure 2. Figure3 illustrated the images of the surviving MCF-7 cells after 24 hours incubation period. Finally, it was concluded that solvent partitioning might have a positive effect on a wide range of human cell lines screening as well as characterisation.

Gum arabic (GA) is a well-known biopolymer compound with antioxidant properties, nephroprotective ability, and other effects that have been highlighted in some recently conducted studies. Its function on the lipids metabolism as well as the beneficial effect in the treatment of certain degenerative illnesses such as kidney failure, gastrointestinal [8,18], and cardiovascular [7] related diseases have also been reported. Thus, GA is considered to be one of the most effective natural products for treating serval diseases, including cancer.

However, there have been no indications about the comprehensive mechanism of GA towards the anticancer activity. Therefore, in this study, the high anticancer activity of the ASG and PTC methanol, and acetone crude extract and its different fractions may have attributed to their high gum bioactive compounds. The effectiveness of different fractions of ASG and PTC has different levels of cytotoxic activity against the MCF-7 human cell lines. Overall, the data are consistent with the traditional use of GA in the treatment of some cancer types and considered as potential sources for anticancer compounds.

For detecting the bioactive compounds (BCs), the components from all methanol crude extracts (MCE) and active fractions were determined by GC-MS/MS as described under the methods section. GC-MS/MS chromatograms for MCE and active fractions extracted through solvent partitioning with major bioactive compounds are shown in table 3. In Table 3, it can be clearly seen that the major constituents in the ASG and PTC were found to be Isovitamin C (42.37%), Crypton (5.86%), Hydroquinone (4.86%), Triacetic acid lactone

(2.67%), 2,4-Di-tert-butylphenol (2.67%), Cyanidin cation (2.05%), Apigenin 7-glucoside (1.9%), Benzoic acid (1.83%), (+)-α-Tocopherol (1.58%), Methyl catechol (1.42%), and 2,6-dimethylol-p-cresol (2.16%). However, these same components were almost doubled in PTC compared to ASG, as presented in Table 3.

Nine compounds namely; Crypton (7.83%), Chromone, 5-Hydroxy-6,7,8 l,-trimethoxy-2,3-dimethyl (7.01%), Phe-1,4-diol, 3,6-dimethyl (6.65%), Hydroquinone (5.31%), Ferulic acid(5.84%), Isopinocampheol (3.06%), Benzoic acid (2.02%), Isovitamin C (1.34%), and β-carotene (1.21%), were found to be significantly high and present in both ASG-AF and PTC-MF respectively. However, Vanylglycol, Quercetin 3-D-galactoside, Vitexin, Gengkwanin, Gallic acid, Retinoic acid, Zearalenone, '4',7-Dimethoxyisoflavone, and flavone, '4'-methoxy-6-acetyloxy, were calculated in methanol fraction (MF) only.

One of the most significant compounds detected by GC-MS/MS was flavonoids; this helps in understanding the most fundamental mechanism of action. For example, Isovitamin C was detected in methanol crude extract of ASG, and PTC (benzoic acid, Crypton, Hydroquinone, Patchoulol, Fisetin, α-Bisabolol, and resveratrol) was ubiquitous. These results suggest that flavonoids are not the only compounds affecting the anti-proliferative effect of Acacia gum extract, since they are present also in the extract with weak inhibition activity, sometimes in higher contents. Also, flavonoids do not indicate the leading role of inhibition of the MCF-7 cell line alone. Following Table 1 and Figure 2, the acetone fraction of both ASG and PTC shows no significant influence on the anti-proliferative effect. Therefore, further investigation is needed in order to understand the mechanism of action with regards to methanol extraction of gum Arabic. This will enhance the potential application of ASG in suppressing MCF-7 cell lines.

In an earlier study, the most abundant constituents present in the volatile fractions of gum arabic were not reported[19]. However, in this study, most of the identified bioactive compounds (BCs) were reported as polyphenols, hydrocarbons, phenolic acids, fatty acids, and several different constituents as clearly shown in Table 3. Various identified compounds have already been reported as pharmacologically active. For instance, iso-vitamin C, to copherol, and Resveratrol have shown antitumor activity in Hep3B hepatocellular carcinoma cells, as reported by Yiang et al[20], and anti-inflammation [21]. Thus, iso-vitamin C and Tocopherol may be considered as the main bioactive compound in ASG.

Furthermore, there are numerous reports about the effects of resveratrol on tumor suppressor gene and transcription factor (p53). For instance, it was proclaimed that resveratrol-induced apoptosis occurred only in cells expressing wild-type p53, not in p53 deficient cells [22]. These results demonstrated for the first time that resveratrol induces apoptosis through the activation of p53 activity. In another study conducted by Aggarwal et al., in 2006, showed that resveratrol inhibited proliferation of pulmonary artery endothelial cells, which correlated with suppression of cell progression through Sand G2-phases of the cell cycle and was accompanied by increased expression of p53 and elevation of the level of the Cdk inhibitor p21Cip 1/WAF1[23]. Thus, ASG extract demonstrated roughly 1% with resveratrol in some fractions alongside with flavonoids.

Several mechanisms have been proposed regarding the effectiveness of flavonoids, including the initiation of process of carcinogenicity promotion and influences on development and hormonal activities[24]. Flavonoids have a molecular mechanism of action namely; down regulation of mutant p53 protein, inhibition of heat shock proteins, tyrosine kinase inhibition, cell cycle arrest, inhibition of expression of R as proteins, and estrogen receptor binding capacity.

