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Highlights

Properties of Turmeric Powder

Nutrient Profiling of Tropical Soybean

Discovering Thoughts, Inventing Future

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Reversible Antifertility Effect of Withaferin-A from *Withania somnifera* in Male Albino Rats

By Dr. Ashish Ranjan Singh & Dr. Maheep Kumar
University of Rajasthan

Abstract- *Background:* Currently world population crosses the 7.30 billion and increasing continuously day by day. There is a great need to support an individuals in family-planning since increasing growth rate of world's population caused negative impact on sustainable, economic growth and increased poverty especially in developing countries. However, there is still no method available in the field of male contraception that satisfies the essential criteria of safety, efficacy, economy and complete reversibility. Clearly, there is a need for development of reversible contraceptive from natural resources. Therefore, we have performed the present study to examine the effect of Withaferin-A on the sexual hormone levels and to consider the effect of alkaloid of *Withania somnifera* on changes of glucose, cholesterol and triglyceride serum levels in male rats and can prevent the fertility of male albino rat by evaluating some andrological parameters such as sperm motility, sperm counts, rate of fertility and morphology which are some of the indices that determine the ability of a male to produce viable spermatozoa.

Keywords: *Withania somnifera, Withaferin-A, Contraceptive, Antifertility, Sperm Motility.*

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Reversible Antifertility Effect of Withaferin-A from *Withania somnifera* in Male Albino Rats

Dr. Ashish Ranjan Singh [α] & Dr. Maheep Kumar [σ]

Abstract- ***Background:*** Currently world population crosses the 7.30 billion and increasing continuously day by day. There is a great need to support an individuals in family-planning since increasing growth rate of world's population caused negative impact on sustainable, economic growth and increased poverty especially in developing countries. However, there is still no method available in the field of male contraception that satisfies the essential criteria of safety, efficacy, economy and complete reversibility. Clearly, there is a need for development of reversible contraceptive from natural resources. Therefore, we have performed the present study to examine the effect of Withaferin-A on the sexual hormone levels and to consider the effect of alkaloid of *Withania somnifera* on changes of glucose, cholesterol and triglyceride serum levels in male rats and can prevent the fertility of male albino rat by evaluating some andrological parameters such as sperm motility, sperm counts, rate of fertility and morphology which are some of the indices that determine the ability of a male to produce viable spermatozoa.

Method: The Withaferin-A was separated by column chromatography; compound was finally purified by crystallization and identified with the help of NMR and administered orally at different dose levels. Animals were distributed equally into five treatment groups containing ten animals in each. Animals were administrated orally with Withaferin-A at the dose of 10, 20 and 40 mg/kg. body wt./day to male albino rats daily for 60 days. A marked dose-dependent decrease in the count and motility of sperms of treated rats was observed as compared with controls in a randomized controlled study and followed by 30 days recovery period.

Results and discussion: This plant-based contraceptive inhibited male fertility, after administration of Withaferin-A from *Withania somnifera*. A marked reduction in counts and motility of caudaepididymal sperm in a dose-dependent manner was observed in treatment group but after 30 days withdrawal of treatment all these changes were reversibly observed in recovery group.

Conclusion: The oral administration of Withaferin-A (*Withania somnifera*) in male albino rats produced a reversible antifertility effect.

Keywords: Withania somnifera, Withaferin-A, Contraceptive, Antifertility, Sperm Motility.

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I. Introduction

Currently population explosion is one of the biggest problems facing by world. It's inevitable consequences are employment, education, housing, health care, economy and environment. At the present growth rates, the population of economically developed countries would double in 120 years. Overpopulation led to serious social and environmental problems such as poverty, overcrowded slums, crime, pollution of air & water and depletion of the protective ozone layer (Vogelsong, 2005) and all around human development especially in developing countries like India (Akbarsha *et al,* 2001,). India is also only the second country to achieve a population of 1.32 billion. Our future well-being depends on increased access to family planning and reproductive health services in developing countries and decreased consumption by people in wealthy countries (Speidel, 2000).

India is first among the countries which adopted an official family planning programmed, as early as 1950. However, fifty years later this has not prevented the population touching the one billion mark (Qian *et al,* 1995). It is obvious that despite good intentions and concerted efforts we have failed in controlling our population. Since the major responsibilities of pregnancy, birth, and child rearing fell on women, they found methods for controlling fertility and aborting unwanted children, and they have passed down this knowledge as an oral tradition that survives worldwide. It is obvious now that there cannot be an ideal contraceptive (Moudgal and Rao, 1984; Joshi *et al,* 1977) suitable for everybody.

About 90% of the world's contraceptive users are women. This gender-based usage has occurred due to the emphasis of family planning programs and contraception research (Hazarika and Sarma, 2007).The only male-specific contraceptive methods currently available are withdrawal, condoms, and vasectomy. As concerns regarding side effects and convenience of these existing methods prevent (Beckman *et al,* 1996; Moore, *et al,* 1996) their universal acceptance. There is an urgent need for development of male contraceptives drug to prevent unintended pregnancy, of which 80–90 million occur annually (Amory, 2016). The development of additional male methods of fertility control can provide tremendous social and public health benefits. Because methods that require infrequent administration

have the lowest typical (user) failure rates (Potter, 1996), considerable research has focused on development of a herbal male contraceptive.

Fertility regulation comprising contraception and management of infertility forms an important (Pankajakshy and Madambath, 2009) component of reproductive health. Though considerable progress has been made in the development of highly effective, acceptable and reversible methods of contraception (Njar*et al,* 1995) among females. Progress and possibilities on male are still slow and limited, with recent progress towards a better understanding of male reproductive physiology; there is a need to develop new contraceptive modalities for male. Several potential approaches for induction of infertility have been investigated over a long period including hormonal, chemical, and immunological, approaches. However, no suitable method has emerged that is effective and free from side effects (Montanari *et al,* 1998).

Clearly, there is a need for development of reversible contraceptive from natural resources. The chemical compounds affecting testicular function include steroidal and non-steroidal but application of these compounds has serious reversible antifertility effect (Kulshreshtha and Mathur, 1990; Jensen, 2002). The present study was undertaken therefore, to determine and examined whether plant might have any effect on male reproductive organs, spermatogenesis, and serum hormone levels in rats. Although contraceptives containing estrogen and progesterone are effective and popular, the risks associated to the drugs have triggered the need to develop newer molecules from medicinal plants (Swerdlloff *et al,* 1998). Hence, there is a need for searching suitable product from indigenous medicinal plants that could be effectively used in the place of pills. More over the phytochemical even today are important resources for medicine (Sundaram and Kumar, 2000).

Plant drugs have been used since time immemorial for their effects upon sex hormones particularly for suppressing fertility, regularizing menstrual cycle, relieving dysmenorrhea, treating enlarged prostate, menopausal symptoms, breast pain and during and after childhood (Williamson *et al,* 1996). Specific biological effects under the division of fertility regulating category are non- specific contraceptive or antifertility effects, abortifacient, uterine stimulant and uterine relaxants, labour induction and labour inhibition oxytocic and anti- oxytocic, oestrogenic and anti-oestrogenic, progesterogenic and anti-progesterogenic, ovulatory and anti- ovulatory, androgenic and anti-androgenic, spermicidal and anti- spermatogenic effects (Soejarto, *et al,*1978). The flavonoids, phytosterol, and terpenoid present in the alcoholic extracts of *Piper betle* may be responsible for significant antifertility activity (62.2%) (Shah andJhade, 2018).

Antifertility agents may therefore exert their effort at this level either by disrupting (Bullock *et al*, 1995, WHO, 1990) hormonal function of the hypothalamus or the pituitary, or by interrupting the neural pathway to the hypothalamus that control the liberation of gonado-tropins releasing hormones.

The investigation of plant constituents with antifertility properties represents a potential alternative approach to birth control from the existing available methods. If an estrogen from a local source could be shown to be active in humans, it would be of great value as a fertility-regulating agent (United Nations, 1998).The development of new fertility regulating drug from medicinal plants is an attractive proposition, because from times immemorial humans have relied on plants and their products as sources of drugs and therapeutic agents, although in recent times, synthetic drugs are used extensively(Tuxhorn, 2002) in modern medicine. However many modern medicines are developed through the clues obtained from phytochemical.

In view of the importance of plants in the traditional Indian system as a positive health promoter, it was decided to carry out work on the chemical profiling of W. somnifera on the basis of Withaferin-A (Roja *et al,*2006, Ganzera *et al,* 2003, Sengupta P*et al,* 2018).

Nonstandardized herbal preparation have not found acceptance in the global market; therefore, there is a need to be chemically standardized on the basis of isolated constituents, preferably bioactive ones. Presently, formulations standardized on the basis of a maximum possible number of biomarkers are accepted readily in the global market. Thus, as part of a long-term evaluation of potential antifertility plant, we have conducted these studies on the effects of *Withania somnifera* extractand their alkaloids (Withaferin-A) on the fertility of rats.The present investigation elucidated the association of biological activities with specific secondary metabolites known as Withaferin-A present in the *Withania somnifera.* The aim of present study was to evaluate safety and reversible contraceptive efficacy of alkaloids from this plant to search for an inexpensive, orally effective and reversible male contraceptive. The present study will help in the development of reversible male oral contraceptive from natural resources and to determine whether plant drugs (phytochemical and phytosomes) might have any effect on male reproductive organs, spermatogenesis and serum hormone levels in rats. The availability and use of acceptable male contraceptive methods could reduce the burden traditionally placed almost exclusively on the female partner.

II. Material and Methods

a) Collection of Plant Materials and Preparations of ethanol extract

The plant *Withania somnifera* Dunal (Family: Solanaceae), also known as Ashwagandha, Indian ginseng, winter cherry has been used in Ayurveda, since ancient times to increase longevity and vitality (Mishra *et al,* 2000). All the parts of the plant *Withania somnifera* have shown remarkable of pharmacological activities are menstrual troubles, dropsy, rheumatism, sexual and general weakness, asthma and bronchitis, diabetes and inflammation (Al-Hindawi *et al,* 1992; Andallu and Radhika, 2000; Sree *et al*, 2008) treatment. The active pharmacological components of *Withania somnifera* are steroidal lactones of the withanolides and the principal Withanolides in Indian *Withania somnifera* are Withaferin -A and Withanolide -D (Gupta *et al*, 1996).

The *Withania somnifera*was identified for authenticity (Herbarium No. RUBL, 19445) in the Department of Botany, University of Rajasthan, Jaipur. The required amounts of *Withania somnifera* was collected from different places around Jaipur. The shade dried and finely crushed plant materials as well as stem; leaves were extracted with 50% of ethanol 8 hours thrice. The extract was filtered and concentrated under the reduce pressure, where a dark brown mass was obtained. The concentrated extract were washed with chloroform for the removal of chlorophyll, washed extract further concentrated under the reduce pressure, and finally chlorophyll removed extract were used for fractionation.

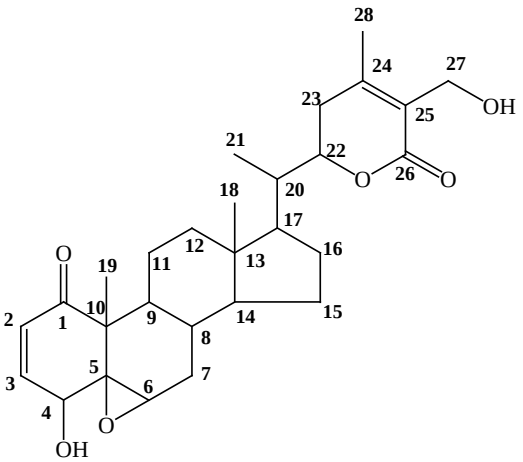
b) Isolation and identification of Withaferin-A

The air-dried root powdered of *Withania somnifera* (2.4 kg) was extracted with ethanol: water (1:1, 4 L) for 8×3 hours, at 32 °C .The ethanol was evaporated under the vacuum. The eight times extract were combined and finally concentrated to 1/8 of the original volume (590 gm.) under reduced pressure at 50 ± 5°C. The concentrated extract was stirred with chloroform to remove chlorophyll content. The chloroform extract was subjected to four times column chromatography on silica gel (1.2 mt., 250 mesh, 600×4g, 0.580×4L), eluted with a stepwise gradient of increasing order (5 to 10% each step and 2 L solvent eluted in each fraction) ethyl acetate (Khajuria *et al*, 2004; Baraiya *et al,* 2005; Mishra *et al*, 2008) concentration in pet ether was eluted. White crystals (5.4 mg) in ethyl acetate were obtained from ethyl acetate: Petroleum ether (1:3) fraction. The repeated crystallization in acetone from fractions was observed for Thin Layered Chromatography (Merck's silica gel $60F_{254}$ precoated glass plate) in system chloroform: ethyl acetate (1:1).

The comparisons of IR (3445; O-H stretching, 2930, 2880, 1719; α,β-unsaturated-δ-lactone, 1665; α,β-unsaturated six-membered ketone, 1447, 1370, 1295, 1130,1025; epoxy), ^{1}H NMR spectral data (300 MHz, $CDCl_3$) δH: 5.85 (1H, dd, J = 9.8 Hz, H-2), 6.53 (1H, dd, J = 10.2 Hz, H-3), 2.48 (1H, m, H-4), 3.19 (1H, d, J = 3.5 Hz, H-6), 2.25 (2H, dd, J = 3.1Hz, H-7), 1.90 (1H, m, H-8), 4.47 (1H, dd, J = 12.7, 3.24 Hz, H-22), 2.25 (1H, m, H-23), 2.68 (1H, m, H-23'), 4.38 (3H, s, H-27), 1.98 (3H, s, H-28), 1.25 (3H, s, H-21), 0.97 (3H, s, H-18), 1.22 (3H, s, H-19), ^{13}C NMR (75 MHz, $CDCl_3$) δC^{13}: 200.5 (C-1), 140.1 (C-2), 135.1 (C-3), 64.8 (C-5), 63.2 (C-6), 52.8 (C-7), 48.5(C-10), 25.1 (C-12), 44.0 (C-14), 10.1(C-19), 31.3 (C-20), 18.3(C-21), 78.2 (C-22), 149.3 (C-24), 168.3 (C-26), 58.7 (C-27) data. Melting point (247.50°C) of single spotted compound with previously reported showed the presence of Withaferin-A in availability of other compounds (Withaferin A, Withanone, Withanolide D).

The comparisons of IR (3445; O-H stretching, 2930, 2880, 1719; α, β-unsaturated-δ-lactone, 1665; α,β-unsaturated six-membered ketone, 1447, 1370, 1295, 1130,1025; epoxy).

The H^1 NMR spectrum (δ ppm, $CDCl_3$) displayed a double doublet at 5.85 (J = 9.8 Hz) for one proton present at H-2 position. Proton present at C-3 position form double doublet at 6.53 (J = 10.2 Hz). Multiplet at 2.48 showed many proton nearby at C-4, doublet at 3.19 for C-6, double doublet at 2.25 for C-7 and 4.47 for C-22 showed two type of proton on different environment. Singlet for C-27, C-18, C-19 in the range of 0.97-1.98 showed an isolated proton without neighboring protons. Complex multiple pattern for C-8, C-23, C-27 in the range of 1.90-4.38 showed presence of more proton at neighboring position. On the basis of above discussion compound was identified as Withaferin-A.



Withaferin-A

Withaferine A [4β, 27-dihydroxy-1-oxo-5β, 6β, epoxy witha-2, 24-dienolide]

(M.P: 245 to 252°C)

c) Animal Model

Colony bred, healthy, fertility proven adult wistar rats (*Rattusnorvegicus*) 60 days aged. Before using the animals for experiments, their initial body weights was recorded and blood samples was examined. Supervision of qualify Veterinarian was available throughout the study. The animals were housed in well-ventilated animal room and kept in plastic cages under controlled conditions (12 hrs. light: 12hrs dark).The rats was maintain on pallet standard rat feed supplemented with soaked gram and wheat and water was provide.

d) LD_{50} of Alkaloids

Withania somnifera extract has been found to be safe up to 2500mg/kg. Withaferin-A, therapeutic marker of WS3 is reported to have LD50 of 80 mg/kg. This suggests comparatively safe profile of WSE over Withanolide (Sharada *et al*, 1996).

e) Treatment Protocol

The experiments, namely an antifertility effect and androgenicity, nature of the alkaloid was conducted during the course of study with Withaferin-A. The experiments suspension were prepared daily (10, 20, 40 mg /ml) before administration. The required drug was administered orally at different dose levels for a period of 60 days. Animals were equally distributed into Five treatment groups containing 10 animals in each, as follows:

f) Experiment

Group-A This group was given alone sterile distilled water orally for 60 days. This group was serves as control group.

Group-B Animals of this group were fed with Withaferin-A at the dose level of 10 mg/kg.body wt./day for 60 days, so this group serves as treated group. A suspension of the Withaferin-A was made daily in DMSO for the administration. Freshly prepared drug was administered orally (oral gavages) with a glass syringe fitted with a feeding needle.

Group-C Animals of this group were fed with Withaferin-A at the dose of 20 mg/kg.body wt./day for 60 days.

Group-D Animals of this group were fed with Withaferin-A at the dose of 40 mg/kg.body wt./day for 60 days.

Group-E Animals of this group were receive Withaferin-A 20 mg/kg.bodywt./day for 60 days followed by 30 days recovery period. These groups were serves as recovery group.

g) Study parameters

i. *Body and Organ Weights*

The initial and final body weights of the animals were recorded. Testes, epididymis, seminal vesicles and ventral prostrate were dissected out, freed from adherent tissues and weighed to the nearest milligram on an electronic balance.

ii. *Sperm Motility and Density*

For sperm motility and density, 50 mg of cauda epididymis was minced in 1 ml of physiological saline. Within 5 minutes after scarification, 1 drop of evenly mixed sample was applied to a glass slide under a cover glass. The percent motility was determined by counting both motile and immotile spermatozoa per unit area. After that caudaepididymal sperm density was made by routine procedure and express as millions/mm^3 suspension (WHO, 1983).

iii. *Fertility Test*

To check fertility of animal mating was carried out with all the animals 5 days prior to sacrifice (male female ratio 1:2). The mated females were allowed to complete the gestation. The number of pups was recorded and litter size and percent fertility was calculated (WHO, 1983).

iv. *Hormone Assay*

Blood samples were also collected for serum separation to estimate testosterone, FSH and LH by radioimmunoassay. Serum samples were separated by standard procedures and stored at -20° C for subsequent analysis. Serum levels of testosterone were assayed in duplicate using radioimmunoassay kit (WHO, 2000).

v. *Tissue Biochemistry*

The testis, epididymis, seminal vesicles and ventral prostrate were dissected out, freed from adherent tissues and weighted at nearest milligram balance. Cholesterol (Mann, 1964), Protein, (Lowry *et al*, 1951), Sialic acid (Warren, 1957), Ascorbic acid(Roe and Kuether,1943) and Fructose (Foreman *et al*, 1973) were estimated in right side of testis and other accessory reproductive organs.

vi. *Histological Study*

Contra laterial side of the testis, epididymis, seminal vesicle, kidney and liver were fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Sections were cut at 6 μ was stained with Harris's hematoxylin and eosin to observe under light microscope. The same sections were used for percentage of normal tubules. Similarly, seminiferous tubules and inter-tubular areas were observed, finally expressed of seminiferous tubules, inter-tubular area and Sertoli cell nuclear area (Abercrombie, 1946) was also determine.

vii. *Statistical Analysis*

Data are expressed as mean ± S.E. and analyze for statistical significance by using student's "t" test. The data considered as significant and highly significant at $P \leq 0.01$, respectively (Gupta, 1978).

h) Ethical Aspects

The study was carried out under the supervision of the ethical committee of the Department of Zoology, University of Rajasthan, Jaipur, India and CPCSEA

(ICMR, 2006) guidelines were followed for maintenance and use of the experimental animals.

III. Results

a) Body and organ weight

The isolated alkaloid Withaferin-A was dissolved in 10 % DMSO and administrated orally in male albino rats for period of 60days. Withaferin-A was administrated orally to intact control vehicle treated rats. The weight of Testes, Epididymitis, and Vasa deferens of rats treated with Withaferin-A at the dose level of 10 mg/kg. b. wt., 20 mg/kg. b. wt. group (Group-B and Group-C,) were non-significantly changed while treatment at the dose level of 40 mg/kg. b. wt. (Group-D) weight of testes significantly reduced ($P \leq 0.01$) as compared to control intact rats (Group-A). Testicular weight of recovery rats after the treatment (Group-E) however altered non-significantly ($P \leq$ ns) (Table 1) Whereas the weight of seminal vesicle, Ventral Prostrate, Kidney, Liver, Heart, an Adrenal gland of Withaferin-A treated rats was non-significantly decreased ($P \leq$ ns) in all treated groups (Group-B, Group-C, Group-D and Group-E) as compared to the control intact rats (Group-A) (Table 1), while treatment with Withaferin-A did not altered body weight of the animals when compared with control group animals.

b) Sperm motility and density

Caudaepididymal sperms motility and density were significantly diminished in treatment with Withaferin-A. In recovery group, changes in sperms motility and density were reversibly observed (Table 2).

c) Fertility test

It was observed that the fertility was significantly reduced in Withaferin-A treated rats at different dose levels. The Withaferin-A treatment reduced fertility of rats respectively by, 64%, 51 % & 35% in dose depended manner while fertility of rat of recovery revealed that after 30 days withdraw the treatment the percentage of pregnancies were increased up to 72 % (Table 2).

d) Hormone Assay

i. *Luteinizing and Follicular Stimulating Hormones (LH and FSH)*

The levels of Luteinizing and Follicular Stimulating hormones in Withaferin-A treated rats was significantly decreased at the dose of 40 mg /kg b. wt. as compared to control rats, while LH and FSH levels in other groups were non significantly changed after treatment. The withdrawal of the treatment show normal concertation of hormones in the rats. (Figure 1).

ii. *Testosterone*

Withaferin-A treatment for 60 days at the dose level of 10 and 20 mg/kg. b. wt., dose level the testosterone levels in serum was non-significantly decreased while 40 mg/kg. b. wt., testosterone levels was decreased highly significantly and in recovery group showed non-significantly changes as compared to control intact rats (Figure 2).

e) Tissue Biochemistry

i. *Changes in Cholesterol and Glycogen level-Testis, Liver and Heart*

The cholesterol content was a marked decreased in Withaferin-A treated rats after the 60 days treatment. It was observed that level of cholesterol in testis and Adrenal gland were non-significantly altered at the dose level of at10 mg/kg. b. wt. while treatment at the level of 20 and 40 mg/kg. b. wt. were significantly decreased cholesterol content in dose depended manner. However in rats of recovery group treatment cholesterol contents were altered up to normal range as compared to cholesterol level in testis and adrenal gland of control intact rats. Observation of cholesterol level in rats treated with Withaferin-A to control intact rats did not altered in liver, heart as compared to control rats (Table 3).

ii. *Ascorbic acid and Fructose*

Data of ascorbic acid contents of adrenal gland and fructose level of seminal vesicle of rats follows Withaferin-A treatment at different dose level to intact rats show normal changes while rats treated at 40 mg/kg. b. wt. show significantly decreased the both contents (Table 3).

f) Protein and Sialic Acid (Testis, Epididymis (Cauda), Seminal Vesicle, Ventral Prostate, Vas-deferens)

The Protein and Sialic Acid levels of Testis, Epididymis (Cauda), Seminal Vesicle, Ventral Prostate, Vas-deferens in rats follows Withaferin-A treatment at the dose level of 10 and 20 mg/kg. b. wt. was non-significant change while both contain level in treated rats at the dose levels of 40, mg/kg. b. wt. was significantly decreased. However recovery rats show minorchange in both contain level of all reproductive organs as compared to control intact rats (Fig 3a and 3b)

g) Histopathology of testes

Histological studies of control rat's testes showing well develop structure of highly convoluted seminiferous tubules lined by a stratified germinal epithelium, which contained all sprmatogenic cells (Spermatogonia, primary spermatocytes, secondary spermatocytes, spermatid and mature spermatozoa) and Sertoli cell with their distinctive nuclei present in the basal lamina.The spermatogonia can see close to the basal lamina. The seminiferous tubules are well developed and supported by loose connective tissue containing Leydig cells, blood vessels and nerves.

