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Pharma, Drug Discovery, Toxicology & Medicine

Properties of Essential Oils

Moringa Oleifera Seed Protein

Highlights

Treatment for Psychodermatology

Antioxidant and Cytotoxic Properties

Discovering Thoughts, Inventing Future

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Antioxidant and Cytotoxic Properties of Essential Oils from Native Brazilian Lauraceae Species

By Fabiana Lima Silva, Amanda Espírito Santo, Paola Cristina Branco, Letícia Veras Costa-Lotufo, Maria Claúdia Marx Young, Cynthia Murakami, Inês Cordeiro, Sueli A. Nicolau, Leticia Megumi Ishibaru & Paulo Roberto H. Moreno

Universidade Paulista

Abstract- Analysis of the leaf and stem essential oils from three Brazilian Lauraceae species showed that the major constituent of O. odorifera was safrole (57.1 and 88.5% for leaves and stems, respectively) while for O. indecora leaves the major ones were the α-pinene (12.8%) and β-pinene (12.4%) and β-bisabolol (12.2%) in the stems. For the third species, Persea venosa, the main components of leaf and stem oils were spathulenol (27.8 and 14.7%, respectively) and humulene epoxide II (11.3 and 5.1%, respectively). The bioactivity results indicated that the leaf essential oil from O. indecora was the most active for both radical scavenging activity (IC50=0.142 mg/mL) and cytotoxicity against human tumor cells, with growth inhibitions for the colon tumoral line HCT-116 (100%) and breast cancer cell line MCF-7 (99.2%), at 5 μg/mL concentration. This activity might be related to the presence of α-pinene and β-pinene in the leaf essential oil of O. indecora. However, further studies with the isolated compounds are necessary to fully understand these bioactivites.

Keywords: *Ocotea odorifera, Ocotea indecora, Persea venosa, essential oil, biological activities.*

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Antioxidant and Cytotoxic Properties of Essential Oils from Native Brazilian Lauraceae Species

Fabiana Lima Silva [α], Amanda Espírito Santo [σ], Paola Cristina Branco [ρ], Letícia Veras Costa-Lotufo [ω], Maria Claúdia Marx Young [¥], Cynthia Murakami [§], Inês Cordeiro [χ], Sueli A. Nicolau [ν], Leticia Megumi Ishibaru [θ] & Paulo Roberto H. Moreno [ζ]

Abstract- Analysis of the leaf and stem essential oils from three Brazilian Lauraceae species showed that the major constituent of *O. odorifera* was safrole (57.1 and 88.5% for leaves and stems, respectively) while for *O. indecora* leaves the major ones were the α-pinene (12.8%) and β-pinene (12.4%) and β-bisabolol (12.2%) in the stems. For the third species, *Persea venosa*, the main components of leaf and stem oils were spathulenol (27.8 and 14.7%, respectively) and humulene epoxide II (11.3 and 5.1%, respectively). The bioactivity results indicated that the leaf essential oil from *O. indecora* was the most active for both radical scavenging activity (IC_{50}=0.142 mg/mL) and cytotoxicity against human tumor cells, with growth inhibitions for the colon tumoral line HCT-116 (100%) and breast cancer cell line MCF-7 (99.2%), at 5 µg/mL concentration. This activity might be related to the presence of α-pinene and β-pinene in the leaf essential oil of *O. indecora*. However, further studies with the isolated compounds are necessary to fully understand these bioactivites.

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I. Introduction

Essential oils from herbal sources are used as food flavours, perfumes and pharmaceuticals purposes (Burt, 2004). Leaves and barks of some Lauraceae species are popular spice ingredients and flavoring agents, such as cinnamon and laurel (Joshi et al., 2010). Additionally, the essential oils from some species within the genera *Aniba* Aubl., *Cinnamomun* Spreng., *Nectandra* Rottb. and *Ocotea* Aubl. have been largely used in the industry (Marques, 2001). Lauraceae comprises about 55 genera and over 2000 species mostly found in tropical, subtropical and mild temperate regions (Takaku et al., 2007). Due to their commercial importance, some Lauraceae species have already been studied regarding their essential oil contents and biological activities, however there are still many neglected species regarding their chemical composition.