The p53 mutation is among the most common genetic abnormalities in human cancers. The inhibition of the expression of p53 may lead to the arrest of the cancer cells in the G2-M phase of the cell cycle. For this reason, flavonoids are found to down regulate the expression of a mutant p53 protein to nearly undetectable levels in human breast cancer (MCF-7) cell lines[25]. Tyrosine kinases (TK) are a family of proteins located in or near the cell membrane (CM). They allow transduction of growth factor (GF)and signals to the nucleus. Their expression is thought to be involved in on cogenesis via an ability to override standard regulatory growth control (RGC). Drugs inhibiting tyrosine kinase (TK) activity are thought to be possible antitumor agents without the cytotoxic side effects that are seen in conventional chemotherapy. Quercetin (detected in methanol fraction only) for example, was the first tyrosine kinase inhibiting compound tested in a human phase II trial [26].Thus, Quercetin can possess a cure for cancer cells.

Flavonoids are known to inhibit the production of heat shock proteins in several malignant cell lines, including breast cancer (MCF-7), leukemia, and colon cancer [25]. Interestingly, in this study, the authors believe that the flavonoids in ASG extract are not only responsible for inhibiting MCF-7 cells lines but also suppressing other cells, and therefore, further investigation will be needed.

Previously, it has been reported that flavanol epigallocatechin-3-gallate can inhibit fatty acid synthase (FAS) activity, and lipogenes is in prostate cancer cells, which is strongly associated with growth arrest and cell death[27]. Up regulation of FAS occurs early in tumor development and is further enhanced in more advanced

tumors[28].Thus, the role of polyphenolic compounds in curing tumors is necessary.

In the present study, the Quercetin and other phenolic acid have revealed the same values in almost all crude/fractions, as shown in Table 3. Moreover, Quercetin is well-known to produce modulators of cell cycle arrest (MCCA) in proliferating lymphoid cells (PLC). Also to its antineoplastic activity, Quercetin exerted growth-inhibitory effects on several malignant tumor cell lines in vitro. These included P388 leukemia cells, gastric cancer cells (NKN-7, HGC-27, NUGC-2, and MKN 28), colon cancer cells (320 DM), human breast cancer cells, human squamous, gliosarcoma cells, and ovarian cancer cells[25]. Markaverich et al[29]suggested that tumor cell growth inhibition (TCGI) using Quercetin may have integration with nuclear type II estrogen binding sites (EBS). This has been experimentally proved, increased signal transduction in human breast cancer (MCF-7) cell line is dramatically decreased by Quercetin when acting as an anti-proliferative agent[30].

Moreover, hydroquinone exhibit a superior ability to inhibit MCF-7 and MDA-MB-231 breast cell growth compared to the standard cisplatin [31]. The maximum consumption of phytoestrogens, involving flavonoids and other is of lavones groups, has shown important protection against prostate cancer risk[32]. It was confirmed during the oxidative stress period, cancer initiation may take place, and thus potent antioxidants show potential to combat the progression of carcinogenesis. The positive impact of antioxidant as an anticancer agent depends on its competence as an oxygen radical in activator and inhibitor[33].Therefore, diets rich in radical scavengers would diminish the cancer-promoting action of some radicals[34]. Thus, gum extracts have a promising natural inhibitor for breast cancer.

Also, Crypton and Hydroquinone are best known to have potential antifungal and antibacterial activities[35, 36]. Furthermore, long-chain unsaturated fatty acids (LCUFAs), such as triacetic acid lactone, also show higher antibacterial activity and are considered to be the essential ingredients of antimicrobial, food additives and some antibacterial activities[37]. Moreover, Calder [38] has reported a similar investigation as an anti-inflammatory agent for these compounds. Furthermore, benzoic acid, ferulic acid, and β- carotene also show anticancer and antioxidant activity [39-41]. Thus, the presence of such bioactive compounds in the gum arabic solvents is considered to play an extremely crucial role in the everyday pharmacological activities as shown by methanol, acetone crude, and its fractions. This finding turns a strong candidate for further in-depth studies about the anti-proliferative activity. Thus, the revaluation of Acacia gum (E414), as a food additive as well as an emulsifier, is exceptionally crucial.