Photomicrograph of testis of treated rats at the dose level of 10 and 20 mg/kg. b. wt. of Withaferin-A show testicular lesions and degenerative changes in germinal epithelium of seminiferous tubules, number of spermatids and spermatozoa was reduced and lumen devoid of mature sperms. The dose level of 40 mg/kg.

b. wt. of Withaferin-A treatment spermatogenesis was completely arrested and atrophied in treated rats. Cellular debris appears in the lumen normal the seminiferous tubules were reduced and inter-tubular in between seminiferous tubules space increase. The testes of the treated animals revealed the arrest of spermatogenesis. Vacuolization was observed in the Sertoli cells, spermatogonia and spermatocytes. Germ cell proliferation beyond the level of the spermatocyte was also affected. The lumen contained sloughed debris and few germ cells. Leydig cell nuclei diameter area and seminiferous tubular diameter were significantly reduced in treated rats. The testes of the recovery animals showed all successive stages of spermatogenesis, and lumen was filled with sperm. Leydig cells were situated in-between the tubules with prominent nuclei (Plate-1).

i. *Cauda*

Photomicrograph of control rats (Group-A) showing normal histoarchitecture of cauda epididymis, lumen is large, lined with pseudostratified epithelium and columnar cells and full with sperms. Oral administration of Withaferin-A in control intact rats reduced lobular size. Lumens of lobules were appeared narrow and inter tubular stroma was in conspicuous. Epithelium was degenerated as compared to control of rats,however Photomicrograph of recovery rats showed normal histoarchitecture of cauda epididymis (Plate-2).

IV. Discussion

Progress to develop a safe orally effective and reversible male contraceptive is moving at a very slow pace. The development of a safe acceptable reversible contraceptive method for man is important steps to increase option for couples who wish to control their family size (Jenson, 2002).The goal of male contraceptive is focused on the inhibition of spermatogenesis process through suppression of the hormones especially androgens (Nieschlag *et al*, 2000). An ideal male contraceptive would be acceptable to large segments of the population, and would contribute to stabilize of population growth (Anawalt and Amory, 2001). The observations of the study are sufficient to establish the fact that Withaferin-A reduce fertility in male rats due to contraceptive like action.

The testicular weight was reduced in Withaferin-A treated rats due to the inhibition spermatogenesis of seminiferous tubules particularly spermatid and spermatozoa in the seminiferous tubules (Jones, 1977; Jain et al, 2012). The protein deficiency in reproductive tract of treated rats might be also responsible for decreased testicular weight and arrest of spermatogenesis at spermatocyte or spermatogonial stages (Okamura et al, 2004; Gupta et al, 2012). So decreased protein level of epididymis sperm is possibly responsible for the decreased in weight of epididymis after the treatment of alkaloids (Paulsen, 1978).

Since the weight of testes is known to as index of FSH secretion, it is suggest that both steroidal and non-steroidal agent inhibit Pituitary gonadotropins either acting directly on the pituitary (Morse et al, 1973; Nair, and Bhiwgade 1990) or through the hypothalamus, hypophyseal axis . The decreased in the weight of testes and sex accessories in rats treated with Withaferin-A probably due to suppression of androgen production by Leydig cells in testes (Narayana et al, 2000; and Wang *et al,* 1999).

The Follicular stimulating hormone responsible for development and function of Sertoli cells by structural proteins and an androgen binding protein, are secreted in the extracellular fluid surrounding the germinal epithelium by Sertoli cells. Androgen binding protein are responsible for the transporting the androgen to the lumen of the epididymis. FSH influences the development of interstitial tissue including LH receptors on Leydig cells. Therefore the reduction in seminiferous tubules and Leydig cell is indicative of reduction in level of FSH and androgen in rats fallows treatment of Withaferin-A. The deleterious effect of Withaferin-A treatment on spermatogenesis of rats suggest impaired Leydig cells functions as evidenced in photomicrographs of testis decreased androgen production arrest spermatogenic process at the primary spermatocytes or spermatid stages (Wu *et al,* 2004).The decrease in the germ cells number in the germinal epithelial of the testes after the administration of Withaferin-A indicates that the site of inhibition is the testes. The increased inter-tubules space in seminiferous reflects the impairment of Leydig cell also affected their tubules function which may be lead to different androgen production.

It has been known for a long time that sperm concentration is related to male fertility (Craft *et al*, 1993). Low concentrations are associated with low fertility. The epididymis spermatozoa undergo morpho-logical, physiological and biochemical changes culminating in their functional maturation. Epididymis provide favorable milieu for the storage and survival of spermatozoa. Androgens are essential for the maturation motility and survival of spermatozoa in the epididymis (Kachhawa *et al*, 2012; Gupta *et al,* 1974).

The decreased of sperm motility suggests structural defects caused by oral administration of Withaferin-A by changing their membrane permeability (Rao, 1979; Kumar *et al,* 2012).

These observations of the study suggest that a strong interaction between the alkaloids and plasma membrane of sperm cell. Sialic acid found free or bound to proteins as sialo mucoproteins secreted by the epididymis and its level are considered to be androgen dependent (Warren, 1959; Stanley *et al,* 1993; Morse *et*

al, 1973). Sialic acid may play role in stabilization of structural integrity of the membrane of spermatozoa, development and maintenance (Rajlakshmi, 1977; Azmeera *et al*, 2012) of fertility ability of spermatozoa.

The density of testicular and epididymis spermatozoa, was reduced significantly in Withaferin-A treated male rats might be due to a consequence of impaired sperm production (Melis, 1995; Lucinda, *et al,* 2011) in the testes. The decreased epididymal sperm density in alkaloids treated rats might be due to reduced level of testosterone since the sperm production in testis and maturation in epididymis are under the control of testosterone (Jana *et al*, 2006).

The result of Withaferin-A treatment in rats marked alteration in sperm counts in dose dependent manner. The androgen deprivation affects sperm density, motility and mature sperms in alkaloids treated rats (Sarvmanglae*t al,* 1983). The decrease in both testicular and epididymal sperms in rats following treatment suggest inhibition of spermatogenesis process by androgen suppression. Since androgen binding proteins are required to maintain intra-tubular androgen concentration and cytological differentiation in epididymis. The decreased proteins and sialic acid in epididymis suggests that the number of sperms was reduced and suppression of androgen with alkaloids treatment. A decreased testicular and epididymis sperm count in rats followed alkaloids administration suggests inhibition of androgen might affect androgen binding protein by Sertoli cell via action on FSH. FSH and testosterone hormones are required for maintaining normal spermatogenesis in rats. It is shown that testosterone alone could restore qualitatively but not the number of sperms. Optimum level of FSH is required to restore the quantity production sperm (Mudgal *et al,* 1997; Almenara *et al*, 2000).

An increase in testicular cholesterol was due to tissue damage increased or decreased the cholesterol has been considered physiological significant.Since cholesterol level involve in inhibition or stimulation of sperm production (Eik-Nes1975). The increased levels of cholesterol in the testes may be considered significant, since it is known to be precursor in androgen biosynthesis in testes and its level is intimately related to fertility and sperm output (Dorfman 1963). Change in level of cholesterol after the Withaferin-A treatment caused degenerative changes in treated rats, might be due to inhibition of steroidgenesis. Adrenal is the main site of steroids synthesis (Saxena and Paul, 1991). Since supplementation of ascorbic acid increased the epididymal sperm concentration and plasma testosterone level and also accelerated degeneration of seminiferous epithelium (Latchomycandane and Mathur, 1999). Therefore decrease ascorbic acid contents in the alkaloids treated rats caused degenerative changes in germinal epithelium of seminiferous tubules resulted decreased number of spermatocyte and sperms in lumen. The fructose level of seminal vesicle alkaloids treated rats was significantly decreased after the treatment at different dose level might be responsible to decreased sperm motility and fertilizing capacity. Therefore androgen supersession effects of treatment may reduce sperm motility and fertility of rats fallows alkaloids treatment (Akbarsha 1995; Rao1988, Mann, 1964). After Withaferin-A treatment of different dose level normal changes were observed in all the hematological indices and serum biochemistry parameters which are show Withaferin-A (WS3) treatment is free from any side effect.

IV. Conclusion

This plant-based contraceptive inhibited male fertility, after administration of Withaferin-A at different dose levels. A marked reduction in counts and motility of caudaepididymal sperm in a dose-dependent manner was observed in the treatment group, but after 30 days withdrawal of treatment, all these changes were reversibly observed in the recovery group.

Significantly decrease in fertility (72% negative) was observed in male rats treated at level of dose 40 mg/kg b. wt. of Withaferin-A, and full recovery were also obtained by withdrawal of alkaloids treatment. Since treatment caused reversible antispermatogenic effects and no adverse orside effect was observed on the general health of the treated animal. In view of above scientific evidence and discussion, Withaferin-A may use as antifertility agent whereas further detail study required for the development of an ideal male contraceptive.

Acknowledgement

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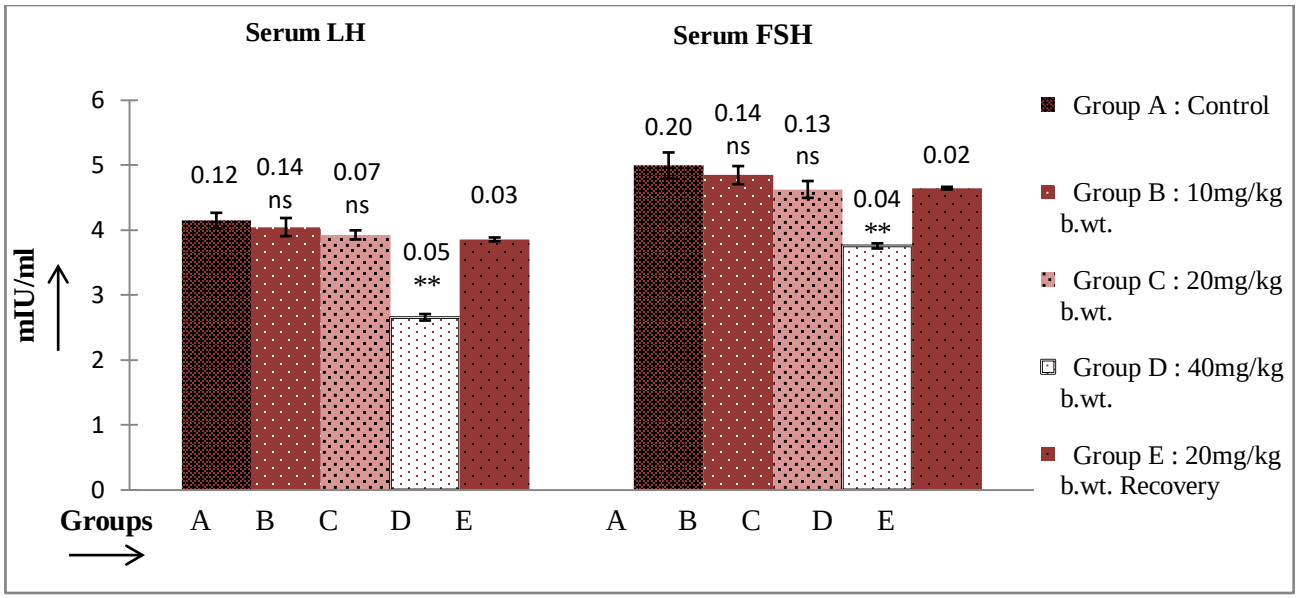

Figure 1: Changes in LH and FSH levels in control and 60 day treatment of Withaferin-A(WS3)in male rats

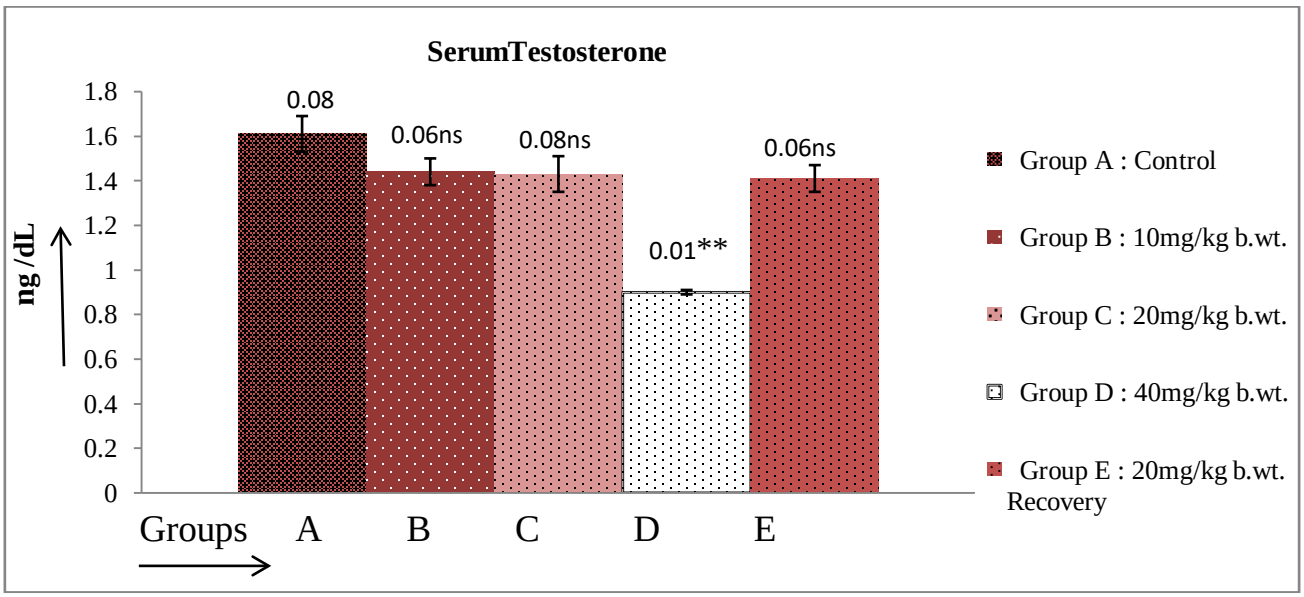

Figure 2: Changes in Testosterone level in control and 60 day Withaferin-A(WS3) treated male rats

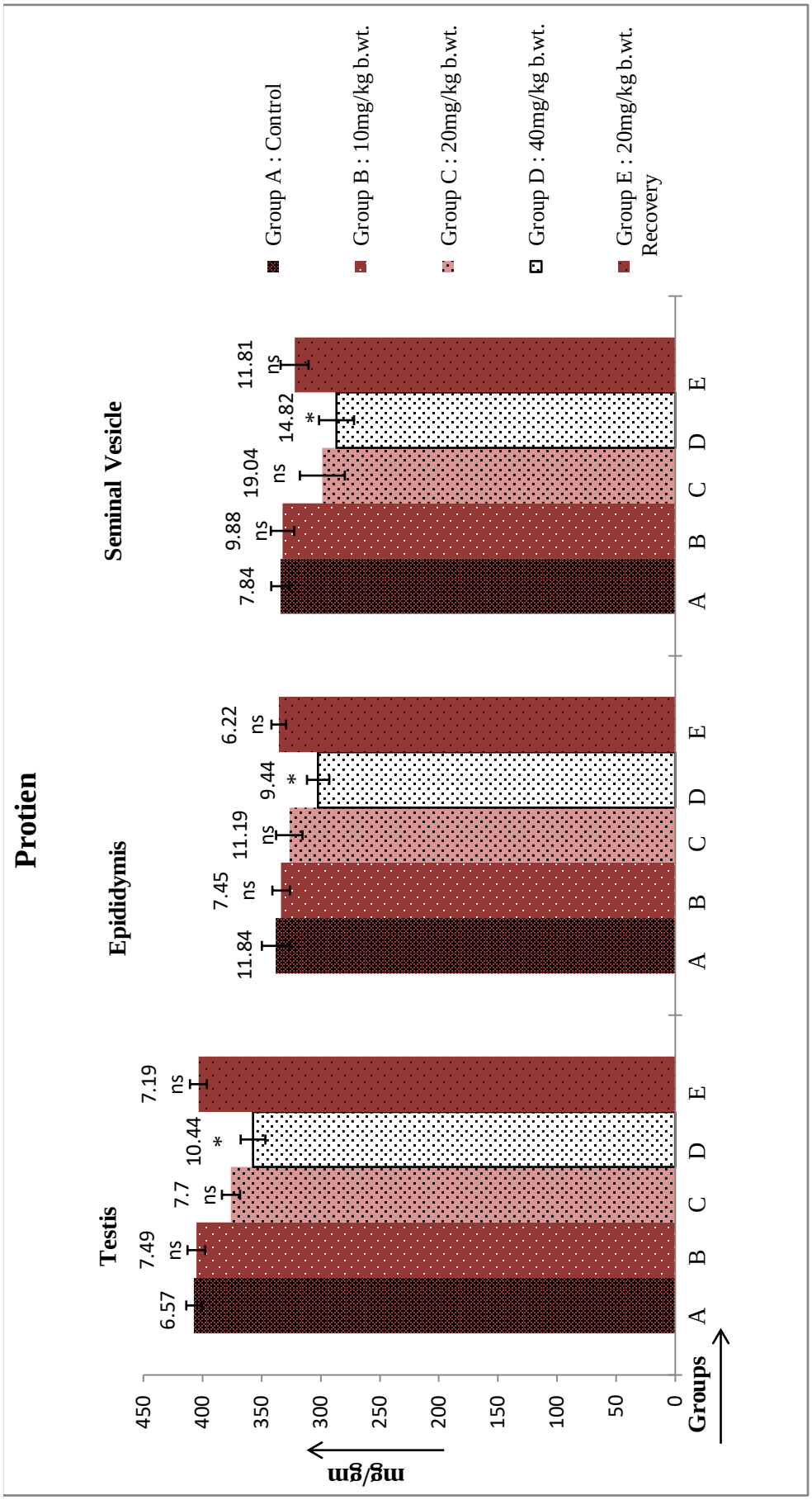

Figure 3a: Changes the protein contents in Testis, Epididymis and Seminal Vesicle of rats after the 60 days treatment of Withaferin-A (WS 3)

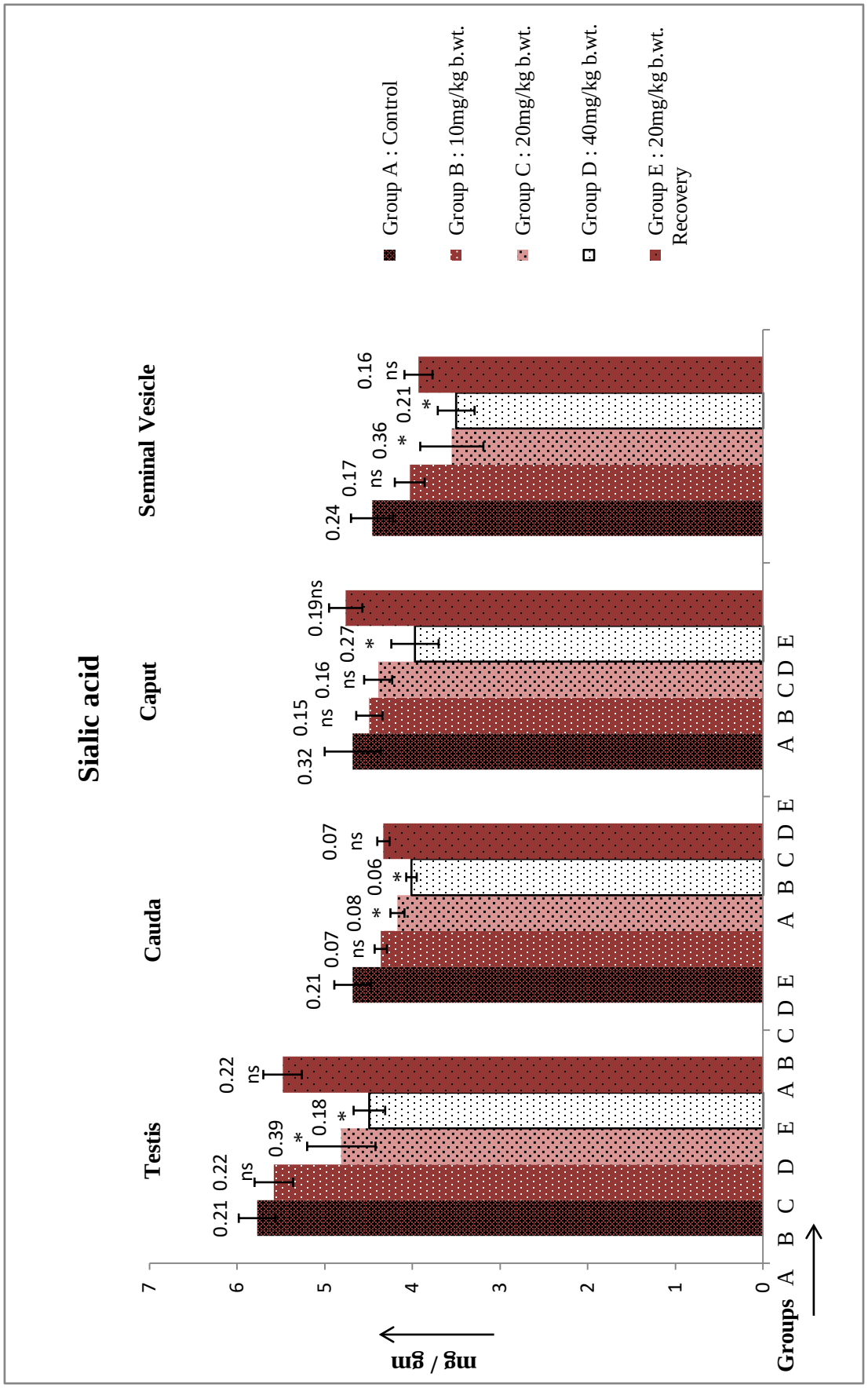

Figure 3b: Changes in Sialic acid contents in Testis, Cauda, Caput and Seminal vesicle of rats after the 60 days treatment of Withaferin-A (WS 3)