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Ocotea is one of the largest genera in the Neotropics, containing *ca*. 350 species from which 170 are found in Brazil (Brotto et al., 2013). Among the native Brazilian *Ocotea* species, *O. odorifera* (Vell.) Rohwer is a tree found in the Atlantic Rainforest, and it is popularly known as 'canela-sassafrás, producing a highly valued essential oil by the cosmetic and pharmaceutical industries due to the high concentration of safrole. The commercial importance had led this species to near extinction and currently it is federally protected (IBAMA, 1992). On the other hand, there are also some species that have not yet been chemically or biologically studied, such as *O. indecora* (Schott) Mez., 'canela-cheirosa', whose barks are commonly used in traditional medicine as sudorific, antirheumatic and anti-syphilic (Marques, 2001). Although it is a native species widely distributed along the Southeastern and Southern Atlantic Rainforest (Brotto et al., 2013), there is only one previous report on the leaf essential oil composition of *O. indecora* (Gonçalves et al., 2018). To the best of our knowledge, there is no previous study about the biological properties of this species.

Persea Mill. is known as the oldest Lauraceae genus (Scora and Bergh, 1992). It is typically represented by the avocado (*P. americana* Mill.), the most important edible species within the genus. The Neotropical *Persea* species are distributed from Brazil and Chile in South America to Central America and Mexico (Moraes et al., 2014). In Brazil, about 30 *Persea* species are found dispersed among the biomes Amazon, Cerrado and Atlantic Rainforest (Flora do Brasil 2020, 2017). *P. venosa* Nees & Mart. is a native Brazilian species, popularly known as 'pau-de-andrade' and 'canela-sebo', it is found in Minas Gerais, São Paulo, Paraná and Santa Catarina (Flora do Brasil 2020, 2017). This species is also used in the traditional medicine for treating wounds and skin ulcers (Mazza, 2000). Although it is a rare species, the tree was extensively harvested for its wood which has put it in a high threat of extinction (Biodiversitas, 2019).

As a part of a research on aromatic species of the Brazilian Atlantic Rainforest aiming to aggregate value to them, in order to increase the interest in their sustainable use. The present study deals with the chemical analysis of the essential oils obtained from leaves and stems of *Ocotea odorifera*, *O. indecora*, and

P. venosa. The oils' biological activity was assessed by evaluating their antioxidant capacity and cytotoxic activity.

II. Materials and Methods

a) Chemicals and cell lines

The linear alkane mixture (C_6-C_{40}), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl-sulfoxide (DMSO) and methanol were obtained from Merck (Darmstadt, Germany).

Human colon (HCT-116) and breast (MCF-7) adenocarcinoma cell lines were cultivated in DMEM/F12 (SK-Mel-19) or DMEM Glutamax (RPE) medium with 10% fetal bovine serum (v/v), 2 mmol/L glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin at 37 °C under 5% CO_2 atmosphere.

b) Plant Material

Leaves and stems of three species were collected at different areas from Minas Gerais, Brazil along Atlantic Rainforest areas, the specific collection sites are presented in Table 1. The plant materials were identified by Dr. Inês Cordeiro and Dr. Sueli Nicolau (Instituto de Botânica, São Paulo, Brazil). Voucher specimens were deposited in the Herbarium of the same institution. The leaves and stems were separated and dried at room temperature. The dried stems were pulverized in a hammer mill.

Table 1: Collection sites of the Lauraceae species and Voucher numbers

Species	Voucher number	Collection site	GPS Localization
Ocotea odorifera (Vell.) Rohwer	S. Nicolau 3885	Morro Grande, Caldas	21.92° S and 46.39° W
Ocotea indecora (Schott) Mez.	Cordeiro 3113	Serra do Selado, Poços de Caldas	21.79° S and 46.56° W
Persea venosa Nees & Mart.	S. Nicolau 3876	Morro Grande, Caldas	21.92° S and 46.39° W

c) Chemical Evaluation

i. Essential oil extraction

Essential oils were obtained from leaves and stems by hydrodistillation using a Clevenger apparatus. The extractions were carried for 4 h and the oils were dried over anhydrous sodium sulfate and stored in a freezer (-20 °C) until further use. The essential oil yields were calculated based on the dry weight of each sample (Table 2).