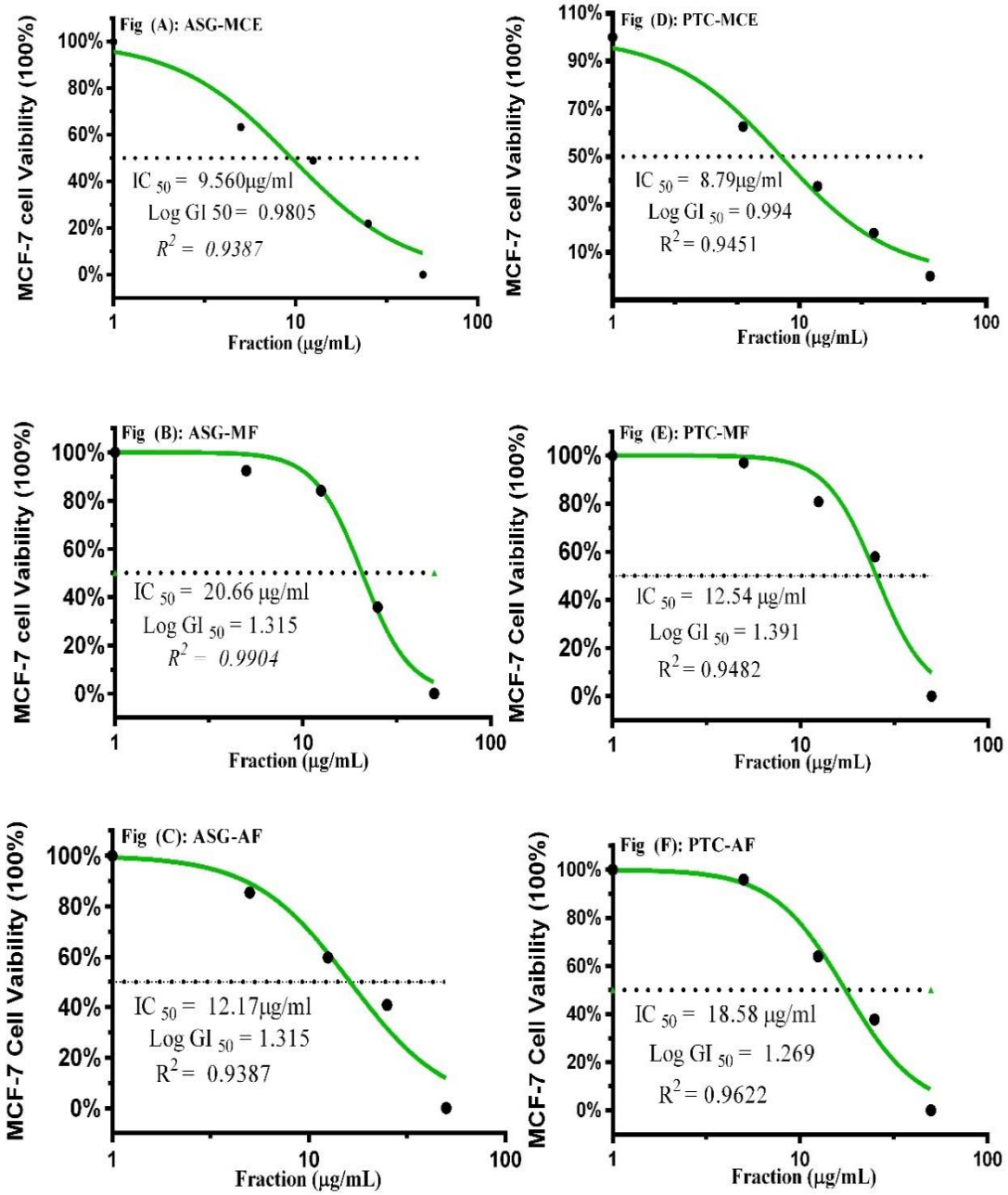


Figure 2: The percentage of MCF-7 cell viability vs. concentrations of *Acacia seyal* gum and Prebio-T commercial (PTC) methanolic crude extracts and their active fractions showed 50% cell kill against cell lines of Human Tumor Carcinoma at the concentration of µg/ml respectively. MF: Methanol fraction and AF: Acetone fraction. Data is based on triplicate experimental sets (N=3±S.D).

Table 1: Extract concentration (μg/mL) needed to 10%, 50% and 90% of growth inhibition of breast carcinoma (MCF-7) cell lines

Plant source	Active Fraction	IC10, IC50, and IC90 inhibitors of MCF-7 Cell lines a				Mean Log GI 50b
		IC50	IC10	IC90	R-square	
Taxol (+ve)[c]		0.13	0.13	0.13		-1.26 P
DMSO(-ve)[d]		0.045	0.045	0.045		
A. seyal gum raw	ASG-MCE	9.56±0.047	1.81±0.012	50.39±6.01	0.9387	0.980 M
	ASG-MF	20.66±0.01	10.87±0.13	39.27±4.13	0.9904	1.315 W
	ASG-AF	12.17±0.08	1.56±0.016	95.24±7.45	0.8343	1.085 W
Prebio-T *A.seyal* gum Commercial	PTC-MCE	8.79±0.046	1.51±0.02	51.08±9.02	0.9451	0.944 M
	PTC-MF	24.54±0.03	11.35±0.13	53.05±5.19	0.9482	1.391 W
	PTC-AF	18.58±0.03	6.62±0.11	52.13±4.23	0.9622	1.269 W

Abbr: [a]Cell lines: MCF-7: mammary.[b]National Cancer Institute criteria [14], 1: inactive, mean log GI 50>1.5;W: week activity, mean log GI 50=1.10-1.5; M: moderate activity, mean log GI 50= 0-1.10; P: potent activity, mean log GI 50=0. 'positive control'; and DMSO is 'negative control'.

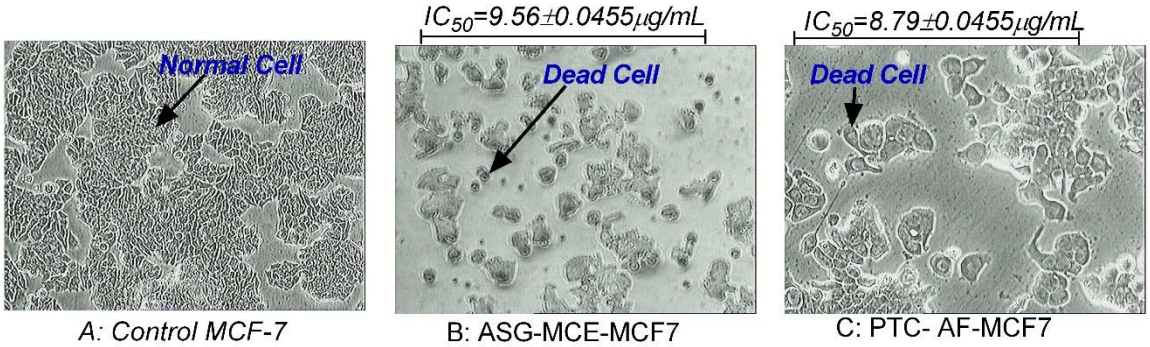


Figure 3: Demonstrative images show the potent surviving MCF-7 cells at 24 hours incubation following the treatment of *A. seyal* gum (ASG) and Prebio-T (PTC) crude extract from methanol, and their active fraction/s respectively.