PLATE-1A
(Testis)

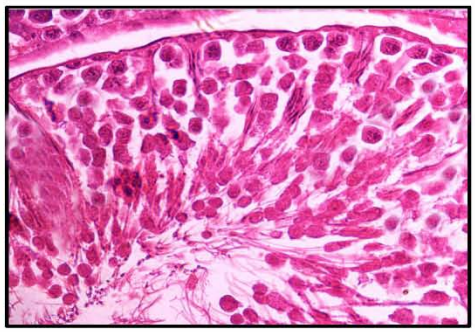

Fig. 1 Group A (Control intact 400X HE)

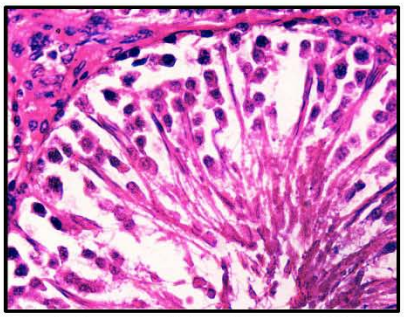

Fig. 2 Group B (10mg/kg.b.wt.400X HE)

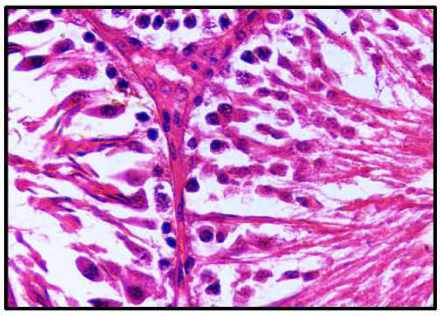

Fig. 3 Group C (20mg/kg.b.wt.400X HE)

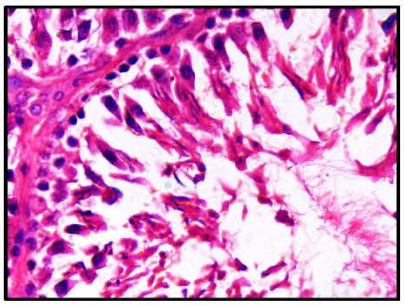

Fig. 4 Group D (40mg/kg.b.wt.400X HE)

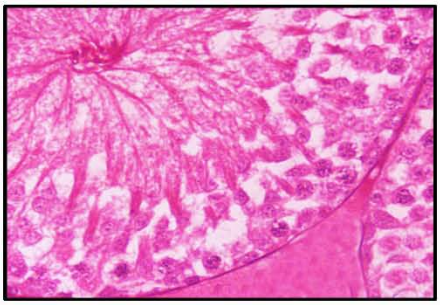

Fig. 5 Group E (20mg/kg.b.wt. Recovery 400X HE)

Plate 1: Photomicrograph of testis of rat fig-1 showing normal histoarchitecture of seminal vesicle and spermatozoa clearly visible in lumen, fig-2-4 showing degenerative changes in dose dependence manner whereas fig-5 showing normal histoarchitecture of spermatogenesis.

PLATE-2
(Cauda Epididymis)

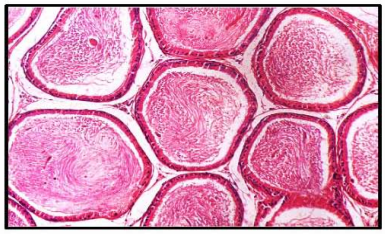

Fig. 1 Group A (Control intact 100X HE)

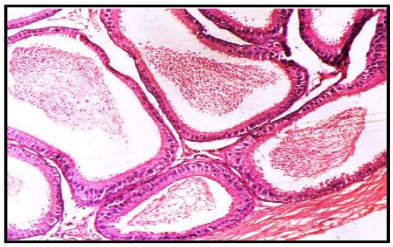

Fig. 2 Group B (10mg/kg.b.wt.100X HE)

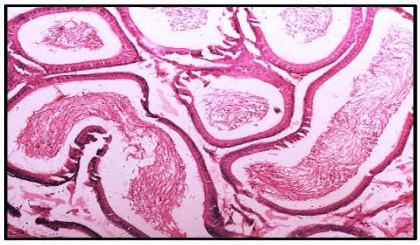

Fig. 3 Group C (20mg/kg.b.wt.100X HE)

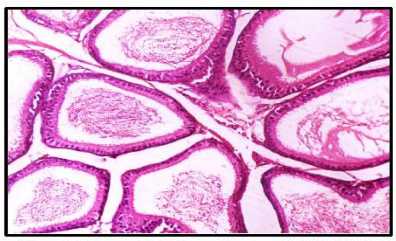

Fig. 4 Group D (40mg/kg.b.wt.100X HE)

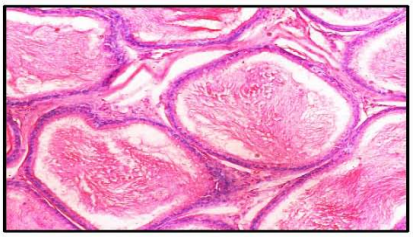

Fig. 5 Group E (20mg/kg.b.wt. Recovery 100X HE)

Plate 2: Photomicrograph of Cauda Epididymis of rat fig-1 showing normal histoarchitecture of germinal epithelial and stereocillia and lumen filled with sperm. Fig-2-4 showing degenerative changes in lumen dose dependence manner, whereas fig-5 showing normal histoarchitecture of cauda.

Table 1: Changes in the body weight and various organ weights of male rats after 60 days treatment of Withaferin–A (WS3)

Treatment	Initial B. wt. (gm.)	Final B. wt. (gm.)	Testes (mg/100 gm b. wt.)	Epididymides (mg/100gm B. wt.)	Vas-deferens (mg/100gm B. wt.)	Seminal vesicle (mg/100gm B. wt.)	Ventral Prostate (mg/100 gm B. wt.)	Kidney (mg/100 gm B. wt.)	Heart (mg/100 gm B. wt.)	Liver (mg/100 gm B. wt.)	Adrenal (mg/100gm B. wt.)
Group A Control Intact	106.50 ± 1.50	157.0 ± 2.00	1155.12 ± 14.71	507.48 ± 16.24	163.89 ± 2.11	425.00 ± 14.27	96.51 ± 1.84	580.72 ± 12.84	274.67 ± 4.84	2454.68 ± 33.91	20.48 ± 0.54
Group B 10mg/kg. b. wt.	105.00 ± 1.67^{ns}	155.0 ± 2.24^{ns}	1144.30 ± 13.53^{ns}	503.21 ± 10.09^{ns}	162.37 ± 3.21^{ns}	423.66 ± 14.17^{ns}	96.49 ± 2.13^{ns}	612.73 ± 15.82^{ns}	271.13 ± 4.78^{ns}	2449.18 ± 24.74^{ns}	20.23 ± 0.41^{ns}
Group C 20mg/kg. b. wt.	102.00 ± 1.33^{ns}	159.0 ± 1.94^{ns}	1137.57 ± 20.20^{ns}	492.99 ± 10.90^{ns}	158.54 ± 2.64^{ns}	421.55 ± 14.30^{ns}	95.75 ± 2.12^{ns}	551.76 ± 7.86^{ns}	268.31 ± 7.76^{ns}	2415.94 ± 35.64^{ns}	19.74 ± 0.34^{ns}
Group D 40mg/kg. b. wt.	102.00 ± 1.33^{ns}	155.0 ± 1.67^{ns}	1105.35 ± 17.87*	464.77 ± 10.62*	153.41 ± 3.33*	399.05 ± 15.77^{ns}	94.32 ± 2.06^{ns}	551.74 ± 12.83^{ns}	240.63 ± 14.71^{ns}	2412.85 ± 27.45^{ns}	19.68 ± 0.43^{ns}
Group E Recovery 20mg/kg. b. wt.	104.00 ± 1.63^{ns}	156.0 ± 1.94^{ns}	1142.34 ± 13.27^{ns}	498.87 ± 10.84^{ns}	159.98 ± 3.41^{ns}	423.63 ± 13.99^{ns}	95.86 ± 2.22^{ns}	573.26 ± 11.76^{ns}	270.58 ± 4.58^{ns}	2446.01 ± 32.72^{ns}	19.84 ± 0.44^{ns}

Data are expressed as mean ±S.E, and analyzed for statistical significance by using Student's t test for 10 animals.Groups B, C, D and E were compared with Group A. ns = non-Significant, Significant (P≤0.05).*

Table 2: Effect on the sperm motility, Density and Fertility after 60 days treatment of Withaferin–A (WS3) in male rats

Treatment	Sperm motility (Cauda) (%)	Sperm density		Fertility (%)	Number of pups delivered
		Cauda (million/mm^3)	Testes (million/mm^3)		
Group A Control Intact	74.57 ± 0.97	15.15 ± 0.19	3.42 ± 0.17	100 % (+ve)	70
Group B 10mg/kg. b. wt	73.89 ± 0.75^{ns}	14.90 ± 0.11^{ns}	3.11 ± 0.10^{ns}	64% (-36%)	45
Group C 20mg/kg. b. wt	73.17 ± 1.02^{ns}	14.88 ± 0.22^{ns}	2.98 ± 0.05*	51% (-49%)	36
Group D 40mg/kg. b. wt.	68.14 ± 0.87**	14.10 ± 0.14**	1.97 ± 0.08*	35% (-65%)	24
Group E Recovery 20mg/kg. b. wt.	73.31 ± 0.71^{ns}	14.90 ± 0.11^{ns}	3.08 ± 1.98^{ns}	72%(-28%)	50

Data are expressed as mean ±S.E, and analyzed for statistical significance by using Student's t test for 10 animals.Groups B, C, D and E were compared with Group A. ns = non-Significant, Significant (P≤0.05), ** Highly Significant (P≤0.01).*

Table 3: Tissue biochemical changes after 60 days treatment of Withaferin-A (WS3) in male rats

Treatment	Cholesterol (mg/gm)				Glycogen(mg/gm)			Ascorbic acid (mg/gm)	Fructose (mg/gm)
	Testis	Liver	Heart	Adrenal	Testis	Liver	Heart	Adrenal	Seminal Vesicle
Group A Control Intact	12.62 ± 0.40	14.11 ± 0.62	14.80 ± 0.83	9.40 ± 0.42	3.62 ± 0.17	4.55 ± 0.17	4.16 ± 0.15	3.05 ± 0.25	7.56 ± 0.41
Group B 10mg/kg. b.wt.	11.56 ± 1.19^{ns}	14.08 ± 0.50^{ns}	14.49 ± 0.74^{ns}	8.82 ± 0.48^{ns}	3.38 ± 0.10^{ns}	4.20 ± 0.13^{ns}	3.93 ± 0.22^{ns}	2.84 ± 0.20^{ns}	7.49 ± 0.34^{ns}
Group C 20mg/kg. b.wt.	11.44 ± 0.42*	13.75 ± 0.68^{ns}	13.88 ± 0.67^{ns}	8.31 ± 0.53^{ns}	3.25 ± 0.19^{ns}	4.22 ± 0.14^{ns}	3.85 ± 0.07^{ns}	2.66 ± 0.21^{ns}	6.11 ± 0.44*
Group D 40mg/kg. b.wt.	10.81 ± 0.85*	13.58 ± 0.57^{ns}	13.81 ± 0.72^{ns}	8.25 ± 0.36*	2.74 ± 0.12*	4.07 ± 0.40^{ns}	3.57 ± 0.35^{ns}	2.38 ± 0.13*	5.96 ± 0.39*
Group E Recovery 20mg/kg. b.wt.	11.00 ± 1.13^{ns}	13.94 ± 0.54^{ns}	14.50 ± 0.74^{ns}	8.56 ± 0.44^{ns}	3.28 ± 0.10^{ns}	4.12 ± 0.12^{ns}	3.71 ± 0.05^{ns}	2.73 ± 0.19^{ns}	7.45 ± 0.36^{ns}

Data are expressed as mean ±S.E, and analyzed for statistical significance by using Student's t test for 10 animals.Groups B, C, D and E were compared with Group A. ns = non-Significant, Significant (P≤0.05).*

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The Hungry Rural India: Their Cacophony and Silence

By Anannya Chakrabort, SK Acharya & Chandrasekhar Chatterjee

Abstract- In spite of being a nation mostly dependent on agriculture, the scenario of Indian agriculture and its farmers are very poor. Not only the farming community, the non farming community of rural India is also living under a miserable situation. They are even sometimes facing huge struggle to arrange their bread and butter. They are the bleak background behind the silver screen; the ground zero level really of shinning India. Our present study was conducted in two rural localities: one is farm locality and the other is non farm locality. There were 21 independent variables for the farm sector and 19 were for the non farm sector, while the dependent variable(hunger) remaining the same. From each locality 75 respondents were chosen (150 respondents in total) and they were being interviewed through a structured interview schedule. The data were analysed through step down regression method and it was revealed that the parameters of hunger for farm sector were very much different from that of the non farm sector though it was linked with a wire of ignorance and silence.

Keywords: *farm sector, non farm sector, hunger, silence.*

GJSFR-D Classification: *FOR Code: 079999*

Strictly as per the compliance and regulations of:

The Hungry Rural India: Their Cacophony and Silence

Anannya Chakraborty [α], SK Acharya [σ] & Chandrasekhar Chatterjee [ρ]

Abstract- In spite of being a nation mostly dependent on agriculture, the scenario of Indian agriculture and its farmers are very poor. Not only the farming community, the non farming community of rural India is also living under a miserable situation. They are even sometimes facing huge struggle to arrange their bread and butter. They are the bleak background behind the silver screen; the ground zero level really of shinning India. Our present study was conducted in two rural localities : one is farm locality and the other is non farm locality. There were 21 independent variables for the farm sector and 19 were for the non farm sector, while the dependent variable(hunger) remaining the same.From each locality 75 respondents were chosen(150 respondents in total) and they were being interviewed through a structured interview schedule. The data were analysed through step down regression method and it was revealed that the parameters of hunger for farm sector were very much different from that of the non farm sector though it was linked with a wire of ignorance and silence.

Keywords: farm sector, non farm sector, hunger, silence.

I. Introduction

Ever since independence in 1947, agricultural development policies in India have aimed at reducing hunger, food insecurity, malnourishment and poverty at a rapid rate. Keeping this overarching goal in mind, the emphasis, which was initially (for 15 years or so) on keeping food prices low, shifted to macro food-security and subsequently to household and individual food-security. Later, the food security of vulnerable, sustainable use of natural resources, and equity between rural and urban or farm and non- farm population became the issues of dominant discourse related to agricultural development. The policies and programmes related to marketing and trade were obviously guided by the overall objective sought to be achieved from the agricultural development strategy. The changes in marketing environment and production performance of the Indian agricultural sector should, therefore, be viewed in the context of weightage attached to these objectives at different points of time.

Even with the swashbuckling claim on growth and prosperity on the present civilization, the other side of this prosperity is so bleak and disastrous that have no match for the past centuries even. Out of around 7 billion population of the world , 1.5 billion are hungry. They don't have adequate access to food, if it is there, the quality doesn't stand any where near to fulfil their calorie requirement. In India 350 million people are living below the poverty line and of them, 200 million people have become victim to moderate to extreme hunger indexes. 42 per cent of the new born babies are under weight. 60 per cent of the children are suffering from moderate to high level of anaemia experiencing stunted growth.

Beyond the curtain of hunger, there is another problem that is chronic hunger. Based on hunger index we the nation is occupying 100th position in the world (IFPRI Report,2017). The scenario of chronic hunger is even worse and astoundingly it is worse than African nations as well.

Nobel laureate Abhijitt V. Banerjee and Ester Duflo's research finds a large percentage of households with at least one member of the family owning their own business; however entrepreneurship is not a common aspiration of the poor. What they really want is for their children to land government jobs – such as teachers .Duflo recounts a very interesting anecdote about the correlation between Mexican maquiladoras (manufacturing centres) located in a mother's village, and strongly increased nutrition of her children. The additional income the mother earned at the maquiladoras was not enough to explain the substantial increase in the children's nutrition. Instead she proposes that "Perhaps the sense of control over the future that people get from knowing there will be an income coming in every month – and not just the income itself – is what allows these women to focus on building their own careers and those of their children" . A predictable, dependable income separates the poor from the middle class and alleviates the stress that was so additionally detrimental.

Objectives

1. To bring out the parameters caused hunger in rural farm sectors.
2. To bring out the parameters caused hunger in rural non farm sector.
3. Comparative and holistic analysis of farm vs. non farm sector as far as the dependent variable hunger is concerned.

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II. Research Methodology

- The present study has conducted in two separate socio-ecological strata. One study has conducted among the farm families and the other one has conducted on the non farm families. Here we have only selected women at the age group of 15-60 as respondents.
- The farm locality is Beraberi gram panchayat, Habra II Block, North 24 pgs and the non farm locality is Bilkanda gram panchayat, Barrackpur 2 block, North 24 pgs.

III. Materials and Methods

An extensive interview schedule was formed and 75 respondents were randomly selected from each sector. They were interviewed in a wholesome manner. The data was analysed through step down regression method.

IV. Result and Discussion

Table 1: Step down Regression Analysis, hunger vs. all 21 Causal variables to find out the independent variables which are retained at last of this data reduction process. (farm sector)

Sl no.	variables	Beta value	B value	Std. error	t value
1	age	-1.056	-9.424	8.922	-1.056
2	education	-0.054	-6.736	21.666	-0.311
3	Family size	-0.073	-32.350	68.835	-0.470
4	Economic motivation	-0.068	-66.776	175.312	-0.381
5	Risk orientation	-0.457	-1089.172	346.538	-3.143
6	Management orientation	0.085	15.675	27.913	0.562
7	Stress perception on hunger	-0.146	-35.350	34.502	-1.025
8	Stress perception on poverty	0.219	41.973	27.647	1.518
9	Stress perception on voice	0.090	36.269	56.083	0.647
10	Size of holding	0.321	48.793	57.272	0.852
11	Cropping intensity	0.484	3.004	1.078	2.787
12	Livestock count	-0.489	-249.398	76.649	-3.254
13	Livestock yield	0.039	9.483	32.694	0.290
14	Pond and fish	0.031	83.957	437.689	0.192
15	Total crop yield	-0.832	-0.027	0.017	-1.527
16	Cost of cultivation	0.370	0.002	0.003	0.660
17	No. of fragments	-0.078	-9.770	28.743	-0.340
18	Communication variables	0.014	4.940	46.004	0.107
19	Marketed surplus	-0.163	-2.370	2.905	-0.816
20	Energy consumption	-0.127	-25.125	30.471	-0.825
21	BMI	-0.055	-5.613	15.177	-0.370

R square value 50.6 %
SE 0.47

Table 2: Regression Analysis, Hunger (Y1) vs 3 causal variables(x11,X12 and X15) which have been retained at the last step of step down regression.

Sl no.	variables	Beta value	B value	Std. error	t value
1.	Cropping intensity(X11)	0.367	2.274	0.734	3.096
2.	Livestock count(X12)	-0.473	-241.297	58.466	-4.127
3.	Total crop yield(X15)	-0.408	-0.013	0.004	-3.417

R square value 30.3%
SE 0.48

V. Result

Table 1 presents the multiple regression analysis between exogenous variable Hunger (Y)vs. 21 causal variables. It has been found that the variable cropping intensity(X11), livestock count(X12) and total crop yield(X15) has contributed to the substantive variance embedded with the consequent variable Y.

The R^2 value being 0.506, it is to infer that 50.60% of variants in the consequent variable has been explained by the combination of these 21 causal variables.

Table 2 presents the step wise regression and it has been depicted that the 3 causal variables that are cropping intensity(X11), livestock count(X12) and total crop yield(X15) have been retained at the last step.

The R2 value being 0.303, it is to infer that 30.30% of variants in the consequent variable has been explained by the combination of these 3 causal variables.

Cropping intensity is the provider of food and nutrition as well as integrated to the food security. So, the higher is the crop the higher has been the cropping intensity and for the growers, it offers better security for them. The livestock count is an indicator for directing and determining the spill over amount for the livestock raisers' own family.

So these three variables the terms of managing hunger can be traced as marker variables which has contributed collectively 30.30 per cent variance in hunger.

Table 3: Step down Regression Analysis, hunger vs. all 19 Causal variables to find out the independent variables which are retained at last of this data reduction process. (non farm sector)

Sl no.	variables	Beta value	B value	Std.error	t value
1	age	0.575	15.614	6.021	2.593
2	education	0.373	33.200	12.647	2.625
3	Family size	0.297	77.111	41.345	1.865
4	Economic motivation	0.110	54.123	71.593	0.756
5	Risk orientation	0.001	1.128	23.799	.005
6	Management orientation	-0.088	-13.162	20.002	-0.658
7	Stress perception on hunger	0.059	15.146	43.976	0.344
8	Stress perception on poverty	0.056	10.077	24.862	0.405
9	Stress perception on voice	-0.174	-47.557	37.629	-1.264
10	Homestead land	0.016	41.681	35.848	0.116
11	occupation	-0.117	-3.937	7.177	-0.549
12	Ancillary income	-0.156	-0.458	0.421	-1.087
13	expenditure	0.270	0.124	0.071	1.764
14	savings	-0.095	-0.155	0.224	-0.690
15	Bank account	0.180	70.338	34.465	1.078
16	Communication variables	0.089	17.750	31.191	0.569
17	Management variables	-0.006	-0.017	0.428	-0.041
18	Energy consumption	0.049	4.420	13.807	0.320
19	BMI	0.101	5.139	7.362	0.698

R square value 47.9%
SE 0.24

Table 4: Regression Analysis, Hunger (Y) vs 3 causal variables(x1,X2 and X9) which have been retained at the last step of step down regression

Sl no.	variables	Beta value	B value	Std. error	t value
1.	Age(X1)	0.502	13.631	2.780	4.904
2.	Education(X2)	0.330	29.381	9.043	3.249
3.	Stress perception on voice(X9)	-0.199	-54.232	27.240	-1.991

R square value 35.7%

SE 0 .23

VI. Result

Table 3 presents the multiple regression analysis between exogenous variable Hunger (Y)vs. 19 causal variables. It has been found that the variable age(X1), education(X2) and stress perception on voice(X9) has contributed to the substantive variance embedded with the consequent variable Y.