ii. GC-MS analysis

Essential oil samples were dissolved in acetone (0.1% v/v) and injected(1.0 µL) in a gas chromatograph Agilent 6890 Series GC apparatus (Agilent, Santa Clara, CA, USA) with a fused silica capillary column (DB-5, 30 m x 0.25 mm i.d. x 0.25 µm film thickness) hyphenated in an electron ionization system 5973 quadrupole MS detector (Agilent, Santa Clara, CA, USA) operating at 70 eV, with a detector temperature of 250 °C, scan time of 0.1 scans/s, acquisition mass range of *m/z* 35 - 500 and using helium as carrier gas (1 mL/min). For the chromatographic run, the injector temperature was set at 250 °C and the oven temperature was programmed to run from 40 °C (1 min) to 240 °C at 3 °C/min. The essential oil components were identified by comparing their retention indices (RI), calculated in relation to a series of *n*-alkanes (C_6-C_{40}) and by comparison of their mass spectra with those reported in the literature (Adams, 2007; NIST).

d) Biological Assays

i. DPPH Radical Scavenging Assay

The antioxidant assay by the DPPH method was performed as described by Machado et al. (2017) with some modifications. Essential oils were tested at final concentrations ranging 33.75-10,000 µg/mL in methanol. Briefly, in a 96-well microplate was added 160 µL of DPPH methanol solution (0.08 mg/mL) and 40 µL of sample solution of different concentrations. Methanol was used as blank solution and the control consisted of 160 µL of DPPH solution plus 40 µL of methanol. After 30 min of incubation in the dark at room temperature, the decrease in the absorbance was measured at 517 nm using a multi-well scanning spectrophotometer (Synergy HT Biotek, Winooski, VT, USA). The radical scavenging activity was calculated using the equation [(Abs control – Abs sample)/(Abs control – Abs blank)] x 100. The IC_{50} value was calculated by non-linear regression (GraphPad Prism 5.01). The experiment included triplicates for each concentration. Quercetin was used as a positive control. The results of the antioxidant activity were presented as mean±SD.

ii. Cytotoxicity Assay

The cytotoxic activity of the essential oils was measured by reduction of soluble MTT to water-insoluble formazan, as described by Costa-Lotufo et al. (2010). Prior the assay, cells of colon (HCT-116) and breast (MCF-7) tumor lines were seeded into a 96-well microplate at a density of 5 x 10^4 cells/mL per well, separately, and expected to grow for 24h at 37°C under 5% CO_2 atmosphere. The cells were then treated with a final concentration of 50 and 5 µg/mL of each essential oil for 72 h. DMSO was used as vehicle control and diluent of the essential oils. Following the incubation, 150 µL of MTT (5 mg/mL) were added to each well and the cells were incubated for additional period of 3h at 37°C. Differences in the cell viability were measured at 595 nm by using a microplate reader (Multiskan FC,

Fisher Scientific, USA). The inhibition (%) of the cell proliferation was determined using the equation [(1 – Abs sample cells)/(Abs control cells)] x 100. The IC_{50} value was calculated by non-linear regression (GraphPad Prism 5.01). The experiment included triplicate for each concentration and two independent assays.

III. Results and Discussion

a) Chemical Evaluation

i. Essential oil characterization

The essential oil yields (% w/w) for the target species varied from 0.003 to 2.790 % (w/w) (Table 2). The highest yields were obtained for the leaves (2.790 %) and stems (1.870 %) of *O. odorifera* and the lowest for the stems of *P. venosa* (0.003%).

Table 2: Essential oil yields for leaves and stems of *Ocotea odorifera*, *O. indecora* and *Persea venosa.*

Plant species	Part used	Yield* (w/w %)
Ocotea odorifera	Leaf	2.790
	Stem	1.870
Ocotea indecora	Leaf	0.090
	Stem	0.200
Persea venosa	Leaf	0.280
	Stem	0.003

* On a dry weight basis.

The GC/MS analyses of the leaf and stem essential oils from the three species allowed the identification of 82 compounds, accounting for 91.0-100% of the total components (Table 3). Number of components in the oils ranged from 6 in *O. odorifera* stems to 42 in *O. indecora* stems.

The key chemical characteristic for leaf and stem essential oils from *O. odorifera* was the high amounts of safrole, a phenylpropanoid, reaching 57.1 and 88.5%, respectively. In addition to phenylpropanoids, the leaf oil contained still oxygenated sesquiterpenoids (22.7%), sesquiterpene hydrocarbons (3.1%), oxygenated monoterpenoids (1.6%) and monoterpene hydrocarbons (1.6%), where the oxygenated sesquiterpene spathulenol (13.8%) was the second major constituent.