b) *Antioxidant activities of methanol extract of Acacia seyal gum*

i. *Total phenolic content (TPC)*

The methanolic crude extract (MCE) of ASG and PTC samples have shown higher antioxidant activity. As presented in Table 2, the yield of extraction recorded was roughly the highest with methanol fraction (MF) instead of the acetone (AF). Between the crude extract and solvent partitioned fractions, the maximum values of the total phenolic content (TPC) was seen in the PTC samples having an average value of 694.68±3.60 mg GAE/100g DW. On the other hand, the TPC value observed was 155.78±2.58 mg GAE/100g DW for ASG samples. The TPC value of MF was found to be 285.08±3.57 mg GAE/100g DW for ASG, whereas TPC value for PTC was significantly higher ($p \leq 0.05$) at 519.93±1.64 mg GAE/100g DW. Furthermore, the TPC value of AcOH fraction (AF) was 358.57±1.58 mg GAE/100g DW for *Acacia seyal* gum (ASG) compare to the TPC value of 657.81±2.58 mg GAE/100g DW for PTC acetone fraction; this is approximately twice. The results indicated that the crude extract and solvent partitioned fractions values have a descending order of MCE, AF, then MF for PTC and AF, MF, then MCE for ASG, respectively. Both samples depicted a significant difference ($p \leq 0.05$) for antioxidant activity. Present results were in good agreement for phenolic compounds; it can be defined as a secondary metabolite with the role of antioxidants, thus owing to their capability of donating hydrogen (DH), therefore, acting as metal chelators, and quenching singlet oxygen [42]. It has been mentioned that the consumption of phenolic-rich foods or beverages prevents diseases, such as cancer, heart disease, arthritis, inflammation, immune-related diseases, neurodegenerative diseases, and diabetes [43]. This study endorsed the health benefits associated with the presence of phenolic compounds in ASG.

ii. *Antioxidant activity by DPPH assay*

The anti scavenging capacity (DPPH) was investigated for the first time as an antioxidant activity test for GA fractionation, as presented in Table 2. The maximum DPPH value was seen in methanol fraction (MF) at 235.34±1.57 mg TE/100g DW for PTC, in contrast to ASG at 235.35±1.51 mg TE/100g DW, this shows no significant differences. The DDPH antioxidant

activity of both acetone fractions (AFs) was also high, with AF obtained at the mean of 233.78±2.57 mg TE/100g DW and 234.85±1.57 mgTE/100g DW for PTC and ASG, respectively. Within each DPPH method, the mean values revealed significant differences between the crude extract and its fractions, which also significantly ($p \leq 0.5$) affects the antioxidant activity.

In this paper, the determined antioxidant activity is considered to be powerful, as compared to the standard gallic acid (GA), which also exhibits a strong correlation with the total phenolic content. The finding showed the possibility of the presence of polyphenolic molecules in ASG and the higher ability of polar solvents to extract them. Thus, the bulk of the solvent polarity is increased with the ability of extraction and thus reducing DPPH radical scavenging activity, especially methanol and acetone fractions. The findings are compared to results on some rice bran protein hydrolysates as reported recently by Phongthai *et al.*[44]. Since there is no enough data related to DPPH values of crude gum extract and gum fractionations, therefore, it was proposed that gum methanol crude extract and gum fractions could have anti-radical scavenging activity[45]. Thus, more studies are urgently needed regarding antioxidant assays in ASG.

Table 2: The antioxidant properties of different active fractions of *A. seyal* gum (natural exudate) (ASG) and *A. seyal* gum Prebio -T commercial (PTC) obtained after Kupchan-partitioning of the crude methanolic extract and its fractions as presented by Elnour et al.[5].

Plants	Extraction/ Fraction	Antioxidant activity of methanol crude and it is active fractions	
		TPC mg GAE/100g	DPPH mg TE/100g
A. seyal gum (ASG) (natural)	ASG Crude Extract (CE)	$155.78^{f} \pm 2.58$	$205.10^{d} \pm 1.50$
	ASG MF	$285.08^{e} \pm 3.57$	$235.35^{a} \pm 1.51$
	ASG AF	$358.57^{d} \pm 1.58$	$234.85^{ab} \pm 1.57$
A. seyal gum; Prebio - T (commercial)	Prebio-T-Crud Extract (CE)	$694.68^{a} \pm 3.60$	$229.01^{c} \pm 3.58$
	Prebio-T-MF	$519.93^{c} \pm 1.64$	$235.34^{a} \pm 1.57$
	Prebio-T-AF	$657.81^{b} \pm 2.58$	$233.78^{b} \pm 2.57$