The R^2 value being 0.479, it is to infer that 47.9% of variants in the consequent variable has been explained by the combination of these 19 causal variables.

Table 4 presents the step wise regression and it has been depicted that the 3 causal variables that are age(X1), education(X2) and stress perception on voice(X9) have been retained at the last step.

The R^2 value being 0.357, it is to infer that 35.70% of variants in the consequent variable has been explained by the combination of these 3 causal variables.

Age and education are the two important factors as far as the hunger status of an individual is concerned. In case of the non farm sector women are involved in tailoring, sells, shop owning and most of them are mere housewives. So as a youngster they can earn more and spend more on nutrition, but as they grow older their ability to earn become less, so as their nutrition status. As far as the education is concerned, higher education means higher chances to get employed and better understanding about nutrition. Another parameter which is crucial in this regard is stress perception on voice. This clearly indicates that the women who cant cry out for their needs and desires are very much under stress and this stress leads to many physical and mental illnesses.

VII. Similarities and Differences in between Farm and Non Farm Sector

As we can see in the results that the parameters responsible for hunger in farm sector are different than that of the non farm sector. In case of farm sectors the parameters are mostly of agricultural sectors which is quiet different from the non farm sector where the parameters are related to individual development cognitive and stress due to unuttered words.

VIII. Conclusion

The causes of hunger is embedded within the socio economic, ecological and economic factors of an individual. In the rural areas where agriculture is predominating occupation, the core agricultural factors are responsible for hunger. Here the voices are better cried and better heared. But in case of the non farm sectors, the stakeholders are less vocal, so automatically the government is less bothered. Farmers and the farming community is spreading their wings of fire. After thousand years' of silence, they finally cry out in an outrageous manner. The example is right in front of us, the Maharashtra outrage where nearly one lakh farmers marched to words Delhi to draw the attention of the government and policy makers.

Some reasons of being hungry vis a vis poor

1. No or less voice for the rights and against the injustice.
2. Low cropping intensity and low yield.
3. Less emphasis on the agricultural allied sectors.
4. Low educational status.

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Nutrient Profiling of Tropical Soybean (*Glycine Max*) Core Collection

By Tonny Obua, Julius P. Sserumaga, Fredrick Nganga,
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Abstract- Soybean (*Glycine max* (L.) Merrill) is a highly nutritious legume with enormous potential to improve dietary quality for humans and livestock. However, the development of varieties with improved nutritional traits has been affected by the negative correlation that exists among the different traits and the high cost of the phenotypic assessment. The objectives of this study were: (1) to quantify the total protein, total oil and fatty acids of 52 soybean genotypes from different sources, (2) to identify correlations among total protein, total oil content and fatty acids. The total protein content was determined using the Modified Folin-Lowry Method. In contrast, the total oil and fatty acids methyl esters were determined using the chloroform/methanol gravimetric method and Gas Chromatography–Mass Spectrometry. The analysis of variance revealed that the studied traits varied significantly depending on genotypes and origin.

Keywords: food composition, total protein, total oil content, palmitic acid, stearic acid, oleic acid, linoleic acid.

GJSFR-D Classification: FOR Code: 860799

Strictly as per the compliance and regulations of:

Nutrient Profiling of Tropical Soybean (*Glycine Max*) Core Collection

Tonny Obua [α], Julius P. Sserumaga [σ], Fredrick Nganga [ρ], Phineas Tukamuhabwa [ω], Thomas L. Odong [¥], Josiah Mutuku [§] & Nasser Yao [χ]

Abstract- Soybean (*Glycine max* (L.) Merrill) is a highly nutritious legume with enormous potential to improve dietary quality for humans and livestock. However, the development of varieties with improved nutritional traits has been affected by the negative correlation that exists among the different traits and the high cost of the phenotypic assessment. The objectives of this study were: (1) to quantify the total protein, total oil and fatty acids of 52 soybean genotypes from different sources, (2) to identify correlations among total protein, total oil content and fatty acids. The total protein content was determined using the Modified Folin-Lowry Method. In contrast, the total oil and fatty acids methyl esters were determined using the chloroform/methanol gravimetric method and Gas Chromatography–Mass Spectrometry. The analysis of variance revealed that the studied traits varied significantly depending on genotypes and origin. Total protein content ranged from 30.07% to 50.40 %, while total oil content ranged from 14.94% to 23.48%. Total oil content varied significantly between origins with genotypes from the USA having the highest mean of 20.43%, while those from AVRDC had the lowest mean of 18.32 %. Palmitic acid (16:0) content ranged from 10.58% to 21.18%; 4.93% to 22.20% for stearic acid (18:0), 22.69% to 39.95% for oleic acid (18:1) and 30.60% to 51.72 % for linoleic acid (18:2). Genotypes from Uganda had the highest percentages of oleic acid, followed by genotypes from Japan. Six negative and three positive correlations were found to be significant in the current study further. The current study identified soybean genotypes with elevated protein and oil content above the average that can be used to improve the nutritional properties of soybean in Uganda and across the East African region hence boosting the soybean industry.

Keywords: food composition, total protein, total oil content, palmitic acid, stearic acid, oleic acid, linoleic acid.

I. Introduction

Soybean (*Glycine max* (L.) Merrill) has become an important ingredient in the diets of both the human and livestock due to its high nutritional value and low cost (Friedman and Brandon 2001; Singh et al. 2008; Drago et al. 2011). The total world soybean production is estimated at 348.7 million metric tons (MT). The world's leading producer is the USA, which produces about 35% of the world soybean (123.7 million MT). Brazil is second with 34% of soybean produced (117.9 million MT) while Argentina is third with 11% (37.8 million MT), and China is fourth with 4% (14.2 million MT). The remaining countries account for 16% (55.2 million MT) of the global soybean output (FAO 2018). In Africa, total soybean production rose from 1.4 million MT in 2008 to 3.6 million MT in 2018, representing 1.0% of the world production. The three leading African countries in soybean production are South Africa (1,316,000MT), Nigeria (730,000MT), and Zambia (351,416MT) (FAO 2018). Soybean grains contain about 40% protein, 20% oil, an optimal supply of protein,and high-calorie value (Singh et al., 2008). Additionally, soybean oil is composed of approximately 16% saturated fatty acids (palmitic [C16:0] and stearic [C18:0]), 24% monounsaturated fatty acids (oleic [C18:1]), and 60% polyunsaturated fatty acids (linoleic [C18:2] and linolenic [C18:3]) (Drago et al. 2011).

The processing capacity for soybean has significantly increased in Uganda and across the East African region that has been triggered by the growing interest of the farmers to grow the crop as a main source of cash because of the available superior varieties(Tukamuhabwa et al. 2019). These established processing plants make different food and feed products. However most consumers are interested in soy-based food products with improved protein and oil content to meet special food applications (Singh et al. 2008; Miladinovic et al. 2011). In the past decade, the key focus for most soybean breeding programs in Tropical Africa has been on improvement of traits such as yield, resistance to pod shattering and lodging, high pod clearance, resistance to pests and diseases. In contrast, the traits related to seed composition have received very little attention (Tukamuhabwa et al. 2019; Tukamuhabwa and Oloka 2016; Bashaasha 1992). Moreover, the development of varieties with improved nutritional properties has been further affected by the negative correlation that exists among the different traits. For example, several studies have reported a significant negative correlation between oleic acid and palmitic acid (Qin et al. 2014; Ahire 2012; Alt et al. 2005; Rebetzke et al. 2001). Similarly total oil has been reported to exhibit a strong negative correlation with oleic acid (Rani et al. 2007; Bachlava et al. 2008).

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Likewise, a negative correlation between oil and protein has also been reported in several studies (Marega et al. 2001; Qin et al. 2014). Therefore during the selection of soybean genotypes for a particular food application or breeding program, it is fundamental to understand the relationships that exist among these quality attributes. However, these relationships have not been widely assessed on tropical soybean germplasm. Additionally one of the requirements in any successful plant breeding program is to create variability to make selection. One way of creating variability in a breeding program is the importation of germplasm from other sources. For example, in Uganda, a lot of soybean germplasm has been introduced from other parts of the world to increase the genetic base. However, there is inadequate information on the nutritional properties of these introduced germplasm.

Knowledge of the relationship among the different nutritional traits in soybean would be very vital in developing soybean varieties with improved nutritional properties. Therefore the objectives of this study were: (1) to quantify the total protein, total oil and fatty acids of 52 soybean genotypes from different sources, (2) to identify correlations among total protein, total oil content, and fatty acids. Therefore the findings from this study could provide an opportunity to develop soybean varieties with improved nutritional traits.

II. Materials and Methods

a) *Plant Materials and Sample Preparation*

Fifty-two soybean genotypes were planted at the National Crops Resources Research Institute (NaCRRI), located in Central Uganda in the first season of 2016. The soybean genotypes were planted in one location to minimize the effect of the environment on the chemical composition of the soybean grain. The 52 soybean genotypes were planted in an alpha lattice design with three replicates. The plot size was 5m long with a spacing of 60 cm × 5 cm. The grain from each plot was harvested and sun-dried to below 10% moisture content. The seed samples for each genotype were divided into three replicates and about 300g ground through a 2 mm screen using Cyclotec™ 1093 mill (FOSS, 2011). The milled samples were stored in the cold room at a temperature -4°C at BecA – ILRI Hub, Nairobi Kenya, where all the subsequent analysis was performed.

b) *Total Protein Content*

Total Protein content was determined using the Modified Folin-Lowry Method (Lowry et al. 1951). 100 mg of the milled samples were weighed in duplicate into 25 mL culture tubes. 5 ml 5% sodium dodecyl sulphate (SDS) was added, vortexed, and left to stand for 2 hours at room temperature and centrifuged at 2000 ppm for 10 min. 50 µl aliquot was taken and diluted with 950 µl of water and made to 1000 µl in separate culture tubes. 100 ul aliquot of the diluted extract was taken for analysis. Blank (100 µl of distilled water), standards (0, 100, 200, 300, 400, 500 µl of Bovine serum albumin) and samples (100 µl) were pipetted into glass culture tubes, and all made to 1000 µl.

1 ml Reagent A (0.4 volumes of water, 1 volume CTC reagent, 1.6 volumes of 5 % SDS and 1 volume of 0.8 M NaOH) was then added to each of the tubes and immediately vortexed; at 20 seconds interval. 500 µl of Reagent B (1 volume of Folin-Ciocalteu phenol reagent, 2 Aldrich 9252, and 5 volumes of distilled water) was added to each tube and immediately vortexed and left to stand for 30 minutes for color development. An ultraviolet-visible spectrophotometer was used to measure the absorbance of the standards and samples versus the blank at a wavelength setting of 750 nm. The absorbance values of the standards versus their corresponding protein concentrations were plotted to prepare a calibration curve, and the protein concentration of the samples were determined.

The total protein content in the residue was calculated using the formulae:

$$\text{Total Protein (g/100 g)} = \frac{C \text{ X } 100 \text{ XDF}}{10^6 \text{ X } W}$$

Where;

C=Concentration obtained from the calibration in µg/ml

100 = Conversion factor to report results in g/100g

DF = Total dilution factor (1000)

10^6= Conversion from µg to g

W= Weight of the sample in grams

c) *Total Oil Content and Fatty Acid Profiling*

Total oil content was determined using the chloroform/methanol gravimetric method (Bligh and Dyer, 1959). 2.0 g of the milled sample for each soybean genotype was weighed in duplicate into 50 mL culture tubes (W_1). 32 ml of Clarase solution was added, the tubes capped and gently shaken until the sample was well mixed with the enzyme solution. The sample was incubated for one hour in a 45°C water bath while gently mixing by inversion after every 20 minutes. All the extract was transferred to a 250 ml polypropylene bottle, capped and centrifuged at 2000 rpm for 15 minutes to clarify the chloroform. The top aqueous phase was carefully removed and discarded with a tap aspirator pump leaving a 2-4 mm thick layer on the chloroform. A hole was cautiously broken into the surface crust with a glass rod, and 20.0 ml of the chloroform extract was pipetted into a pre-weighed 50 ml beaker (W_2). Further, a 20 ml aliquot of the chloroform extract was taken and stored at -20°C for fatty acids methyl esters (FAMES) analysis with Gas Chromatography – Mass Spectrometry (GC – MS). The solution was evaporated to dryness by leaving it overnight in a fume hood; the beaker placed in an oven at 102°C for 30 minutes,

removed and cooled in an evacuated desiccator for 1 hour. The beaker and the total oil was weighed on a microbalance to the nearest 0.1 mg (W_3).

The total oil in the residue was calculated using the formulae:

$$\text{Total oil (g/100 g)} = \frac{(W_3) - (W_2) \text{ X } 100 \text{ X } 4}{(W_1)}$$

Where; W_1 - Sample weight (g)

W_2 - Weight of beaker

W_3 - Weight of beaker + total oil

FAMES were analyzed using a DB-5 column, on an HP 5890 Series II GC equipped with an HP 7673 auto sampler (Hewlett Packard, Sunnydale, CA). Peak areas were recorded using Chem Station software (Hewlett Packard, Sunnydale, CA). Identification of individual FAMES was performed by calculating the Kovats linear retention index. The linear retention index was subsequently compared with obtained values from the National Institute of Standards and Technology (NIST) and Pherobase databases in cases where standards were absent. Quantification of individual FAMES was performed by the area percent method.

d) Statistical Analysis

Statistical differences among the different soybean genotypes were estimated from ANOVA test at the 5% level (P=0.05) of significance for all the parameters evaluated, using Genstat, 13th Edition (Payne et al. 2010). Whenever ANOVA indicated a significant difference, a pairwise comparison of mean by Least Significant Difference test (LSD) was carried out. Correlation analysis was performed using the corrplot package for graphical display using the Pearson method.

III. Results

a) Total Protein Content

Genotypes from Japan had the highest protein content of 43.47%, followed by the USA (42.42%), and genotypes from Uganda had the lowest protein content of 40.19% (Table 1). Genotypes Sline 5.18, BSPS 48A-8 and BSPS 48A-27-1 had the highest protein content of 50.40%, 48.88%, and 48.08% respectively (Table 2). On the other hand, NIIXGC 17.3 and Nam II had the lowest protein content of 30.07% and35.57% (Table 2).

b) Total Oil Content

Genotypes from the USA had the highest oil content of 20.43%, followed by Seedco (20.11%) and AVRDC had the lowest (18.32%) (Table 1). G32B, Roan and AGS 338 had the highest oil content of 23.48%, 23.47% and 23.26% respectively while Signal had the lowest content of 14.94% (Table 2).

c) Fatty Acids Content

Soybean genotypes from Uganda had the highest oleic fatty acid content (33.85%), followed by genotypes from Japan (33.17%), and Seedco had the lowest (30.68%) (Table 1). Genotypes BSPS 48A-25, G7955 and BSPS 48A-5 had the highest oleic fatty acid content of 39.95%, 38.95%, and 38.66% respectively while NG 14.1-16 had the lowest content of 22.69% (Table 2).

Genotypes from the USA had the highest palmitic acid content of 15.00%, followed by AVRDC (14.71%), and SEEDCO had the lowest (13.23%) (Table 1).Genotypes Nam II, Siesta, and Namsoy 3 had the highest palmitic acid content of 21.18%, 20.18% and 19.68%, respectively (Table 2).On the other hand, G7955 and K-Local had the lowest palmitic acid content of 10.58% and 10.95%, respectively (Table 2).

For stearic acid content, significant differences were observed among both genotypes and origin of the genotypes (Table 1). Genotypes from AVRDC had the highest stearic acid content of 9.05%, while those from Japan were the lowest (7.42%). Genotypes NG 14.1-16, AVRDC SRE-B-11-13, and Sequel had the highest stearic acid content of 16.76%, 15.58%, and 14.57% respectively, while Sline 16.2 had the lowest content of 4.93% (Table 2). Additionally, Saga had the highest linoleic acid content of 51.72%, while Maksoy 5N had the lowest content of 30.60% (Table 2).

Table 1: Variation of Total Protein (%), Total Oil (%) and Fatty Acids (%) of soybean genotypes from different origins

Origin and No. of genotypes		Total Protein	Total Oil	Oleic Acid	Palmitic Acid	Stearic Acid	Linoleic Acid
AVRDC	Mean	41.05 **	18.32 ***	32.40	14.71 *	9.05 ***	43.64
6	Range	36.88- 46.06	11.59-23.27	22.94-38.35	11.14-19.12	5.46-15.38	36.59-48.60
	%cv	9.9	6.9	15.8	14.84	14.22	8.16
	Se	4.052	1.273	4.974	2.217	1.287	3.617
Japan	Mean	43.47 ***	18.73 ***	33.17 *	13.44	7.42 **	45.52*
6	Range	39.88- 50.40	15.35- 20.65	31.13-36.17	11.91-13.31	4.93-7.06	35.69-48.66
	%cv	5.1	5.1	1.16	4.7	4.39	1.03
	Se	2.199	0.948	0.3872	0.6164	0.299	0.4739
SEEDCO	Mean	42.18	20.11 ***	30.68 **	13.23 **	8.59 ***	46.82
10	Range	37.93- 44.88	14.94- 23.47	22.68-36.79	10.47-20.39	5.02-14.56	40.47-53.19
	%cv	10.9	10	8.55	14.93	16.29	8.64
	Se	4.589	2.004	2.632	2.026	1.388	4.017
UGANDA	Mean	40.19 ***	19.11 ***	33.85 ***	13.57 ***	8.24 ***	43.94***
25	Range	30.07- 48.88	15.27-22.58	22.70-39.66	10.93-21.05	4.94-16.75	30.25-51.65
	%cv	16.8	8.7	9.23	10.56	30	4.26
	Se	6.739	1.658	3.17	1.374	2.324	1.904
USA	Mean	42.42 *	20.43 **	31.69 **	15.00	8.70 *	43.89
5	Range	36.1- 44.68	17.19- 23.48	26.83-35.31	11.66-17.33	5.74-12.02	39.85-47.26
	%cv	10.1	6.9	0.91	20.42	8.83	9.58
	Se	4.296	1.419	0.2866	2.955	0.7764	4.299

, ** and * indicate significance at p ≤ 0.05, p ≤ 0.01, and p ≤ 0.001 respectively*

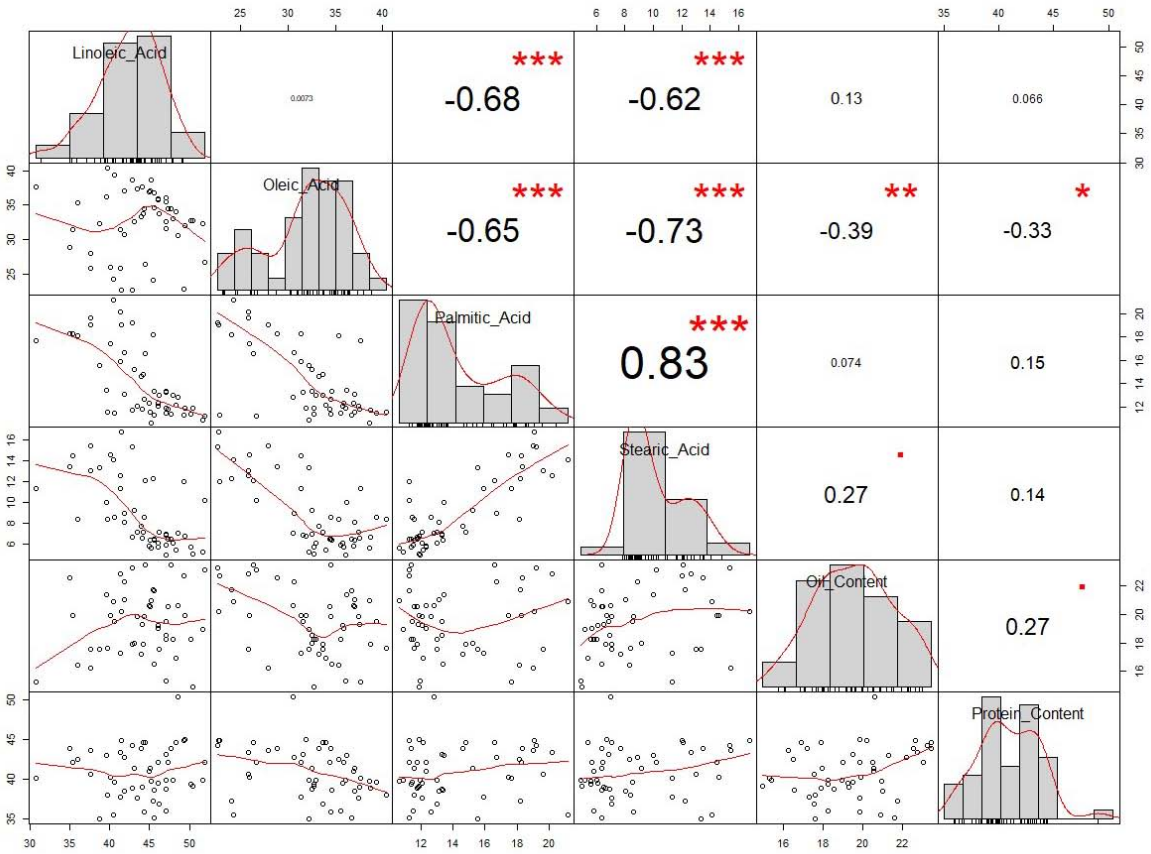
Table 2: Composition of Total Protein (%), Total Oil (%) and Fatty Acids (%) of soybean genotypes

Genotypes	Total Protein	Total Oil	Oleic Acid	Palmitic Acid	Stearic Acid	Linoleic Acid
AGS 338	37.97	23.26	35.88	11.80	6.54	45.77
AVRDC G2843B	37.32	21.75	24.12	18.21	12.26	45.42
AVRDC G7956	36.88	17.55	33.35	13.06	7.09	46.48
AVRDC GC00138-29	41.43	17.96	32.74	15.20	9.17	42.90
AVRDC GC84051-31-1	42.55	16.46	35.51	18.13	8.32	35.89
AVRDC SRE-B-11-13	43.58	16.23	27.90	19.08	15.58	37.41
BSPS 48A-25	37.11	17.61	39.95	11.65	8.41	39.97
BSPS 48A-27-1	48.08	19.87	37.38	12.08	7.07	43.47
BSPS 48A-3B	37.84	19.30	37.56	11.84	6.54	44.06
BSPS 48A-5	41.37	17.87	38.66	11.78	6.67	42.89
BSPS 48A-8	48.88	17.59	32.64	15.44	13.23	38.54
G32B	44.68	23.48	26.49	16.58	12.15	44.28
G42	43.87	17.19	33.67	14.51	7.56	43.84
G45	36.10	18.27	35.53	11.51	5.84	47.11
G7955	46.06	20.97	38.95	10.58	5.47	45.05
Gazelle	44.57	17.89	34.1	14.68	6.979	44.08
K-Local	39.89	18.94	32.26	10.95	5.167	51.63
Kab 1	39.17	15.59	34.58	11.49	5.36	48.57

Kuntz	39.88	22.24	30.65	16.76	9.01	41.65
Maksoy 2N	43.86	22.58	28.88	18.38	13.57	34.86
Maksoy 4N	45.32	21.34	36.32	13.43	10.01	39.50
Maksoy 5N	40.11	15.27	37.63	17.72	11.46	30.60
MNG 12.4	35.86	19.85	34.49	12.63	8.54	44.36
Nam II	35.57	20.91	23.94	21.18	14.14	40.71
Namsoy 3	40.68	17.54	25.78	19.68	13.13	37.41
NG 14.1-16	44.93	20.20	22.69	19.05	16.76	41.50
NGDT 4.11-4	37.66	21.00	37.04	13.14	8.03	41.79
NGDT 8.11-4	42.11	21.63	36.52	11.35	6.47	45.64
NII X GC 11.2	39.64	18.54	32.51	13.42	7.07	46.97
NII X GC 17.3	30.07	20.53	34.42	11.79	6.47	47.32
NII X GC 20.3	38.90	17.28	31.35	15.99	11.42	41.21
NII X GC 28.2B	38.65	18.26	32.97	13.00	6.04	47.99
NII X GC 30B	42.82	20.58	36.91	12.35	5.80	44.94
NII X GC 32.6	41.01	20.55	37.04	11.57	6.36	45.02
NII X GC 43.2	39.54	19.88	31.95	11.92	6.75	49.38
NII X GC 44.2	38.80	18.27	32.76	11.42	5.64	50.18
NII X GC 7.2	39.46	16.54	34.63	13.37	6.32	45.69
Roan	43.84	23.47	34.32	11.45	6.49	47.71
Saga	42.12	23.07	27.09	11.16	10.07	51.72
Saxon	40.95	17.91	36.95	12.22	6.94	43.90
Sentinel	37.93	15.81	33.50	11.45	5.47	49.57
Sequel	40.27	19.92	26.13	17.42	14.57	40.00
Siesta	43.42	22.79	25.86	20.18	12.62	41.34
Signal	39.17	14.94	32.71	11.93	4.97	50.39
Sline 13.2A	42.19	19.88	31.04	18.29	14.51	35.69
Sline 16.2	39.88	15.35	36.04	11.95	4.93	47.07
Sline 4.21	42.12	19.00	36.26	12.26	5.55	45.98
Sline 5.18	50.40	20.60	30.49	12.90	7.15	48.39
Sline 6.22	44.67	16.90	33.67	12.19	5.45	48.67
Sline 7.11	43.14	20.65	31.56	13.30	6.87	47.08
Soprano	40.11	19.24	35.54	11.84	5.68	46.99
Squire	44.88	22.66	22.82	11.28	11.96	49.44
Mean	40.71	19.44	32.79	13.88	8.50	44.38
LSD	9.33	2.82	3.28	3.32	3.09	3.72
CV%	14.30	9.00	6.10	14.60	22.20	5.10

d) Correlation Analysis for Total Protein, Total Oil and Fatty Acids

A total of nine correlations were found to be significant; six negative and three positive (Figure 1). Negative correlations were found between palmitic acid/linoleic acid, stearic acid/linoleic acid, oleic acid/palmitic acid, oleic acid/stearic acid, oleic acid/total oil and oleic acid/total protein. The positive correlations were found between palmitic acid/stearic, stearic acid/total oil and total oil/total protein.



, ** and * indicate significance at p ≤ 0.05, p ≤ 0.01, and p ≤ 0.001 respectively; values with ns are not significant at P < 0.05.*

Figure 1: A Corrplot showing distribution and correlation between the different nutritional traits

IV. Discussion

In this study, we used 52 soybean genotypes that were carefully selected based on origins from a core set of 504 accessions from five countries (USA, Japan, Taiwan, Zimbabwe and Uganda). We investigated the variability of different nutritional traits and correlations among these traits that were from different countries. High protein content has been one of the most key traits for most soybean improvement programs. The results of this study revealed that soybean genotypes from Japan had the highest protein content. The high protein content of genotypes from Japan is in agreement with a study conducted by Grieshop et al. (2001), who compared the nutritional properties of soybean from Brazil, China, and the USA and found out that soybean genotypes from China had the highest protein.

Similarly, Karr-Lilienthal et al. (2004) compared the nutritional composition of soybean grain and subsequent soybean meal from Argentina, Brazil, China, India, and the United States. This study reported that soybean from China had the highest protein content compared to the other four countries. Therefore in the current study, it is not surprising that soybean genotypes from Japan, which is geographically closer to China, had high protein content. The high oil content among genotypes from the USA also agrees with previous studies (Baize 1999; Grieshop et al. 2001) who reported that soybeans from the USA had higher oil content those soybeans from China. The high protein content in soybean genotypes from Japan and USA was high because of the extensive breeding for high protein and high consumption of soybean protein in these two countries (Fukushima 2011).

The proportion of palmitic acid observed in the current study was in general agreement with previous studies conducted on different soybean genotypes (Maestri et al. 1998; Cardinal et al. 2007; Rani et al. 2007; Bachlava et al. 2008; Ahire 2012). However, there were several soybean genotypes that showed an elevated proportion of palmitic acid that could be used for the development of high-palmitic soybean varieties. Soybean oil with high proportions of palmitic acid has industrial application for production of plastic fats like shortening and margarine (Fehr et al. 1998). For plastic fat to remain stable, it is desirable to have a fatty acid composition of about 15% or more of palmitic acid (Fehr et al. 1998). In the present study, palmitic acid showed a positive correlation with stearic acid that was in

agreement with earlier reports (Stoltzfus et al. 2000; Rahman et al. 2003; Bachlava et al. 2008; Ahire 2012).

Further, the use of soybean oil having a relatively high content of saturated fatty acids (palmitic acid and stearic acid) allows the production of more desirable plastic fat. Palmitic acid averages approximately 11% of the total fatty acids, whereas stearic acid averages about 4% of total fatty acids present in conventional soybean oil. Additionally, it is highly desirable to develop soybean varieties having elevated palmitic acid and stearic acid contents to produce stable plastic fat. A separate study by Rahman et al. (2003) reported that palmitic acid and stearic acid are inherited independently; therefore, the trait of elevated saturated fatty acids in one genotype can be easily achieved by conventional breeding.

Furthermore, we also observed a significant negative correlation between palmitic acid and oleic acid. This negative correlation has been reported previously in several studies (Rebetzke et al. 2001; Qin et al. 2014; Ahire 2012; Alt et al. 2005). Qin et al. (2014) reported a significant negative correlation between oleic acid and palmitic acid. Ahire (2012) also detected a negative correlation between oleic acid and palmitic acid among selected mutants from soybean variety MACS 450. Alt et al. (2005) showed that oleic acid of 88 F2:3 lines was negatively correlated with palmitic acid ($r=-0.470$).

The negative correlation between oleic acid and the saturated fatty acids (palmitic and stearic) coupled with a positive correlation between palmitic acid and stearic acid offer the opportunity to develop soybean varieties with improved oil quality. In the present study, results indicated that selection for higher oleic acid results in lower palmitic and stearic acids hence improvement in the quality of the soybean oil (Pham 2011; Qin et al. 2014). Oleic fatty acid is a monounsaturated fatty acid that can improve the oil quality and self-life of products processed using such oil.

In soybean, oleic fatty acid has been reported to exhibit a strong negative correlation with total oil content that is in agreement with the present study (Rani et al. 2007; Bachlava et al. 2008). The significant negative correlation between oleic acid and total oil suggests that it would be challenging to breed soybean varieties with high oleic fatty acid as well as high total oil content. A similar trend was observed between oleic acid and total protein, where there was a significant negative correlation. This implies that it is difficult to develop a soybean variety with high oleic acid and protein. The study suggests that the breeding program should aim at overcoming the negative correlation between oleic acid and total oil and total protein content using advanced techniques like genetic transformation.

V. Conclusions

Soybean genotypes originating from different sources have different nutritional properties like total protein, total oil, and fatty acids. Soybean genotypes from Japan had the highest protein content, while genotypes from the USA had the highest oil content. This study identified soybean genotypes with protein content up to 50.40% and oil content up to 24.23% that are way beyond the average nutritional properties of most soybean genotypes of 40% and 20%, respectively. Such genotypes with elevated protein and oil content can be used by soybean breeders in Uganda and across the East African region to improve the protein and oil content of soybean varieties. The significant negative correlation of oleic acid with both total oil and total protein indicates that more efforts would be required to develop soybean varieties that contain high oleic acid as well as high total oil and total protein using conventional breeding. There is a need to explore other options of increasing oleic fatty acid content without altering the other nutritional traits.

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Author Contributions

PT, TLO, JM, and NY: conceived and designed the experiments; TO, FN, JM, and NY: performed the experiments; TO, JPS: analyzed the data; TO, JPS: wrote the paper.

Conflict of interest: The authors declare no conflict of interest.

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An Experimental Study on Particle and Physiochemical Properties of Turmeric Powder Ground at Different Temperatures

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Abstract- Turmeric was ground at different grinding temperatures viz. -80°C, -40°C, 0°C and 40°C. The powder obtained at different grinding temperatures were analyzed to classify the powder. For this reason, we determined the particle size distribution, flowability, surface morphology, mineral composition and physicochemical properties of the turmeric powders at different grinding temperature. Particle size shows inverse relation with grinding temperature and, flowability shows a direct correlation with the increase in grinding temperature. We analyzed the elemental content of the samples by using SEM EDX and, the result shows that turmeric powder imparts more bioavailability of minerals with a decrease in grinding temperature. There is good retention of physicochemical properties at lower grinding temperature (-80°C) such as volatile oil (47.61%) and color (more brightness) as compared with those obtained at ambient temperature (40°C). Color parameters also significantly varied with a decrease in grinding temperature.

Keywords: turmeric powder, grinding temperature, flowability, physicochemical properties, surface morphology, mineral composition.

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Abstract- **Turmeric was ground at different grinding temperatures viz. -80°C, -40°C, 0°C and 40°C. The powder obtained at different grinding temperatures were analyzed to classify the powder. For this reason, we determined the particle size distribution, flowability, surface morphology, mineral composition and physicochemical properties of the turmeric powders at different grinding temperature. Particle size shows inverse relation with grinding temperature and, flowability shows a direct correlation with the increase in grinding temperature. We analyzed the elemental content of the samples by using SEM EDX and, the result shows that turmeric powder imparts more bioavailability of minerals with a decrease in grinding temperature. There is good retention of physicochemical properties at lower grinding temperature (-80°C) such as volatile oil (47.61%) and color (more brightness) as compared with those obtained at ambient temperature (40°C). Color parameters also significantly varied with a decrease in grinding temperature.**

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I. Introduction

Spices have occupied an important place in culinary and health purposes of people since ancient times due to their characteristic flavor, aroma, color and antioxidant properties. Global spice production has increased rapidly since 1960, from 1.7-million metric tons in 1965 to 6.6-million metric tons in 2005. India is home to a variety of spices and produces around 75 types spices out of the 109 listed with ISO [1]. Turmeric (*Curcuma. long*) is one of the most studied spices because of its promising health benefits. It is known as the "golden spice" as well as the "spice of life" [2]. It belongs to the *Zingiberaceae* family, and it is mainly grown in tropical and subtropical regions [3]. India is the highest producer of turmeric as well as consumer of turmeric [4], since ancient times it is used as an Ayurvedic medicine in India [5]. In addition to this, turmeric is used in Indian cuisine as a food additive to increase shelf life and taste [6].

Few spices are used in their natural form, however, most of the spices are made into powder form by grinding. Grinding of spices plays a key role for easy transportation, storage, mixing of components and, extraction of bioactive compounds. Curcumin is the important chemical compound present in turmeric, which is studying by many researchers. Nowadays, in food and pharma industries, there is a high demand for curcumin for its medicinal and other value addition properties. To meet the demand of curcumin in market, many industries have also started curcumin extraction units. Curcumin is present inside the cell of turmeric rhizome tissue along with oleoresin. So, before the extraction process of curcumin, turmeric rhizome needs to be ground into powder form. It is reported that finer the particle size greater the extraction efficiency for any bioactive compounds[7]. Since finer particles have more surface area, that is in contact with solvent during extraction [7]. However, a higher amount of mechanical forces is required to achieve the fine particle size, resulting in higher energy requirement and heat generation that is harmful to the flavoring, nutritional and medicinal properties of the spices [8][9]. These hindrances of the conventional grinder can be eliminated by using a cryogenic grinding technique. Cryogenic grinding is an improved version of conventional grinding. It grinds the spices with the help of cryogens like liquid nitrogen (LN_2) [8] and maintaining an inert atmosphere inside the grinding chamber [9]. It is reported that by using cryogenic grinding, there is an improvement in the yield of volatile oil, color, good texture, uniformity of powder spices [13]. There are also a reduction in air pollution and no risk for overheat as of cryogenic grinding as compared with conventional grinding [10].

Flowability has a vital significance in handling and processing operations like storage, transportation, mixing, compression and, packing. At the industrial level, poor flowability leads to an increase in wastage and, machinery problems. Bulk density, Haunser ratio (HR), and angle of repose (α) are used in industrial-scale to measure the flow ability of powder to design the industrial equipment, storage, and transportation requirements. Moisture content and water activity of the powder plays a crucial role in shelf life and storage. Color, minerals, and volatile oil are the important properties that influence the various properties like flavor, taste, etc. during grinding. To retain various

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properties of spices, a novel grinding method like cryogenic grinding is required.

Few reports are available in the literature on the effect of grinding temperature on quality of the ground black pepper [11], fenugreek [12], king chili [13], and coriander powder [14]. However, the effect on grinding temperature on the quality of the turmeric powder was not reported yet. Therefore, the main objective of the present work is to study the changes in the particle size, particle distribution, flow ability, and physicochemical properties of turmeric ground at different grinding temperatures (-80°C to 40°C). Knowledge in the various changes in turmeric powder during different grinding temperatures helps to optimize industrial operations like storage, extraction, transportation, mixing, compression, and packing. In addition to this, the design of equipment like feed, hopper, conveyors, storage, and materials to be handle can be easily estimated.

II. Materials and Methods

a) Sample preparation

Dried turmeric (Curcuma longa L.) rhizomes of Pragati variety were collected from the Indian Institute of Spices Research (IISR) Calicut, Kerala, India for, this work. Only good quality and mature rhizomes were randomly selected. The moisture content of the turmeric samples was determined by the oven drying method (Entrainment, US ISO 989) [8][15] and was 7.21% wet basis. The sample was maintained at constant moisture content by sealing in moisture resistant polythene and stored at 4 ± 1 °C. Before starting each grinding experiment, the samples kept at refrigerator temperature were brought into ambient conditions. The weighed turmeric rhizome samples (250 g) were used for grinding.

b) Grinding

The cryogenic grinding system, which consists of hammer mill with outlet sieve openings of 250 µm (ASTM-E11 60), screw conveyer type pre-cooler to get the desired temperature of the fed sample, vibratory feeder to maintain the feed rate into the pre-cooler and pressurized Dewar to pump liquid nitrogen for cooling the sample. Fig. 1 shows the schematic view of the cryogenic grinding system. The speed of the hammer mill was adjusted to 1000 rpm for all the experiments. Digital thermocouples with an indicator of ±0.01°C accuracy were used for measuring the temperature by putting at feeder and hopper of the grinder. The sample was fed at the rate of 40 g/min into the grinder. The grindings were done at four different regular interval temperatures viz. -80°C, -40°C, 0°C and 40°C (ambient temperature) for the testing of turmeric powder. Each experiment was replicated three times. The turmeric powders sample was stored at 4°C in moisture resistant polythene till they were analyzed for particle size distribution (PSD), flowability, and other physicochemical properties.

c) Analysis of particle, particle size distribution (PSD) and specific surface area

Laser diffraction particle size analyzer (Fritsch GmbH Analysette 22; MicroTech Plus) was used to measure particle size, PSD, and specific surface area of the different powder. It can continuously measure particle size with a range from 0.08 to 2000 µm. For control and data evaluation, Laser diffraction particle size analyzer was connected to a computer. The computer was operated with FRITSCH MaS control software. Fraunhofer theory, a preprogram theory to Mas control was selected for calculating statistical parameters like mean diameters, specific surface area, etc.

d) Surface topography, PSD and mineral composition of the sample

To examine surface topography and mineral composition of turmeric powder Scanning Electron Microscope (SEM) (Model EVO 60; Carl ZEISS SMT, Germany) was used. Before starting the investigation, turmeric powder was coated with palladium alloy by using sputtering equipment (SC7620, England) to make sample conductive. The characteristics like surface roughness, regularity, shape, and size of the particles can be analyzed by using a software called SmartSEM. Mineral compositions of turmeric powder were determined by using the SEM-EDX technique, which is associated with a software INCA PentaFET × 3 (Oxford Instrument, UK). The samples were measured at different magnification of 250X, and 500X operated at an accelerating voltage of 20kV.

e) Flow ability measurement of powder

Haunser Ratio (HR), Carr Index (CI), and Angle of repose (α) value examined the flowability of ground turmeric sample. The following Eq. (1) [11] can define the HR of turmeric powder.

$$HR=\frac{\rho_t}{\rho_b} \quad (1)$$

where, ρ_t and ρ_b are densities of tapped and bulk, kgm^{-3}.

The bulk density was measured by pouring turmeric powder in a graduated cylinder of volume 50 ml. The upper layer of the powder was leveled with the help of an iron strip. Exact the height and weight of the powder in the graduated cylinder were measured [13, 18, 19]. The bulk density of the turmeric powder was determined by using Eq. (2) [11].

$$\rho_b = \frac{M}{V} \quad (2)$$

where, M is the weight of powder in Kg and V is Volume of powder in m^3

The tapped density was measured by manually tapping the powder in the graduated cylinder [13, 19]. The tapped density of the turmeric powder was determined by using Eq. (3) [11].

$$\rho_t = \frac{M}{V_t} \quad (3)$$

where, M is the weight of the powder in kg, V_t is the volume of tapped powder in m^3

Carr's index shows the compressibility of a powder. It is estimated by following Eq. (4) [11].

$$CI = 100 - \frac{100}{HR} \quad (4)$$

The α was measured as a maximum cone angle form by loosely pile powder on a circular base plate with a diameter of 0.05m. Laboratory setup for measurement of the angle of repose was shown in (Fig. 2). α value can be determined by the following Eq. (5)[11].

$$\alpha = \tan^{-1}\left(\frac{r}{h}\right) \quad (5)$$

where, r is the radius of the plate in m, h is the height of pile in m

f) Physicochemical analysis of the powders

Some properties like moisture content, water activity (a_w), volatile oil (V), the color of turmeric powder will be discussed in this section. Moisture content was measured by using a hot air oven method. a_w is measured by using a water activity meter (Rotornic hygrolab). Volatile oil is extracted by hydro distillation method for 7 hours for 100g of turmeric powder sample in 1000 ml at 85°C [16]. A laboratory setup for extraction volatile oil is shown in (Fig.3).

The chromaticity of the ground turmeric samples was measured using CIELab colorimeter (BYK Gardner GmbH). CIELab colorimeter has four colors for calibration, namely black calibrated standard, white calibration standard with a certificate, green checking reference, and finally by high gloss standard. After calibration, the measurements of L*, a*, b* was done. The color of the turmeric samples was measured to find the values of 'L*' range 0 (luminance) to 100 (lightness) component, 'a*'ranges −120 (green) to +120 (red) axis and 'b*' (ranges −120 (blue) to +120 (yellow) axis. After shaking well, the ground turmeric sample measurement of color was done for three different replicas. Chroma value (C^*), hue angle (h) and yellowness index (YI) were calculated by using the following Eq. (6), (7), and (8).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (6)$$

$$h = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (7)$$

$$YI = \frac{142.86a^*}{L^*} \quad (8)$$

g) Statistical analysis

The experimental results were analyzed with univariate analysis of variance using the least significant difference (LSD) of statistical package for the social science (SPSS) (version 20, IBM, USA). Statistical values were considered significant if $p \leq 0.05$. Each significant value was level with significant value of (a-d). Origin pro 2015 (OriginLab Corporation, USA) was used for graphical plotting.

III. Results and Discussion

a) Mean particle diameters, particle size distribution (PSD) and specific surface area

From Table 1, it is clear that fineness of powder increases with the decrease in grinding temperature (from 40°C to -80 °C). The powder obtained at -80 °C gave a lower value of arithmetic mean diameter, median, and mode by 66.34%, 34.76%, and 50.07% respectively than powder obtained at 40 °C. This shows that the fineness of the particle depends on grinding temperature.

There is a significant ($p \leq 0.05$) increase in the specific surface area of the particle from 7145.16 cm^2 cm^{-3} to 45960.50 cm^2 cm^{-3} as grinding temperature decreased from 40°C to -80 °C as shown in Table 1. When the sample is in liquid nitrogen, the sample temperature falls below the glass transition temperature and then sample change from ductile to brittle state. This lowers the material's ability to resist high mechanical stress behavior as the grinding temperature decreases. Hence the turmeric sample becomes more brittle, easily broke down into small pieces and resulted in the higher specific surface area.