Abbr: ASG-MCE: Crude Methanol Extraction, ASG-MF: methanol fraction, ASG-AF: acetone fraction, ASG-HXF: hexane fraction, and ASG-CHF: chloroform fraction, respectively. Total phenolic content (TPC) expressed as milligram Gallic acid equivalent per 100 grams dry weight of crude/fraction of sample (mg GAE/100g of crude or fraction Dry weight), and DPPH as anti-scavenging capacity expressed in mg Trolox equivalent per 100 grams dry weight of crude/fraction of sample.

iii. *The chemical composition of the solvent extracts using GC-MS/MS analysis*

In an experimental study, the crude and active fractions were extracted from ASG and PTC by solvent-solvent partitions to determine their chemical composition using GC-MS/MS analysis. According to the author's knowledge, there are no reports yet on the GC-MS/MS analysis for gum arabic regarding extraction. Their chemical investigations show that the methanol crude extract (MCE), its methanol fraction (MF) and acetone fraction (AF) of the ASG and PTC are dominated by Isovitamin C amounting to 42 % of the total composition, among the presence of a total of 21 compounds as illustrated in Table 3. The main components in this group (ASG and PTC methanol crude extract) were Isovitamin C (42.37%), Benzoic acid (6.62%), Crypton (5.86%), 2,6-Dihydroxypurine (5.11%), Hydroquinone (4.86%), (+)-α-Tocopherol (3.89%), Thiazolidin (3.38%), Triacetic acid lactone(2.67%), Apigenin 7-glucoside (2.24%), 4-Mercapto-phenol(3.37%), and Resveratrol (0.89 %) respectively. However, similar bioactive compounds were almost doubled in PTC methanol crude extract MCE as illustrated in Table 3. Furthermore, the variation in the bioactive compounds in ASG extraction content and chemical composition is attributed to some well-known factors, including geographical location, solvent polarities, and methods of partitioning employed [46, 47]. Hence, more chemical properties of different fractions of ASG is extremely crucial. Notably, the comparison between the chemical composition of methanol crude extract and its active fraction of ASG is only estimated in the present study. Furthermore, nine compounds, Cyanidin cation (22.29%), Patchoulol (13.06%), Fisetin (5.81%), Crypton (5.90%), Hydroquinone (5.23 %), 2,6-Dimethylol-p-cresol (3.49%), (Dihydrocarvone 2.18%), Iso-vitamin C (2.1%), and Quercetin(0.84%), were found to be significantly higher and present in both ASG-AF and PTC-MF respectively. However, 5,7,3',4'-Tetrahydroxy flavone (8.4%),β-Resorcylaldehyde(2.39%) were estimated in methanol fraction (MF) only. Finally, the compounds in GC-MS/MS analysis were studied based on a comparison of the mass spectra (MS) and retention time (RT) with the references present in the NIST mass spectral library. Therefore, the presence of such components in the gum arabic solvents were thought to play a crucial role in the everyday pharmacological activities as shown by methanol, acetone crude, and its fractions.

Table 3: GC-MS/MS chromatogram of bioactive compounds in *Acacia seyal* gum and Prebio-T methanol crude extractions and their active fractions. ASG-MCE: Crude Methanol Extraction, ASG-MF: Methanol Fraction, ASG-AF: Acetone fraction, PTC-MCE: crude methanol extraction, PTC-MF: methanol fraction and ASG-AF: acetone fraction respectively [5].

		Percentage of the compound in fractions Area Sum (%)								
No	Compound	ASG/ MCE	ASG/ MF	ASG/ AF	PTC/ MCE	PTC/ MF	PTC/ AF	RT	MW	MOF g/mol
1	4-Methylcatechol	1.42	1.43	1.43	3.04	4.25	0.00	3.10	$C_7H_8O_2$	124
2	Thiazolidin	2.49	3.38	3.38	3.02	3.72	1.47	3.56	$C_{10}H_9NO_3S$	223
3	Crypton	5.86	5.90	5.90	4.23	1.24	7.83	5.30	$C_9H_{14}O$	138
4	4-Mercaptophenol	1.11	2.02	2.02	3.37	0.00	1.50	6.29	C_6H_6OS	126
5	Triacetic acid lactone	2.67	1.76	1.76	2.6	2.74		4.54	$C_{10}H_{10}O_3$	178
6	Hydroquinone	4.86	5.23	5.23	5.15	1.33	5.31	7.00	$C_6H_6O_2$	110
7	Isobornyl acetate	1.05	1.89	1.89	0.00	0.00	1.34	7.86	$C_{12}H_{20}O_2$	136
8	Apigenin 7-glucoside	1.90	1.96	1.96	2.24	0.00		8.33	$C_{21}H_{24}O_9$	420
9	Benzoic acid	1.83	0.00	0.00	6.62	0.00	1.49	8.68	$C_7H_6O_2$	122
10	2,6-Dihydroxypurine	1.48	2.26	2.89	5.11	0.00	2.02	9.12	$C_5H_4N_4O_2$	152
11	(+)-α-Tocopherol	1.52	2.89	1.64	3.89	2.81	0.00	9.23	$C_{29}H_{50}O_2$	430
12	β-Resorcylaldehyde	0.00	0.00	0.00	2.39	0.00	6.65	9.34	$C_7H_6O_4$	154
13	2,4-Di-tert-butylphenol	1.10	2.27	0.00	0.00	1.60	2.45	11.12	$C_{14}H_{22}O$	206
14	2,6-Dimethylol-p-cresol	2.16	2.1	3.49	2.63	0.00	0.00	13.56	$C_9H_{12}O_3$	168
15	Isovitamine C	42.37	42.45	2.1	24	1.81	1.34	14.23	$C_6H_8O_6$	176
16	Cyanidin cation	2.05	2.14	2.4	2.39	22.29	1.46	15.89	$C_3H_3N_3$	81
17	Fisetin	0.00	1.90	1.90	0.45	5.81	2.42	16.09	$C_{15}H_{10}O_6$	286
18	Ferulic acid	0.00	7.49	7.49	1.16	0.00	1.34	16.58	$C_{10}H_{10}O_4$	194
19	Resveratrol	2.89	0.64	0.64	0.47	0.71	0.54	16.91	$C_{14}H_{12}O_3$	228
20	β-Citronellol	0.00	1.01	1.4	0.00	0.00	5.84	16.97	C10H20O	156
21	Dihydrocarvone	0.00	2.18	2.18	0.00	4.29	0.075	17.13	$C_{10}H_{16}O$	152
22	Patchoulol	1.21	3.35	3.35	1.74	13.06	1.52	17.21	$C_{15}H_{26}O$	222
23	5,7,3',4'-Tetrahydroxy flavone	0.00	8.4	0.00	0.00	2.11	1.60	17.31	$C_{15}H_{10}O_6$	286
24	Quercetin	0.44	0.39	0.84	0.33	0.46	0.49	19.35	$C_{15}H_{10}O_7$	302