The particle size distributions with a distinct cumulative mass of different particle sizes obtained from different grinding conditions of turmeric samples are presented in (Fig. 4a-d). In (Fig. 4), the colored curves namely red and black curves denote two consecutive measurements of cumulative curve distribution of the powders over the given size range, whereas, the histograms represent the frequency of certain particle size. In (Fig. 2a-d), abscissa represents the particle size (μm), the right ordinate represents the frequency of certain particle size (Q3(×) [%])., and left ordinate represents cumulative curve distributions of powder (Q3(×) [%]).

From (Fig. 4a-d), it is clear that lower the grinding temperature distribution curve attains more skewness towards left (mean particle diameter is on the left) ,which affirms that finer powder is more at lower grinding temperature. Kurtosis increased (from 0.43 to 2.08) with a decrease in grinding temperature (from 40°C to -80°C). This shows that uniformity of particle size distribution increased with a decrease in grinding temperature. The maximum peak and leftmost skewness among the distribution can be seen at the

lowest grinding temperature, i.e., at −80 °C. It can also be concluded that the reduction in grinding temperature showed a decrease in the particle size and more uniform PSD.

b) Surface topography, PSD and mineral composition of the sample

From (Fig. 5a-d), it can be identified that by lowering grinding temperature, there is an increase in fineness and uniformity. And (Fig. 5e-f), shows surface roughness increased with grinding temperature. The highest surface roughness, lowest specific surface area, and uniformity can be seen at 40°C. Hence, results from the SEM micrograph confirm the results of particle size and particle size distribution of the powder.

Macro elements like potassium, iron, manganese, chlorine, and copper were determined by using the SEM EDX analysis present in turmeric. Through the EDX analysis of turmeric powder, it is found that potassium content is highest followed by chlorine, copper, manganese, iron and zinc for all grinding conditions (40°C, 0°C, -40°C, -80°C). During the grinding process at different temperatures, the sample may subject to different oxidation and reduction processes, which may be the reason for the changes in the composition. All the elements present in the powder are less than 1% at all grinding conditions except for potassium.

It was found that the amount availability of elements in turmeric powder increased with a decrease in grinding temperature. This may be because of the increase in surface area imparts more bioavailability of minerals. Potassium has the highest bioavailability of elements followed by chlorine, Manganese, and Iron. Trends can be seen in Table 2., which shows that mineral composition depends on particle size. In (Fig. 6) shows the position of EDX and spectrum of turmeric powder ground at different grinding conditions.

c) Flow ability measurement of powder

From Table 4, it is clear that HR, CI, and α were increased with a decrease in grinding temperature. By comparing Table 3 and 4, HR, CI and α powder obtained from the ambient grinding (40°C) condition can be assumed as good flow characters, and remaining were having fair flow character. In (Fig. 7 & Fig. 8) shows the variation of flow ability properties (Haunser Ratio and Angle of repose) with respect to grinding temperature.

The increase in flow ability with decrease in grinding temperature could be by following reasons: a) with decrease in particle size there may be increased in the force of attraction like Van der waals force between the particles b) Moisture content, volatile oils, curcuminoids, and others mineral composition of the particles are higher at lower grinding temperature, these may an increase the cohesive forces among the particles.

d) Physicochemical properties

In Table 5, moisture content, water activity, volatile oil, and color are compiled and presented. The moisture content and water activity increased by 18.87% and 5.72%, corresponding to the grinding temperature of −80 °C to 40 °C respectively. An increase in the moisture content of the samples may be because of condensation of moisture present in the grinder environment. Shelf life and stability of the food sample decreases with the increase in biochemical reaction and microorganism action on the sample. Biochemical reaction and microorganism action on the sample increases, if water activity is more than 0.6 [17]. Hence, the powder obtained from different grinding conditions are stable and has a longer shelf life due to water activity is less than 0.6.

Volatile oil yield at−80°C was relatively higher as compared to that of powder at 40°C. Volatile oil yield at −80°C was 47.61 % higher than that obtained at 40°C (Table 4). Here it is clear that volatile oil yield depends on grinding temperature significantly as yield decreases with an increase in grinding temperature. Decreased in volatile oil with an increase in grinding temperature can be explained by the following facts. a) Volatile oil may be evaporated at the higher grinding temperature [9][22], b) with an increase in specific surface area, there is an increase in contact surface of powder with solvent (water) during hydro distillation extraction of oil. Therefore, the mass transfer of volatile oil from powder to water will increases [18]. Hence there is a relatively higher yield of volatile oil in cryogrinding as compare to ambient grinding.

The grinding temperature also plays an important role in producing the good color of the turmeric sample. From Table 6, it can be observed that Chroma value, Hue angle and Yellowish index of the powder produced at -80°C were 12.29%, 2.72%, 1.56% and 7.95% respectively higher than that obtained at 40°C. L* and a* were not varied significantly with grinding temperature whereas b* significantly varied with grinding temperature. The color quality of the sample was good with the decrease in grinding temperature, which may be because of the reduction of mechanical forces, heat generation, and burning on the sample at lower grinding temperature. Moreover, changes in color quality may be because of change in particle size [19]. With decrease in particle size the bandwidth also decreases which results in an increase in brightness and yellowness of the particle.

IV. Conclusions

It can be concluded from the above study that grinding temperature greatly affects the quality and quantity of the powder obtained. Finer particle size, larger specific surface, uniform distribution, good color retention, volatile oil yield and mineral composition of

powder are on the side of lower grinding temperatures. It was observed that finer the particle size has significants effect on the flow ability of the powder. This paper will give the ideas about the design of grinding parts (like feed, hopper), determination of grinding temperature for better shelf life (for storage), maximum extraction (for volatile oil), excellent color (as per consumers), and higher mineral compositions.

Nomenclature:

T: Grinding Temperature (°C);

mc: moisture content (%);

a_w: water activity;

ρ_b: Bulk density (kgm^{-3}) ;

ρ_t: Tapped density(kgm^{-3});

HR: Haunser Ratio;

CI: Carr Index;

V: Volatile oil ($ml(100g)^{-1}$)

α: angle of repose(Degree)

C:* Chroma Value

YI: Yellowish index

h: Hue angle

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Hepatotoxic Restorative Potential of *Whitfieldia Lateritia* Leaf Decoction and Vitali Supplement on Phenylhydrazine-Induced Anemic Cockerels

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Abstract- *Background and Objective:* Food supplements and plant extracts are used farmers to boost animal performance. Hepatotoxic restorative potential of Whitfieldia lateritia leaf decoction and vitali supplement on phenylhydrazine-induced anaemic cockerels was investigated.

Materials and Methods: Phytochemical screening of W. lateritia leaf was carried out using standard methods. Sixty (60) cockerels were purchased, acclimatized for two weeks and assigned into 12 groups (coded T1, T2----T12) of 5 birds each, where T2,T3 and T4 received 100, 200 and 400 mg/kg body weight of the decoction, respectively; T5,T6,T7,T8,T9, T10,T11,T12 were induced anaemia using 10 mg/kg body of phenylhydrazine. This was followed by treatment of T5,T6 and T7 with 100, 200 and 400 mg/kg body weight of decoction, respectively, while T8, T9 and T10 were administered with 100, 200, 400 mg/kg body weight of vitali supplement, respectively, the T1, T11 and T12 were the normal control, negative control and baseline control, respectively.

Keywords: *phytochemical, decoction, anaemia, feed conversion, whitfieldia lateritia.*

GJSFR-D Classification: *FOR Code: 079999*

Strictly as per the compliance and regulations of:

Hepatotoxic Restorative Potential of *Whitfieldia Lateritia* Leaf Decoction and Vitali Supplement on Phenylhydrazine-Induced Anemic Cockerels

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Results: Saponin, cardiac glycosides, tannins and flavonoids were found in the *W.lateritia* leaf decoction. The total feed intake was lower ($p<0.05$) in all the text groups except T10 (anaemic cockerels) treated with 400mg supplement which compared with the normal control. The feed conversion ratio was significantly decreased ($p<0.05$) in T10 when compared with the control and groups treated with decoction. There was a significant increase ($P<0.05$) in the weights gain and carcass weights of anaemic cockerels (T10) that received 400ng supplement compared to normal control and decoction test groups. The alanine amino transferase (ALT) activity of the test groups were significantly lower ($P<0.05$) when compared to the normal control, baseline control and negative control, except T4 that was treated with 400mg decoction only which was significantly higher ($P<0.05$) than the normal control. The AST activity of the test groups were significantly lower ($P<0.05$) when compared to the normal control, except T3 that was treated with 200mg decoction only which was significantly higher ($P<0.05$) than the normal control. The ALP activity of test groups were significantly higher ($P>0.05$) than the normal control, baseline control and negative control. Graded healing of the hepatocytes was observed among the different test groups with the anaemic groups (T8, T9, T10) treated with the vitali supplement showing the highest recovery.

Conclusion: The study showed that anaemic cockerels treated with vitali supplement had better hepatotoxic restoration that the anaemic cockerels treated with decoction.

Keywords: phytochemical, decoction, anaemia, feed conversion, whitfieldia lateritia.

I. Introduction

Plants have been a source of medicinal agents for thousands of year because of their nutritional and phytochemical compositions (Mithraja *et al.,* 2012). A remarkable number of modern drugs have been isolated from medicinal plants which have led to sudden increase in the number of herbal drug (Boopathi and Sivakumar, 2011). Medicinal plants with therapeutic properties are used for the treatment of many infectious diseases of humans as they contain many bioactive phytochemical constituents which are of curative effects (Sanaa *et al*., 2012). The medicinal properties of the plants are mainly due to the presence of secondary metabolites like alkaloids, cardiac glycosides, tannins, flavonoids, saponins, reducing compounds, minerals and vitamins However, the secondary metabolites are of great medicinal interest as they have significant biological activities and constitute the major actual active constituents of many crude drugs. Most of these constituents are not expressly safe as most of them can naturally be harmful or become toxic during in-vivo biotransformation, e.g. generation of reactive oxygen species. (Vinoth *et al*., 2011). Reactive oxygen species which create oxidative stress cause human diseases and disorders such as heart disease, inflammation, atherosclerosis, stroke, cancer, diabetes mellitus, (Rackova *et al*.,2007). The secondary metabolites possesses hepatoprotective and antioxidant effects through a scavenging action to the free radicals in the body by donation of electrons and avoiding the disruption of the cell membrane lipid bilayer structures, thus reducing the hepatotoxicity of the liver.). Due to the profitable efficacy of medicinal plants on biological activities, there is a profitable need to regularly investigate their biological activities with a view for

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possible isolation of newer biological compounds from plants that can serve as novel drugs (Gambhire *et al*., 2009; Mirzaei *et al*., 2013). Plants that are under-studied and under-utilized are usually studied, and *W.lateritia* is one the plants used in folklore medicine but have been understudied. It is commonly called blood plant, and is a flowering plant belonging to the family of *acanthaceae* (D'Incalci *et al*., 2005). *Whitfieldia lateritia* is a native plant in Sierra Leone but recently has been observed in several parts of the world like Nigeria. *Aja et al*., (2016) reported that *W.lateritia* contain alkaloids, flavonoid, saponins, tanins etc., and posses hypercholesterolemic property. *Whitfieldia lateritia* leaf is routinely used as cooking vegetable for the purpose of boosting haematological parameters by local people in various local governments' areas like Ikwo, Izzi, Afikpo South and Ivo in Ebonyi State of Nigeria yet no documentary evidence on the hepatotoxic effect (Okorie *et al.,* 2020; Aja *et al.,* 2015). This project was designed to investigate the hepatotoxic restorative potential of *W. lateritia* leaf decoction and vitali supplement on phenylhydrazine-induced anaemic cockerels

II. Materials and Methods

a) Collection of W. lateritia Plant Sample

Fresh leaves of *W. lateritia* plant were collected from izzi, Ebonyi state, Nigeria. The plant was identified by a taxonomist from the University of Port Harcourt. Some leaves were deposited their herbarium.

b) Preparation of W.lateritia Leaf Decoction

The fresh leaves of *W.lateritia* were collected, destalked, weighed and washed under a running tap water. Twenty eight point six (28.6g) of the leaves was weighed with an analytical weighing balance, washed and then boiled with 300ml of distilled water at temperature of 70^0C for 15 minutes in order to extract the active ingredients of the plant in the form of decoction. Thereafter, the decoction extract was decanted into a clean container and stored in a refrigerator, and later used for the biochemical and histological studies..

$$\% \text{ yield} = \frac{weight\ of\ beaker + extract - weight\ of\ empty\ beaker}{\text{initial weight of sample used}} \times 100$$

c) Phytochemical Screening

The phytochemical screening for the presence of tannins, saponins, alkaloids, cardiac glycoside, flavonoids and others were carried out on the aqueous leaf extract of *W.lateritia*.

❖ *Test for the presence of tannins*

This was carried out using Harbone method (1973).

Principle: Tannins are secondary metabolites of plant species and consist of sugar and non-sugar parts. They are capable of undergoing hydrolysis when inserted into dilute acids or boiling water to give rise to products such as poly hydroxyl phenolic compounds. They are reactive following the possession of functional groups called hydroxyl group (OH). They participate in redox reaction to give characteristics color change on the reagent applied.

Procedure: One milliliter (1ml) of crude extract of the sample was collected using syringe and dispensed into test tube. Then, one milliliter (1ml) of ferric chloride ($FeCl_3$) was added to the test tube. A dirty green precipitate was observed which showed the presence of tannins.

❖ *Test for the Presence of Saponins:* This was carried out by the method of Harborne (1973)

Principle: Saponins are glycosides with distinctive foaming characteristics. They consist of a polycyclic aglycone that is either a choline steroid or triterpeniod attached through C_3 and an ether bond to a sugar side chain. The aglycone is referred to as the sapogenin and steroid saponins are called saponins. The ability of saponins to foam is caused by the combination of the non-polar sapogenin and the water soluble side chain (hydrophilic part), which have hydroxyl groups (-OH) as functional group.

Procedures:

Frothing Test: Two milliliters (2 mls) of the extract were diluted with 5 mls of distilled water in a test tube. The mixture was stirred vigorously for about 5mins and was allowed to stand for 30 minutes. Frothing which persisted for this duration indicated the presence of saponins.

❖ *Test For Presence of Alkaloids*

This was carried out by the method of Trease and Evans (1989)

Principle: Alkaloid can be detected as loose complexes following their ability to react with some reagents by producing characteristics color changes depending on the type of reagent used. Alkaloids have an amino group (NH_2) as their functional group as in nicotine.

Procedure: Two milliliters (2 mls) of the extract was collected using syringe and was dispensed into a test tube, the test tube was heated for 2 mins and 5mls of hydrogen (HCl) was added and heated again and allowed to cool. The mixture was divided into A and B. To A, 2 drops of Meyer's reagent was added and white precipitate was observed which showed the presence of Alkaloids. To B, 2 drops of Dragendroff's reagent was added and the formation of red precipitate was observed which confirmed the presence of alkaloids.

❖ *Test for the Presence of Flavonoids*

This was carried out by the method of Harborne (1973).

Principle: Flavonoids are colorless or pale yellow glycosides that are not soluble in non- polar solvents. They are compound that are oxidize by ethyl-acetate. They react with polar solvent to produce color changes

in accordance with the level of redox reactions that are likely to take place. Flavonoids also reacts with sodium hydroxyl group (NaOH) to form a yellow color following the reaction of the hydroxyl group (-OH) with the ketone functional group.

Procedure: Five milliliters (5 ml) of the extract was collected using syringe and was dispensed into a test tube. Exactly 10 mls of distilled water, 5mls of dilute ammonium hydroxide (NH_4OH) and few drops of tetraoxosulphate (VI) acid (H_2SO_4) were added in the test tube. A yellow coloration was observed which showed the presence of flavonoids.

❖ *Test for the Presence of Cardiac Glycoside*

This was carried out by the method of Harborne (1973)

Principles: Cardiac glycosides are organic compounds that are capable of undergoing hydrolysis in the presence of dilute acids, alkali or enzymes.

Procedure: Two milliliters (2 mls) of the extract was collected into a test tube and 5ml of glacial acetic acid was added and then 2mls of $FeCl_3$and 2mls of concentrated ferric acid were added too. A brown ring formation observed at inter phase of the mixture indicated the presence of deoxy sugar characteristics of cardiac glycosides.

d) Design of Animal Experiment

Sixty (60) cockerels were purchased and acclimatized for two weeks after which they were divided into 12 groups of 5 birds each, where Test groups T2 (100mg dc),T3(200mg dc) and T4(400mg dc) while T5 (100mg dc +PHZ),T6 (200mg dc +PHZ), and T7 (400mg dc +PHZ), T8 (100mg VS + PHZ),,T9(200mg VS + PHZ), and T10(400mg VS + PHZ and control groups T1(normal control),T11 (negative control) and T12 (baseline control).The birds were weighed and their initial weights recorded.

(VS: Vitali supplement, dc, decoction, PHZ phenylhydrazine)

e) Induction of Anaemia

Anaemia was induced through the injection of 10 mg/kg body weight of Phenylhydrazine for two consecutive days, after which the group T12 (baseline control). Blood was collected through ocular puncture; liver was collected into formalin and used for histological study.

f) Clinical Signs

Clinical signs or abnormalities observed upon induction of anaemia were: closing of the eyes, weakness, and loss of weight and itching of the eyes.

g) Collection of Serum

After 34 days of treatment, the final weights of the birds were taken before they were sacrificed. Whole blood sample were collected using syringe and centrifuged to get the serum for the biochemical analysis. The liver organ was carefully removed and preserved in formalin before taken to the laboratory for the histopathology analysis.

III. Biochemical Analysis

a) Determination of Alanine Amino Transeferase Activity

Alanine aminotransferase (ALT), formerly called serum glutamate-pyruvate transaminase (SGPT), catalyzes the transfer of amino group from alanine to keto-glutarate with the release of pyruvate and glutamate.

Principle: ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate to form L-glutamate and pyruvate. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD. The rate of change of the absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to NAD^+ and is directly proportional to the amount of ALT present in the sample (Wróblewski and La Due, 1956).

b) Determination of Aspartate Amino Transeferase Activity

Aspartate aminotransferase (AST), formerly known as glutamate-oxaloacetate transaminase (GOT) or serum glutamate–oxaloacetate transaminase (SGOT), catalyzes the transfer of the amino group from aspartate to α-ketoglutarate with the release of oxaloacetate and glutamate.

Principle: The aspartate aminotransferase (AST) test is based on the Karmen rate method (Karmen., 1955) as modified by Bergmeyer (Bergmeyer *et al*., 1977). AST catalyzes the reaction of L-aspartate and α-ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to NAD^+ by the enzyme malate dehydrogenase. The rate of absorbance change at 340 nm / 405 nm caused by the conversion of NADH to NAD^+ is directly proportional to the amount of AST present in the sample.

c) Determination of Alkaline phosphatase Activity

Principle: Alkaline phosphatase hydrolyzes *p*-NPP in a metal-ion buffer and forms *p*-nitrophenol and phosphate. The amount of ALP in the sample is proportional to the rate of increase in absorbance difference between 405 nm and 500 nm.

Procedure

The piccolo chemistry analyzer was turned on and allowed to completely boot.100 ul of the serum was collected using 100UL pipette. The pipetted serum was added into the piccolo cassette through a small dot on the cassette. The piccolo cassette was inserted into the piccolo chemistry analyzer and the result was displayed on the digital meter.

d) Histopathology Analysis on Tissue (Liver)

Histological method of processing tissue: this involves various ways of preparing, cutting, staining, and examination slides for histological report.

Stages in tissue processing:

(1) *Fixation:* The tissue was fixed in 10% formal saline in a container with light fitting lids for 3 day to prevent autolysis; improve staining quality and to aid optical differentiation of cell.

(2) *Dehydration:* The tissue was dehydrated to remove water that is not miscible with xylene and wax using different grades of alcohol ranging from 50% – absolute Alcohol for 30mins each

(3) *Clearing/De-alcoholization:* The dehydrated tissue was cleared by removing the alcohol from the tissue by immersing it through 3 changes of xylene for 30mins each.

(4) *Wax impregnation/ infiltration:* The cleared tissue was impregnated and infiltrated to remove the clearing agent (xylene) in the hot oven temp of 60^{0c} by passing it through three changes of molten paraffin was in a hot air oven for 30mins.

(5) *Embedding:* The infiltrated tissue was buried or embedded with molten paraffin wax in an embedded mould and allowed to solidify.

(6) *Mounting on wooding block:* The paraffin block of tissue was attached to a wooding block with the aid of a hot spatula held in between wood block and paraffin way, the spatula Melts the wax which solidifies when spatula was removed.

(7) *Microtomy:* The block of tissues was sectioned using rotary microtome, it was trimmed to obtain the cutting surface of the tissue at 15 micron and was sectioned at 5micron, and dry in hot plate for staining.

IV. Statistical Analysis

Data were analyzed using the statistical package for social science (Version 17). Difference among groups were measured using one way analysis of variance (ANOVA) followed by Duncan test. The results were expressed as mean ± standard deviation and differences were considered statistically significant at $P<0.05$.