Abbr:Acacia seyal gum methanol (ASG); MCE:Methanol Crude Extract; MF: Methanol fraction; AF: Acetone fraction; PTC: Acacia seyal gum (commercial sample).

iv. *Correlation analysis between methanol crude extract and its active fraction and IC_{50} of MCF-7 cell lines*

Table 4 presents the correlation coefficients of the possible correlation between the methanolic crude extract (MCE), and its active fractions and the human breast carcinoma (MCF-7) cell lines as presented in Table (4). It also shows the correlation between the different antioxidant methods used. The DPPH and TPC exhibited a significant and positive linear correlation ($p \leq 0.05$) with MCF-7. The correlation was a decreasing order of DPPH > and TPC, respectively. These results suggested that the anti-proliferative activity express as (IC_{50} values) is more closely related to DPPH than TPC. A higher positive correlation between DPPH and antiperoxidative properties also proved that the anti-scavenging compounds were the major contributors to the anti-antiproliferative capacity of the MCE and their active fractions of each *A. seyal* gum and Prebio-T (PTC) commercial. Moreover, Pearson correlation analysis of the findings showed a significant and positive correlation between cell lines IC_{50} ($p \leq 0.05$). The highest correlation was found between TPC and MCF-7 ($r=0.656$). However, DPPH anti-scavenging capacity (DPPH) material of MCE and their active fractions

resulted in the highest correlation (r =0.976) towards MCF-7. It indicated that the bioactive compounds in the extracts that could inhibit MCF-7 cell lines and served as anti-proliferative inhibitors. Furthermore, the strong correlation between DPPH and TPC, and MCF-7 respectively cell lines suggests that the antioxidants in the methanolic crude extracts, and it is active fractions react similarly with these antioxidant assays.

Table 4: Person's correlation coefficient of methanol crude extract and its active fraction and IC_{50} of cell lines.

Variables	DPPH [a]	TPC [b]
TPC mg GAE/100g DW	0.773**	
MCF7-IC_{50} (μg/mL)	0.976***	0.656*

Note: Antioxidant activity measured in methanol crude extract and their fractions on [a]DPPH radical - scavenging activity, and [b]FCI assays, correlated with MCF-7 human cell lines, * significant and, *** highly significant at $p \leq 0.05$ and 0.01.

IV. Conclusions

In this study, methanol crude extract (MCE) and its active fraction of both ASG and PTC exhibit anti-proliferative effect against breast cancer adenocarcinoma (MCF-7) cell lines by inducing loss of cell viability via cell death, change of cell morphology, and cell cycle arrest at the G0/G1 phase. This inhibition was selective to the growth of MCF-7 cell, proposing that MCE of ASG possesses selective antitumor towards cancer cells when compared to Taxol as a positive control. It also revealed their potentiality as an antioxidant activity when calculated as Gallic acid and Trolox equivalent using TPC and DPPH, respectively. Furthermore, the MCE of ASG has inhibited MCF-7 cell growth by reducing the number of cell growth inhibition (GI). These results proposed that the MCE of ASG and PTC can be considered as a novel defence-based agent for the prevention and cure of breast cancer. More studies are urgently needed to explore the mechanism of action to peruse the therapeutic impact of ASG extract, in addition to the investigation of bioactive compounds that thought to be responsible for cytotoxicity towards breast cancer.

Conflict of Interest

The authors declare that there is no conflict of interest. The copyright for reusing Table 3 is under license of permission letter (DPL-4821).

Acknowledgements

The authors would like to express gratitude and gratefulness to Dr. Elbasheir Sallam for his continuous and unlimited financial support to the Ahmed. A. M. Elnour while conducting this research. Finally, we would like to thank the American Journal of Pharmacology and Pharmacotherapeutics for the permission letter (DPL-4821) grated to us with regards to Tables 2 and 3.