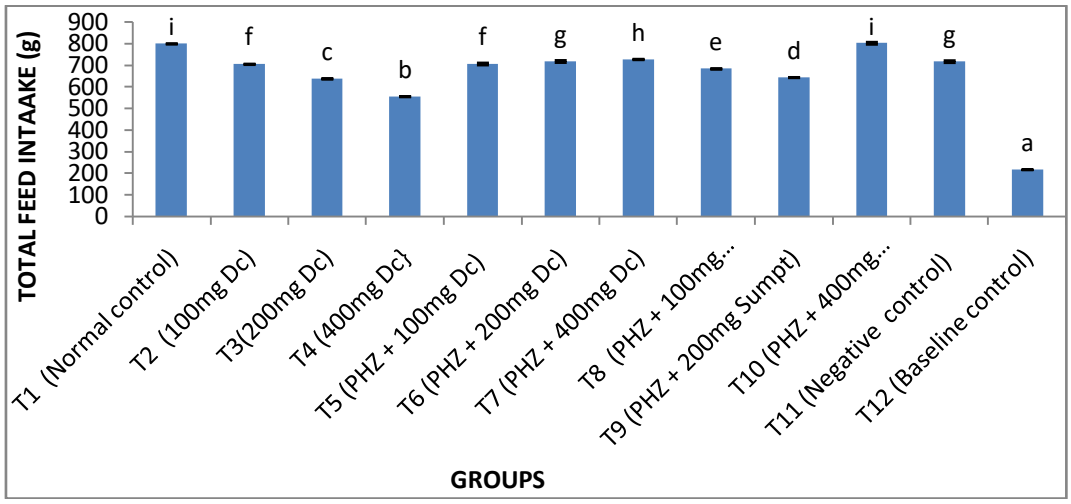
V. Results

Table 1: Phytochemical constituents of *W.lateritia* leaf decoction

S/N	Phytochemicals	Remarks
1	Alkaloids	-
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Cardiac glycosides	+

Keys: + = Present, - = Absent

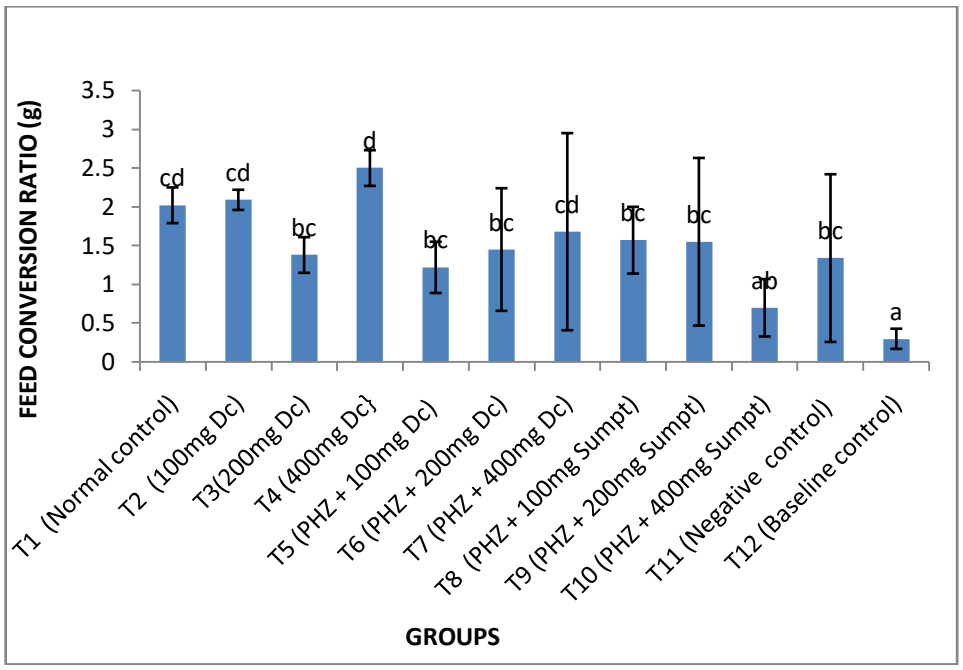
Table 1 showed that the *Whitfieldia lateritia* leaf decoction contained appreciable amount of the following phytochemicals: flavonoids, saponins, tannins and cardiac glycosides. Our results (table 1) revealed the presence of bioactive compounds such as alkaloid flavonoids, saponins, tannins and cardiac glycosides. This observation is in line with the work of Aja *et al.,* (2016) who had observed similar phytochemicals in ethanol extract of *Whitfieldia lateritia* leaf. Previous reports had shown that these bioactive compounds are biochemically active in many respects; eliciting different arrays of biochemical activities (enzyme activation/ inactivation, antioxidant activities etc.). Flavonoids have been reported to possess antibacterial property as they have the capability to associate with soluble proteins and bacterial cell walls (Doss *et al.,* 2011). They also have antioxidant property as they inhibit oxidative stress by mitigation of the generation of free radicals, exhibit anti-inflammatory and anti-cancerous activity (Liu *et al*., 2008; Alsabri *et al.,* 2013). Flavonoids perform major roles in plants such as, protection against ultraviolet radiation, defense against pathogens and pests, pollen fertility, auxin transport regulation and pigmentation (Winkel, 2001). Similarly, saponins are reported to have antimicrobial (Rohit *et al.,* 2012) and anti-cancerous properties (Shi *et al.,* 2004). Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells (Rohit *et al.,* 2012). Tannins have antimicrobial, antidiarrheal, anti-inflammatory and antioxidant activities (Killedar and More, 2010). Cardiac glycosides are used for the treatment of cardiac failure as they can stimulate cardiac output by increasing the force of contraction as a result of their ability to increase intracellular calcium concentrations through binding to the extracellular surface of the ion transport protein in the cell, the membrane-inserted sodium potassium pump (Na^+/K^+-ATPase) (Xie and Askari, 2002).



Mean ± SD, bars with similar superscript are not significantly different (P>0.05) n=5

Fig. 1: Variations in the total feed intake of cockerels treated with *w. Lateritia* leaf decoction and vitali supplement

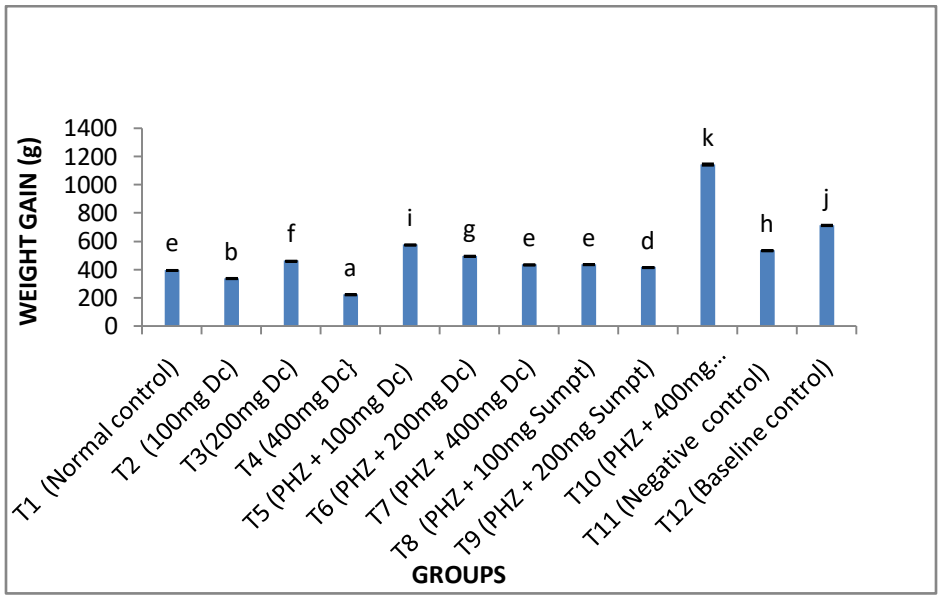
The total feed intake of the control was higher ($p<0.05$) than all the groups except group ten which was anaemic but treated with 400mg supplement. This observation agreed with the work of Machebe *et al.,* (2010) who reported a higher feed intake in birds fed vitalyte supplement than the extract of other leafy vegetables. This suggests that the vitali supplement might have stimulated appetite on the birds or increased their feed utilization leading to increased feed intake. The decreased feed intake witnessed in the decoction is consistent with the report of Chung *et al* (2010) who described decreased feed intake in birds treated with plant extracts due to possible effect of the bioactive components, since some phytochemicals such tannins had previously been reported to alter, negatively, feed intake in animal models. It can equally be inferred that the induction of anemia with phenylhydrazine with the attendant stressors, imposed a challenge on the metabolic capacity of the bird resulting to decrease feed intake, as this inferement had been corroborated by (Peter and Abel, 2006) who imputed that elevation of stress hormones will cause the body reserves to be mobilized to fuel the fight or flight response. Also nutrient absorption and gut motility decreases substantially during the stress response leading a decrease in feed intake.



Mean ± SD, bars with similar superscript are not significantly different (P>0.05)

Fig 2: Variations in the Feed conversion ratio of cockerel treated with *W.lateritia* decoction leaf and V itali Supplement

The feed conversion ratio (figure 2) of the group 4, non anaemic group treated with the decoction only was significantly ($p<0.05$) increased when compared with the control and the group treated with the supplement This finding agrees with the work of Nworgu (2007) which showed that administration of leaf extract of fluted pumpkin can stimulate feed conversion ratio in cockerel. Feed conversion ratio measures the efficiency with which the bodies of live stock convert animal feed into the desired output. Feed conversion ratio is a function of the animal's genetics, age, the quality and ingredients of the feed. The cockerels in T10 had the highest feed efficiency while the other groups, especially those treated with the decoction exhibited low feed efficiency. This could be accounted for, by the possible negative interference of the bioactive components of the decoction on certain metabolic pathways by metabolic stimulating hormones, e.g. cathecholamines. Animals that have a low feed conversion ratio are considered efficient users of feed (Arthur *et al*., 2014).



Mean ± SD, bars with similar superscripts are not significantly different (P>0.05) n=5

Fig. 3: Variations in weight gain of anaemic cockerels treated with *W. lateritia* leaf decoction and vitali supplement

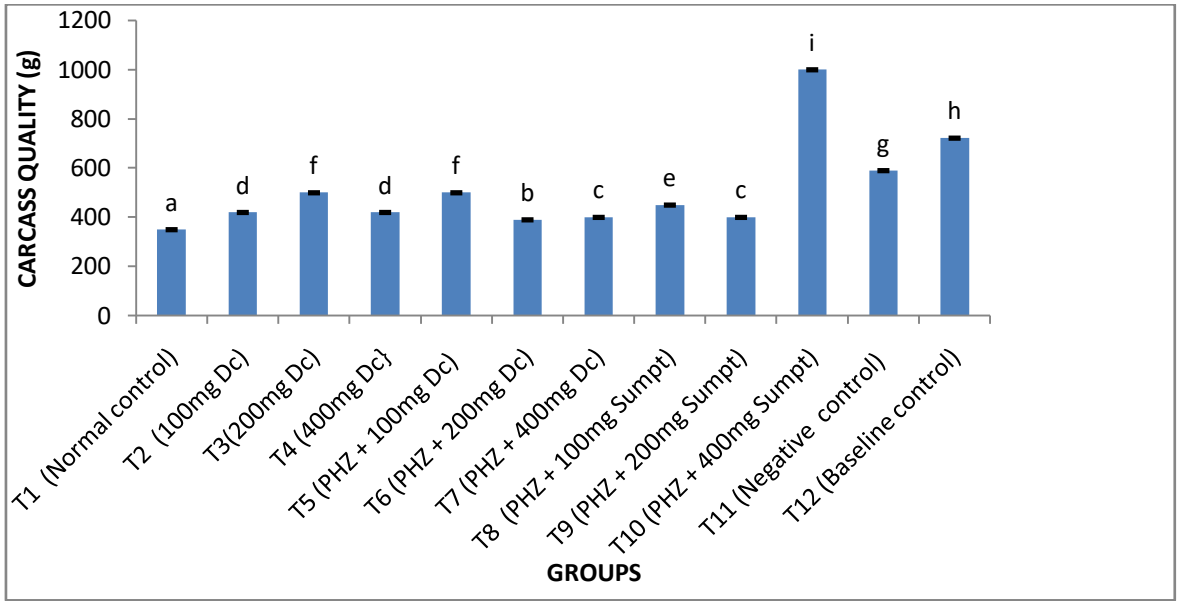
There was a significant increase ($P<0.05$) in the weights of anaemic cockerels (T10) that received 400ng supplement compared to normal control and other test groups (fig.3). The increased weight gains in conformity with earlier reports of Machebe *et al.,* (2011) who observed an increase in weight gain of birds fed vitalyte supplements. The increase in weight gain observed in group T10 can be attributed to the ability of VS to excite the olfactory nerves and taste buds, leading to an increase in feed consumption with the consequent increase in weight gain. An increase in the digestive secretion is one of the most important effects in improving the nutritional status (Platel and srinivisan, 2011). The actions that can produce these effects are increased salivary gland secretion, stimulation of mucus secretion in the stomach and intestine. Also weight gain can be as a result of increased secretion of pancreatic enzymes which accelerate digestion and shorten the time of feed passage through the digestive tract, improved digestion and absorption of lipids (Costa *et al.,* 2018). Furthermore, decreased weight gain noted in group 4 which was treated with decoction could be as a result of impaired feed intake and utilization as well as possible alteration of hormones responsible for animal growth and development.

Table 3: Variations in relative weight of the liver of cockerels treated with *W. lateritia* leaf decoction and vitali supplement

Groups	Relative Liver Weight (g)
T1 (Normal Control)	0.05 ± 0.02^{a}
T2 (100mg Dc	0.05 ± 0.02^{a}
T3 (200mg Dc)	0.04 ± 0.02^{a}
T4 (400mg Dc)	0.05 ± 0.02^{a}
T5 (PHZ+100mg Dc)	0.04 ± 0.02^{a}
T6 (PHZ+200mg Dc)	0.05 ± 0.02^{a}
T7 (PHZ+400mg Dc)	0.05 ± 0.02^{a}
T8 (PHZ+100mg Supmt)	0.05 ± 0.02^{a}
T9 (PHZ+200mg Supmt)	0.05 ± 0.02^{a}
T10 (PHZ+400mg Supmt)	0.02 ± 0.02^{b}
T11 (Negative Control)	0.04 ± 0.02^{a}
T12 (Baseline Control)	0.03 ± 0.02^{b}

Mean ± SD, values with similar superscripts are not significantly different (P>0.05) n=5

The relative weight of the liver of the test groups were not significantly different (p>0.05) from that of the normal control. The groups that were PHZ-induced and treated with decoction were not significantly (p>0.05) different from those that were induced and treated with vitali supplement (p>0.05) except for T10. Increase in the relative weight of animal liver can occur when there is accumulation of tiacyglycerol leading to enlarged liver consequent upon increased influx of fatty acids into the liver or de novo synthesis of the fatty acids in liver and or low capacity of excretion of lipoprotein from the liver resulting from a deficiency of apolipoprotein B synthesis, and ecreased VLDL is one of the causes of lipid accumulation in the liver; this occurred when the protein synthesizing materials (e. g. choline) are in short supply (Ohno *et al.,* 2000).

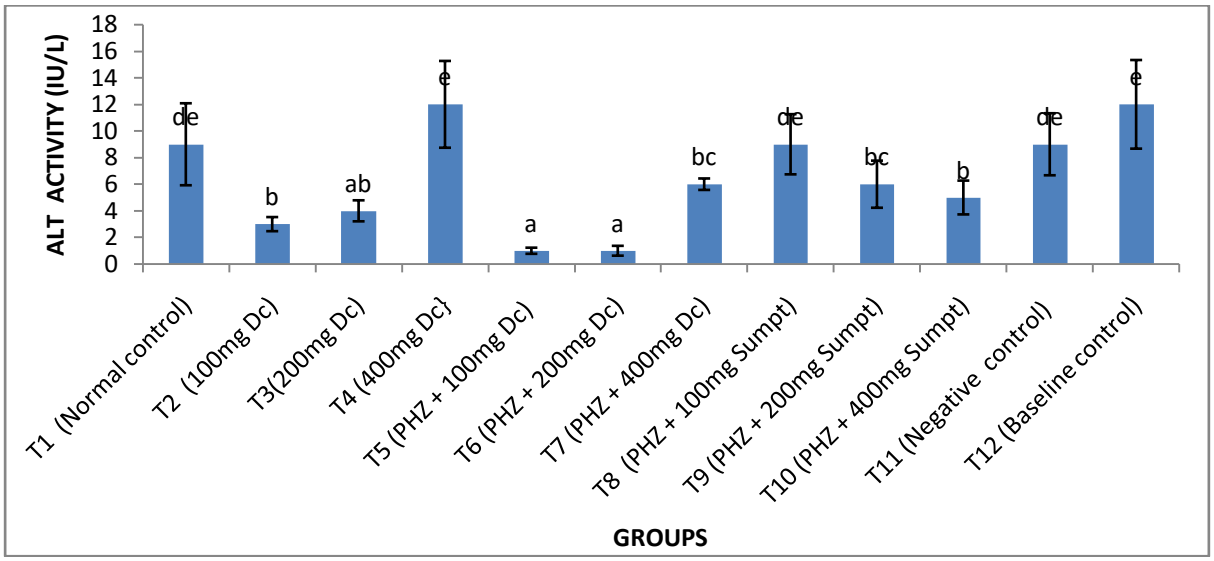


Mean ± SD, bars with similar superscripts are not significantly different (P>0.05) n=5

Fig. 4: Variations in carcass weight of cockerels treated with *W. lateritia* leaf decoction and Vitali supplement

There was a significant increase (P<0.05) in the carcass weight of test group, T10, compared to normal control and other test groups (figure 4). The elevated carcass weight observed in this study was not in conformity with the earlier reports of Esonu *et al.,* (2006) who observed that the administration of neem extract in poultry stimulated carcass weight increase than the group that received supplement. Carcass weight can be affected by dietary protein content of animal feed. A diet with lower protein reduces the yield of meat and increases the fat content of the carcass, also high bird density may induce competition for space, lower feed intake, leading to poor feathering, skin lesions and decreases the carcass quality. Dim lights have been observed to increase fat levels of carcass, as adequate light is responsible for the stimulation of receptors responsible for the release of growth hormones which act as lipolytic agents in the body, however, under dim

lights the concentrations of these hormones is decreased (Garcia *et al* ., 2002).

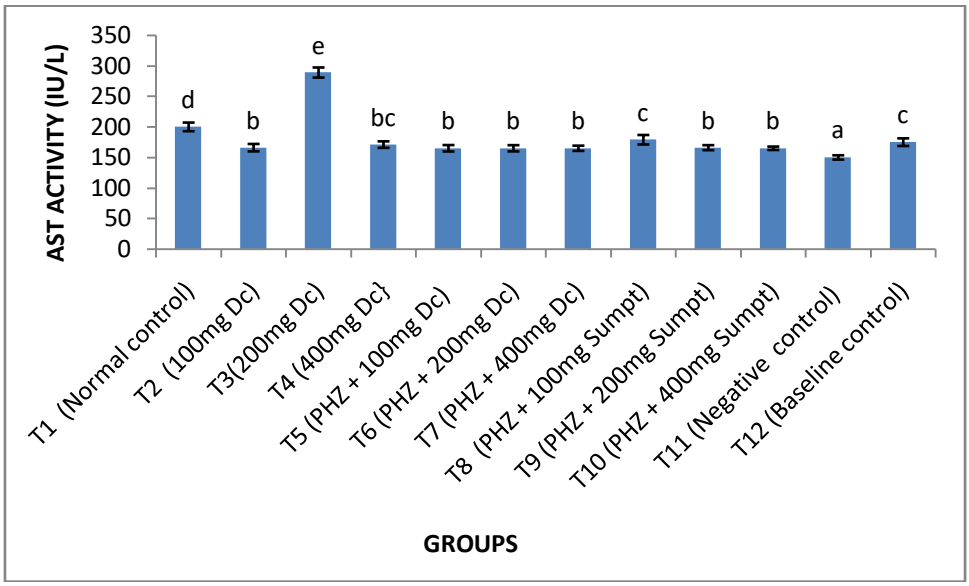


Mean ± SD, values with the similar superscript are not significantly different (P>0.05). n=5

Fig. 5: Variations in ALT activity of cockerels treated with *W. lateritia* leaf decoction and vitali supplement

The alanine amino transferase (ALT) activity of the test groups were significantly lower (P<0.05) when compared to the normal control, baseline control and negative control, except T4 that was treated with 400mg decoction only which was significantly higher (P<0.05) than the normal control. Our current findings (figure 4.5) revealed decreased (p<0.05) alanine amino transferase (ALT) activity of the test groups when compared to the normal control, baseline control and negative control.

The decrease in ALT activity observed in the test groups did not agree with the work of Duru *et al.,* (2018) who observed a significant increase (p<0.05) in ALT activity of rats fed the bark of *Carica papaya extract*, although there was an observed increase (p<0.05) in the test group, T4 which was treated with 400mg decoction only in our present study. The decreased level of ALT activity observed in the decoction treated groups compared with the control could be due to impairment of protein synthesis through various mechanisms such as interference at the gene expression or protein inactivation, of which tannin is a known culprit. The supplement treated grouped which did not differ (p>0.05) from the control could be because the supplements lacks the necessary ingredients required for growth and tissue repair. ALT is an enzyme that is mostly used in clinical biomarker for monitoring liver disease (Ozer *et al.,* 2008). ALT which is responsible for the metabolism of alanine is found in much higher concentration in the liver compared to other organs. The observed increase in ALT activity of T4 and baseline control can be an indication of liver damage (Edgar *et al.,* 1998). When hepatocellular injury such as necrosis or membrane damage occurs, the liver abundant enzyme ALT will leak into the extracellular space and enter the blood where it shows a slow clearance rate (Ozer *et al.,* 2008). However, despite the specificity of ALT levels to hepatocellular disease, the absolute peak of the ALT elevations does not correlate with the extent of liver cell damage (Mathiesen *et al*., 1999). Extrahepatic injury such as muscle injury can lead to an elevation in ALT thereby making ALT not entirely hepatospecific, also fenofibrinate has been found to increase ALT gene expression in the absence of apparent liver injury (false positive), (Edgar *et al.,* 1998).

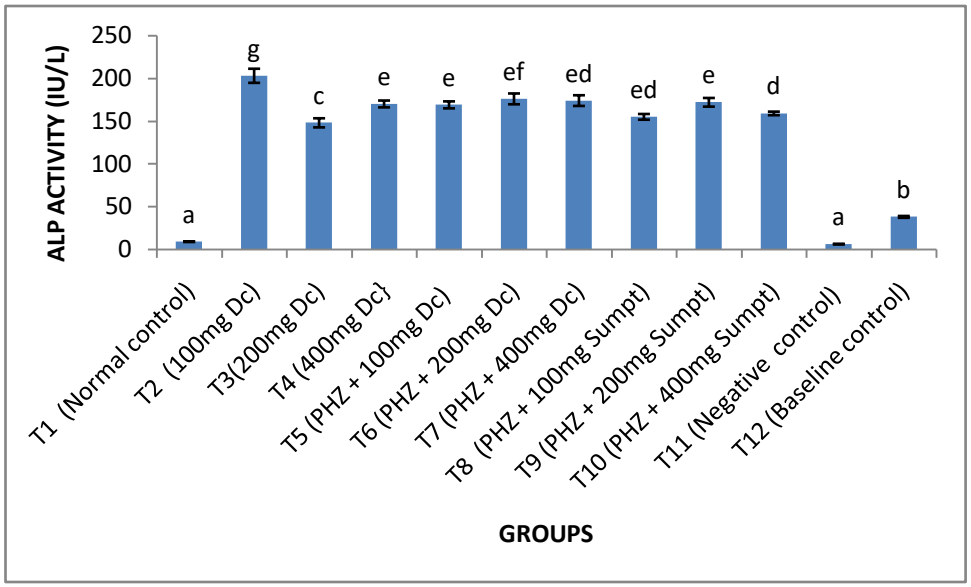


Mean ± SD, bars with the similar superscript are not significantly different (P>0.05). n=5

Fig. 6: Variations in AST activity of cockerels treated with *W. lateritia* leaf decoction and vitali supplement

The AST activity of the test groups were significantly lower (P<0.05) when compared to the normal control, except T3 that was treated with 200mg decoction only which was significantly higher (P<0.05) than the normal control (Figure 6).