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Global Journal of Science Frontier Research: C
Biological Science
Volume 20 Issue 3 Version 1.0 Year 2020
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Avances En La Resistencia Del Género *Conyza* A Distintos Herbicidas Pre Y Posemergentes

By Alejandro Bagnolo, Eduardo Cortés, Ignacio Dellaferrera & Marcos Mitelsky

Introduction- *Conyza sp,* conocida como “rama negra”, es una de tantas malezas que crecieron en abundancia en la mayoría de los campos de la región pampeana. Dentro de este nombre reconocemos en Argentina a 3 especies que se comportan como malezas. *Conyza sumatrensis* (Retz.) E. Walker var. Sumatrensis (Retz.) E. Walker, *Conyza bonariensis* (L.) Cronquist var. bonariensis (Colla) y Conyza bonariensis (L.) Cronquist var. angustifolia (Cabrera) Cabrera. Estas tres especies son capaces de formar comunidades, lo que dificulta su diferenciación cuando se establecen juntas (Olivella et al, 2016).

GJSFR-C Classification: *FOR Code: 069999*

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Alejandro Bagnolo [α], Eduardo Cortés [σ], Ignacio Dellaferrera [ρ] & Marcos Mitelsky [ω]

I. Introducción

Conyza sp, conocida como "rama negra", es una de tantas malezas que crecieron en abundancia en la mayoría de los campos de la región pampeana. Dentro de este nombre reconocemos en Argentina a 3 especies que se comportan como malezas. *Conyza sumatrensis* (Retz.) E. Walker var. Sumatrensis (Retz.) E. Walker, *Conyza bonariensis* (L.) Cronquist var. bonariensis (Colla) y *Conyza bonariensis* (L.) Cronquist var. angustifolia (Cabrera) Cabrera. Estas tres especies son capaces de formar comunidades, lo que dificulta su diferenciación cuando se establecen juntas (Olivella et al, 2016).

Esta maleza presenta varios atributos biológicos asociados, que la posicionan como problemática y que son:

La correcta identificación.
La falta de monitoreo.
El inadecuado uso de herbicidas.
Las aplicaciones fuera de los tamaños recomendados.

Las tres especies son de ciclo anual e inician su germinación en varios pulsos dependiendo de las condiciones climáticas siendo la variedad *Conyza bonariensis* var. angustifolia la que primero se registra en el campo. Luego *Conyza sumatrensis* var. sumatrensis y finalmente *Conyza bonariensis* var. bonariensis. La floración también sigue este orden desde fines de primavera hasta fin del verano.

Imagen 1: izq *C. bonariensis* var. angustifolia; derecha *C. bonariensis* var. bonaeriensis.

En estado vegetativo las 3 se presentan como roseta. Las plantas adultas son erectas, exhiben una raíz pivotante robusta, son híspido-pubescentes, de colores verde-grisáceos o amarillentos, de 30 y hasta 200 cm de altura, con tallos rectos, cilíndricos, sub-leñosos en la base, densamente hojosos, erguidos. Las hojas son alternas, sésiles, pubescentes, y es una de las pocas características vegetativas que pueden diferenciar a *Conyza bonariensis* var. angustifolia con hojas muy estrechas de los dos tipos restantes con hojas lanceoladas (imagen 1).

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II. Situación Actual De La Resistencia

Durante varios años esta maleza pudo controlarse sin problemas con glifosato, sobre plantas en estado vegetativo (*Ustarroz et al*.: 2010), aunque ya en estado reproductivo si presentaba complicaciones, una correcta combinación de herbicidas y momentos de aplicación lograban niveles adecuados de control (*Papa et. al*, 2010). Sin embargo a partir del año 2008 comenzaron los reportes de fallas de control (imagen 2).

Imagen 2: Individuos de *C. bonaeriensis* var. bonariensis con distinta sensibilidad a glifosato.

En el año 2012 se confirma la resistencia de *Conyza bonariensis* a glifosato. Aun así, estos biotipos resistentes podían ser manejados con un programa basado en el uso de la combinación de glifosato y herbicidas inhibidores de ALS en pre y posemergencia (Puricelli *et al* 2015).

Por tanto, si bien el desarrollo de la resistencia a glifosato era un problema a tener en cuenta, se presentaban todavía muchas alternativas para lograr eficaces controles de esta maleza. Es así, que los herbicidas inhibidores de ALS se comenzaron a utilizar con alta frecuencia en la última década debido a su particular persistencia en el suelo y buen control posemergente.

En año 2018, en la zona de San Pedro (Buenos Aires) se detectaron plantas de *Conyza* que presentaban una sensibilidad diferencial en comparación con el resto en donde se aplicó una dosis de diclosulam en posemergencia. En forma simultánea se comenzaron a recibir denuncias de fallas en el control.

A inicios del año 2019, se confirma la resistencia en un biotipo de *Conyza sumatrensis* del departamento Caseros (Santa Fe) determinado por aplicaciones post emergentes de herbicidas ALS (Balasone *et al* 2019).

Esta denuncia no fue una denuncia más, dado que en la actualidad el grupo de estos herbicidas son utilizados ampliamente en barbechos otoñales y primaverales, y las sulfonilureas particularmente son la base herbicida de los cultivos de invierno.

III. Evaluación De Resistencia a Herbicidas Inhibidores ALS Utilizados Como Residuales o Preemergentes

En concordancia con la aparición de poblaciones sospechosas, es que se realizaron ensayos en situaciones controladas en laboratorios de la FCA-UNL de Esperanza. Se trabajó con distintas poblaciones de *Conyza sumatrensis*, provenientes del norte de la provincia de Buenos Aires y del centro de la provincia de Santa Fe.

En todos los casos se realizaron aplicaciones preemergentes de herbicidas pertenecientes a las 3 familias dentro del grupo de inhibidores de ALS (imidazolinonas, triazolpirimidinas y sulfonilureas).

De todas las poblaciones testeadas las provenientes del norte de la provincia de Buenos Aires sobrevivieron a las dosis de uso recomendadas para cada uno de los herbicidas testeados.

Comparadas con los testigos sin tratar, los tratamientos a dosis de uso, mostraban reducción en el número de plantas y en el tamaño de las mismas. Estas plantas sobrevivientes lograron, sin embargo, continuar el crecimiento hasta producir semillas.

Los resultados demuestran que dentro de la familia de las sulfonilureas evaluadas para ambos

herbicidas probados, se necesitan entre 9 y 10 veces más cantidad de herbicida que la dosis de uso recomendada para un control efectivo.

En el caso del Diclosulam (Triazolpirimidinas) se necesitaron 3 veces la dosis de uso para controlar el 80% de la población. Mientras que las imidazolinonas probadas (imazapyc + imazapyr) son las que menos necesitan incrementar la dosis para un control efectivo, siendo esta dosis alrededor de dos veces la dosis de uso.

Así mismo, si se comparan las poblaciones anteriores con poblaciones sensibles estas requieren (para lograr el 50% de reducción de biomasa), incrementar la dosis entre 3 y 6 veces para imidazolinonas y hasta 150 veces para las dos familias restantes.

Por lo tanto, se puede decir que las poblaciones de *Conyza sumatrensis* var. sumatrensis estudiadas se comportan como biotipos *resistentes a los herbicidas inhibidores de ALS, en este caso utilizados como residuales,* lo cual acrecienta más el problema, principalmente en los lotes que se destinaran a cultivos invernales.

IV. Evaluaciones De Herbicidas Alternativos

Anexo a los resultados anteriores, en el Laboratorio LMAgro se evaluaron mediante ensayos de dosis respuesta los herbicidas Flumioxazin y Terbutilazina (como herbicidas pre emergentes de uso en trigo) y Atrazina (utilizado en barbecho a maíz).

Estos herbicidas aplicados a la dosis de uso, fueron capaces de controlar satisfactoriamente los nacimientos de los biotipos de rama negra, determinados como resistentes a inhibidores de ALS en estudio.

Además, se realizaron ensayos de dosis respuesta en Glifosato, Saflufenacil, 2,4D y Dicamba como posemergentes sobre los biotipos de *Conyza* antes citados. Ninguno de los herbicidas posemergentes utilizados fueron totalmente efectivos en el control del biotipo resistente. Todas las dosis necesarias para el control del 80% de la biomasa estuvo por encima de la dosis de uso recomendada *encontrándose estos activos al borde de la pérdida de efectividad.*

V. Consideraciones Técnicas

El panorama actual de *Conyza sp.* referido a la resistencia en distintos herbicidas (confirmadas o en desarrollo), sirve para prevenirnos y estar atentos en nuestros lotes. Con esto no significa que debamos dejar de usar los herbicidas ALS y los hormonales (solos o en mezclas); pero si es importante monitorear nuestros lotes con mayor frecuencia luego de la aplicación de estos activos para ver si se observan fallas de control y poder actuar en consecuencia, más que todo aquellos que se sembrarán con cultivos de invierno.

Por otro lado, es importante ante esta situación ser coherentes en la planificación de rotación de principios activos con distintos mecanismos de acción, ya que el avance de la resistencia se verá acentuado si se repiten las aplicaciones de un mismo grupo herbicida.

El monitoreo de malezas en macollaje del trigo tiene que ser más activo y preciso, debido a la ventana de acción que nos permite controlar escapes o nacimientos. Si tenemos sospechas de resistencia a ALS y ya hemos aplicado un residual del mismo mecanismo de acción, no sería recomendable aplicar nuevamente un herbicida ALS.

Si se presentan sospechas de que los herbicidas ALS utilizados presentan fallas de control y vamos a sembrar trigo, los únicos herbicidas registrados *no ALS* son Flumioxazin (PPO) y Terbutilazina (PSII), cada uno con sus consideraciones de uso. Como se comentó anteriormente, en todos los ensayos de laboratorio y campo, los mismos presentaron muy buenas performances, principalmente la triazina.

Teniendo en cuenta que la evaluación de los herbicidas posemergentes más usados, resulto en una muy baja eficacia para el control de los biotipos en estudio, son las mezclas las que actualmente se recomiendan y permiten el control de esta maleza, sin embargo, se observan algunos escapes a estos manejos que hoy son comunes.

Otra opción en el período de barbecho, es la incorporación del doble golpe (DKD), estrategia útil y que mejora la performance de los tratamientos en malezas poco sensibles a glifosato en situaciones de escapes de las mismas. En particular este manejo sobre especies del género *Conyza* ha resultado en un control efectivo cuando estas alcanzan un tamaño superior al de inicio de roseta.

Considerando que los manejos químicos se hacen cada vez más complejos, debemos conocer en profundidad nuestros lotes y diseñar estrategias de manejo que aporten soluciones, con el fin de reducir la presión de esta maleza, pero teniendo en cuenta la sostenibilidad del sistema.

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7. Este informe está basado en investigaciones realizadas en laboratorio y a campo, desarrolladas por el equipo de la Cátedra de Cultivos Extensivos de la FCA-UNL en colaboración con investigadores y técnicos externos.

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Financial

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Associates are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

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We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template.zip

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Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

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It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
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- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

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Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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ISSN 9755896