In the present study, AST activity (figure 6) was decreased (p<0.05) in the test group when compared with the control. The decreased AST activity reported in the test group relative to the control was not in conformity with the work of Duru *et al.,* (2012) who observed a significant increase (P<0.05) in AST activity of rats fed the bark of *Carica papaya crude* extract relative to their control. The discrepancies in both studies could be as a result of the different treatments of the animals in both studies such as induction anemia, as in the case of our study, different dosages used and duration of studies. AST which is less specific than ALT, is found in highest amount in extra hepatic organs, such as heart and muscle. Increased AST activity in the test group T3 can be as a result of cellular leakage and loss of functional integrity of the cell membrane of the hepatocytes. Of course, this is understandable since alteration of membrane integrity through lipid peroxidation of membrane lipid is a typical toxic effect of phenylhydrazine which was used to induce anemia. This also conformed to the histological analysis that revealed a recovery from hepatic inflammation of the birds. However, diseases such as viral hepatitis, necrosis, cardiac infarction and muscle injury can equally elicit the elevation of AST (Robert *et al.,* 2017, 2000).



Mean ± SD, bars with the similar superscript are not significantly different (P>0.05). n=5

Fig. 7: Variations in ALP activity of cockerels treated with *W. lateritia* leaf decoction and vitali supplement

The ALP activity of test groups were significantly higher (P>0.05) than the normal control, baseline control and negative control (figure 7). But the test groups that were PHZ-induced and treated with decoction were not significantly different (P>0.05) from the test groups that were PHZ-induced and treated with vitali supplement except for T2 which showed highest (P<0.05) ALP activity. In the present study, ALP activity of the test groups agreed was observed to increase when compared with normal control, this is in conformity with the work of Andongma, (2014) who had reported an increase in ALP activity in PHZ-induced anaemic rats that received methanolic extract of *Gnetum africanum* leaves. The increased ALP activity relative to the normal control could be due to the stimulation of protein synthesis cum disruption of cellular membrane of the hepatoctyes, small intestine, and bone. Also, elevated ALP activity seen in the test groups could be as a result of blockage of bile ducts (Robert *et al*.,2017 and Mathew *et al.,* 2000). This observed elevation in ALP is in tandem with the histopatholgy result that revealed hepatoxicity. ALP is found in the kidney, bone, liver and placenta, but its activity is mainly in the liver. Infiltrative liver diseases such as abscesses, granulomatous and amyloidosis may also cause an increase in ALP activity (Fuchs *et al.,* 2008). While the decreased level in the negative control observed in the current study might be attributed to ALP synthesis impairment, zinc deficiency, vitamin B12 deficiency since availability of both nutrients can be compromised in an anaemic condition. Other conditions that could lead to low levels of serum ALP include hypothyroidism and congenital hypophosphatasia which could explain the low ALP activity since phenylhuydrazine and its the metabolic products could act through different mechanism to warrant compromise of materials needed for the development of the bone and thyroid gland (Agbai *et al* ., 2014 and Clermont and Chalmers, 1967).

Histopathology

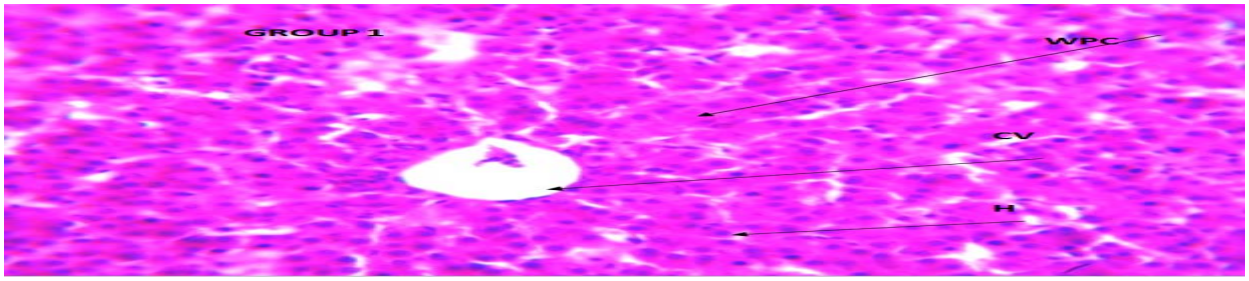


Fig. 8: Group T1: normal control received distilled, water section of liver (x400)(H/E) shows normal well perfused hepatic architecture with central vain (C),cytoplasm (C) and hepatocyte (H).

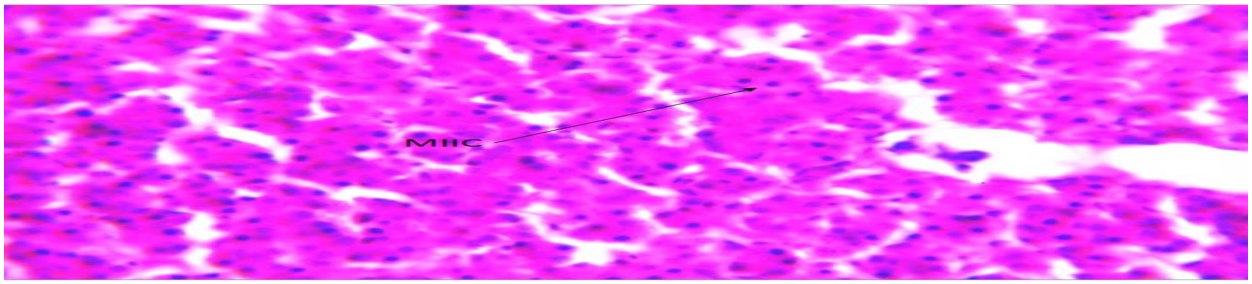


Fig. 9: Group T2 100mg decoction only, section of liver (x400) (H/E)shows hepatic tissue with mild infilteration of inflammatory cell (MIIC) otherwise normal

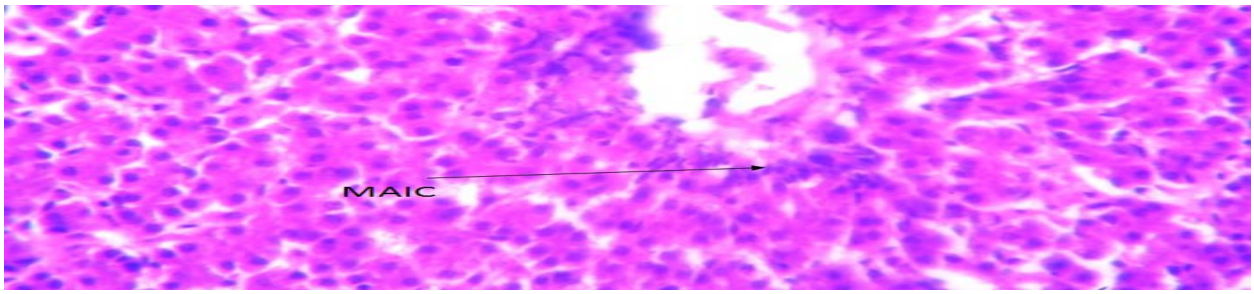


Fig. 10: Group T3:200mg decoction only section of liver (x60,150) (H/E) shows hepatic tissue with mild focal aggregate inflammatory cell (FAIC) around the central vain otherwise normal

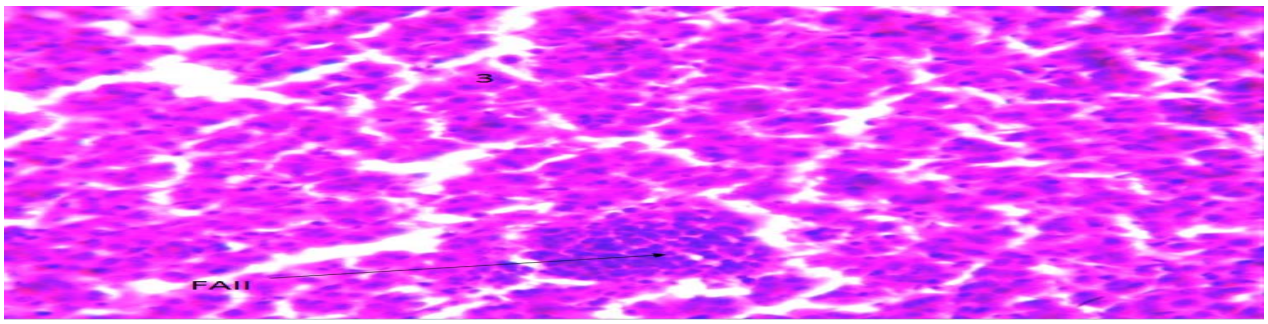


Fig. 11: Group T4: decoction only of section of liver (x400) (H/E) shows hepatic tissue with moderate focal aggregate of intrahepaticinflammatory cell (FAIIC)

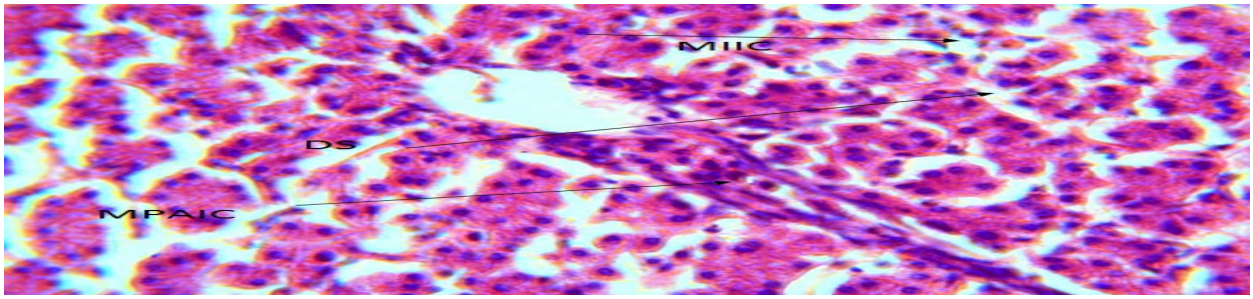


Fig. 12: Group T5: PHZ + 100mg decoction, section of liver (x400) (H/E) shows hepatic tissue with moderate portal aggregate of inflammatory cell (MPAIC) ,dilation of the senosoid (DS) and clumping of the hepatic tissue (CHT)

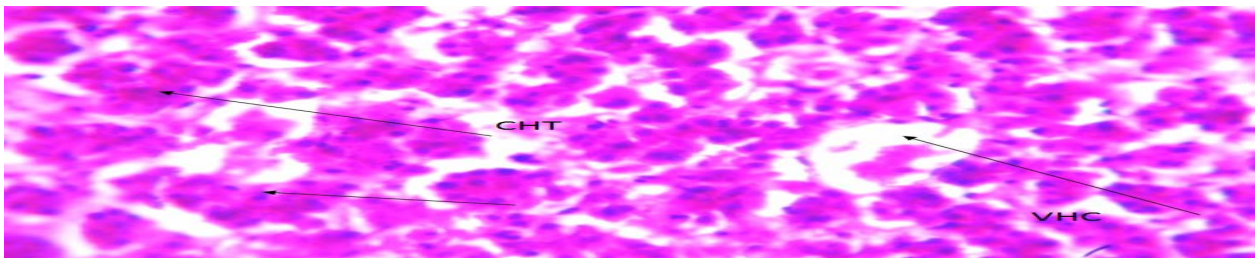


Fig.13: Group T6: PHZ + 200mg decoction, section of liver (x400) (H/E) shows hepatic tissue with clumping (C) moderate infinfilteration of inflammatory cell (MIIC) and vacoulation hepatic tissue(VHT)

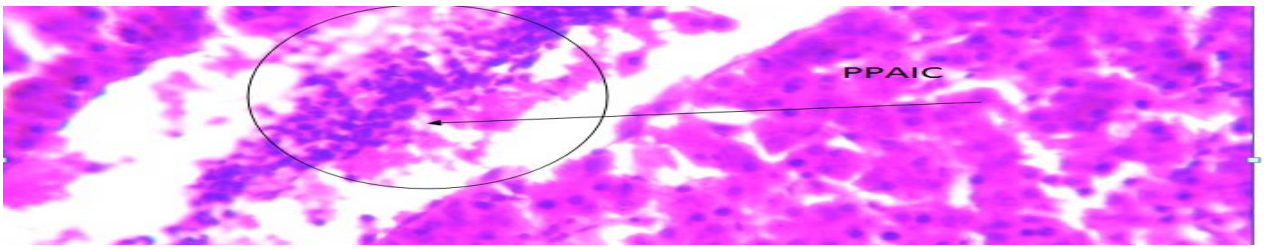


Fig.14: Group T7: PHZ + 400mg decoction, section of liver (x400) (H/E)shows hepatic tissue with peri portal aggregate of inflammatory cell (pPAIC) .

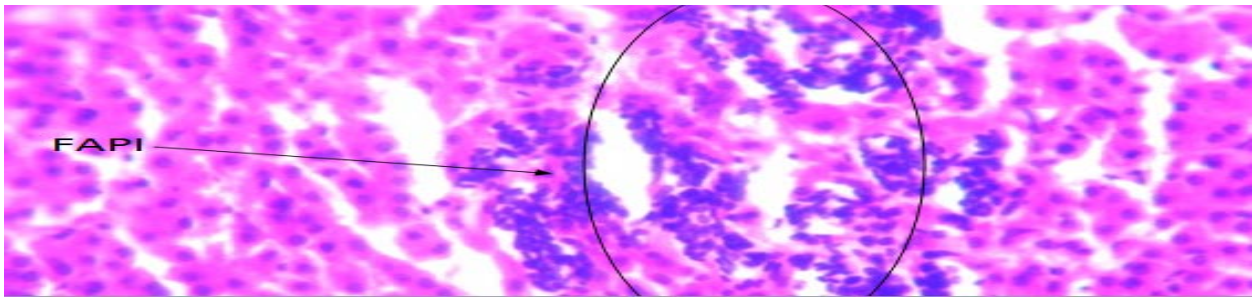


Fig. 15: Group T8: PHZ+100mg vitali supplement, section of liver (x400) (H/E)shows hepatic tissue with moderate to sever peri portal aggregate of inflammatory cell(PPAI)

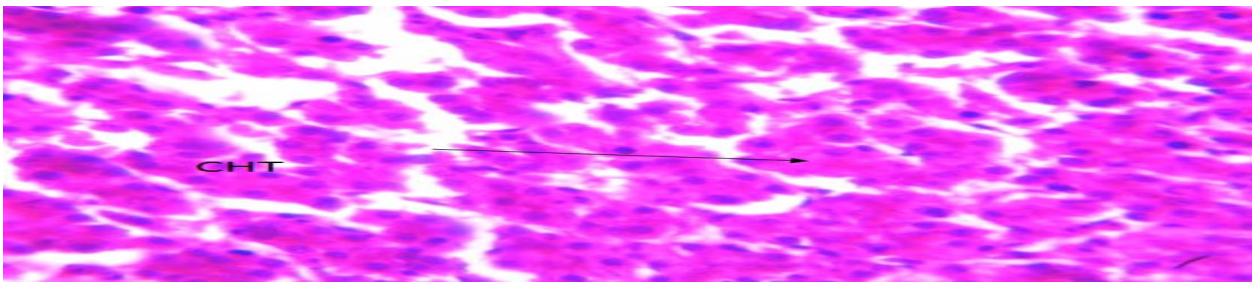


Fig.16: Group T9: PHZ+200mg vitali supplement, section of liver (x400) (H/E)shows hepatic tissue with mild clumping of the hepatic tissue(CHT) otherwise normal

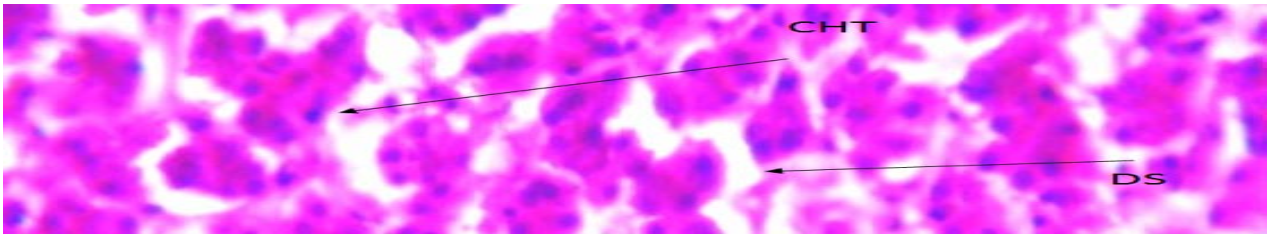


Fig.17: Group T10: PHZ+400mg vitali supplement, section of liver (x400) (H/E)shows hepatic tissue with moderate dilation of the senosoid (DS) and clumping of the hepatic tissue (CHT)

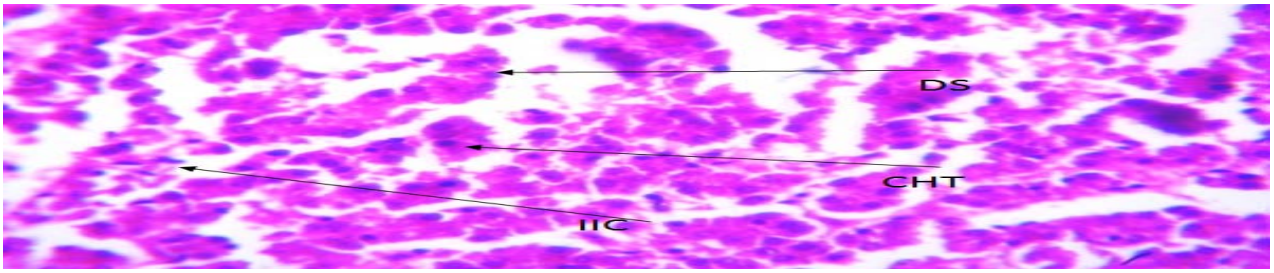


Fig.18: Group T11: PHZ(anaemic, not treatment), negative control; section of liver (x400) (H/E) shows disorganised hepatic tissue with moderate to severe ,dilation of the senosoid (DS), clumping of the hepatic tissue (CHT) and infilteration of inflammatory cell(IIC)

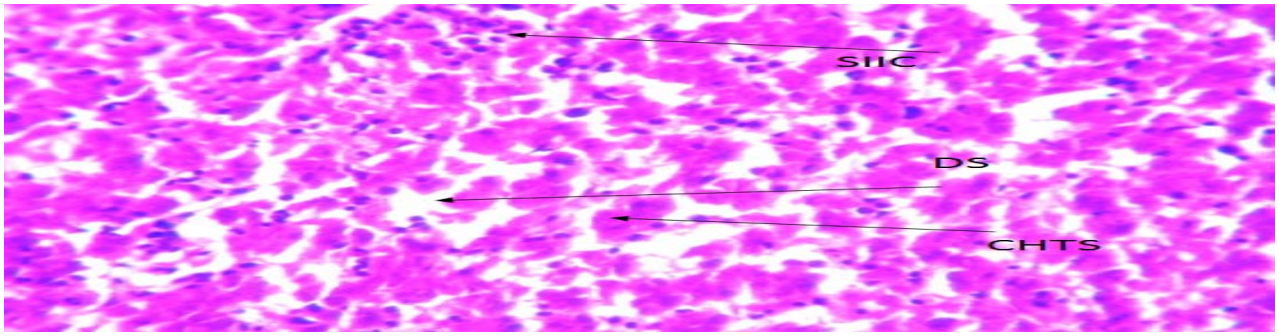


Fig. 19: Group T12: PHZ, baseline control, section of liver (x400) (H/E) shows severe disintegration of hepatic architecture with dilation of the sinusoid (DS), clumping of the hepatic tissue (CHT) and severe infiltration of inflammatory cell(SIIC)

The histopathological results (Figures 8-19) revealed compromised liver integrity in the baseline control (fig 19), negative control (fig 19) as against normal hepatic architecture in the normal control-T1 (fig 8). But in the test groups, graded healing of the hepatocytes was observed among the different test groups with the groups (T8, T9 and T10) that were PHZ-induced but treated with the supplement showing the highest recovery. The histological finding had indicated that the supplement treated cockerels have better hepatotoxic healing potential than the *W. lateritia* leave decoction treated groups.

VI. Conclusion

The results from this study revealed that anaemic cockerels treated with vitali supplement showed better performance, which are evident in the increased weight gain, carcass weight, lower activity of serum liver enzymes and observed liver injury recovery when compared with test groups that were treated with the *W. lateritia* leaf decoction. Groups that were treated with lower dosage of *W. lateritia* leaf decoction showed a better performance than those that were treated with higher doses of the decoction. Finally, vitali supplement have a better protective effect on the cockerels than *W. lateritia* leaf decoction and can be used in improving the general well being of cockerels.

Conflict of interest

The authors declare there is no conflict of interest

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The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.

Benefit

To the institution

Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

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Career | Credibility | Exclusive | Reputation

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Financial

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Organize seminar/conference

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.

Career | Credibility | Financial

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Exclusive | Financial

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Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

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All members get access to 2 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 5 GB free secure cloud access for storing research files.

Associate	Fellow	Research Group	Basic
$4800 **lifetime designation**	$6800 **lifetime designation**	$12500.00 **organizational**	APC **per article**
Certificate, LoR and Momento **2** discounted publishing/year **Gradation** of Research **10** research contacts/day **1 GB** Cloud Storage **GJ** Community Access	**Certificate,** LoR and Momento **Unlimited** discounted publishing/year **Gradation** of Research **Unlimited** research contacts/day **5 GB** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**Certificates,** LoRs and Momentos **Unlimited** free publishing/year **Gradation** of Research **Unlimited** research contacts/day **Unlimited** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**GJ** Community Access

Preferred Author Guidelines

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

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

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2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
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7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

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Plagiarism is not acceptable in Global Journals submissions at all.

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Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
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- Printed material
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2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

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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Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

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.

Format Structure

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).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

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.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- 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.

Approach:

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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	A-B	**C-D**	**E-F**
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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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