The stem oil from *O. odorifera* did not present mono- and sesquiterpene hydrocarbons, but their oxygenated counterparts were found in lower amounts. The most abundant compounds from those classes were spathulenol (4.2%) and 1, 8-cineole (4.0%).

Previous investigation of the leaf oil from *O. odorifera* revealed safrole contents between 36.3 – 42% (Cansian et al, 2010; Mossi et al., 2014; Alcoba et al., 2018). The marked differences in the % of safrole could be attributed to many factors as growing stage or extrinsic factors (Sari et al., 2006).

The oils from *O. indecora* did not contain phenylpropanoids, as safrole or other eugenol derivatives, showing that the biosynthetic pathways in this species mainly favored the formation of terpenoids. In the leaf essential oil from *O. indecora* were identified twenty-five compounds, constituting 93.8% of the sample. Monoterpenoids had a clear predominance in the volatile profile, presenting eighteen compounds that contribute with 53.8% (monoterpene hydrocarbons) and 13.2% (oxygenated monoterpenoids) of the oil, from which α-pinene (12.8%), β-pinene (12.4%) and sabinene (11.02%) were the major components. The third most important class in this oil was oxygenated sesquiterpenes, with spathulenol (9.06%) as the most important.

Compared to leaf oil, the *O. Indecora* stem oil high amounts of oxygenated sesquiterpenoids (41.8%), followed by sesquiterpene hydrocarbons (30.9%), with β-bisabolol (12.2%), α-cuprenene (5.2%) and α-eudesmol (4.0%) as the main constituents.

Unlike our results, a recent study on the chemical composition of the leaf essential oil from *O. indecora* showed that the main component was the sesquiterpene hydrocarbon bicyclogermacrene (29.8%) (Gonçalves et al., 2018), a compound that was not found in our specimen. In the oil of other *Ocotea* species from Costa Rica, the presence of bicyclogermacrene and other germacrene derivatives were also detected, among them germacrene D, considered apparently common to the *Ocotea* species from Costa Rica (Takaku et al., 2007). Still, nine compounds could be apparently common in the leaf oil from *Ocotea spp.* (α-pinene, β-pinene, β-elemene, β-caryophyllene, α-humulene, germacrene D, γ-cadinene, δ-cadinene and α-cadinene). In our case, only two of these compounds (α-pinene and β-pinene) were found in the leaf oil of *O. indecora*.

The essential oil of some *Persea* species have been chemically investigated (Bergh et al., 1973; Scora and Scora, 2000), however, this is the first study conducted with the essential oil from *P. venosa*. The present analysis resulted in the identification of twenty-two and nineteen compounds from the leaf and stem oils, respectively, representing of them 98.8% and 91.0% of the total constituents. Leaf and stem oils of *P. venosa* showed qualitative similarity in oxygenated sesquiterpenes (leaf, 57.6% and stem, 45.6%) content, that were also the major compound class. The major compounds for both leaf and stem oils were spathulenol (27.8 and 14.7%), humulene epoxide II (11.3 and 5.1%) and caryophyllene oxide (7.6 and 4.8%), respectively. Besides these compounds, the leaf oil still contained sesquiterpene hydrocarbons (26.4%) and oxygenated monoterpenoids (10.3%) and does not contain phenylpropanoids. For the stem oil, besides

phenylpropanoids, no monoterpenoids were found, however this oil presented a high amount of a fatty acid ester, methyl octadecanoate (23.7%).

Leaf essential oils from *Persea spp*. can vary a lot, some species presented a higher concentration of monoterpene hydrocarbons, mostly α- and β-pinene, and sesquiterpene hydrocarbons, represented by β-caryophyllene, while in other taxa phenylpropanoids, such as estragole (methyl chavicol) and (E)-anethole, were the main components (Bergh et al., 1973; Scora and Scora, 2000).

Table 3: Chemical composition of the essential oils from three Lauraceae species collected in Atlantic Rainforest areas.

Compounds	RI lit[a]	RI[b]	%					
			1	2	3	4	5	6
4-hydroxy-4-methyl-2-pentanone	831	832	1.6	1.8	1.2	-	-	-
α-thujene	924	923	-	-	1.2	-	-	-
α-pinene	932	929	0.9	-	12.8	1.9	-	-
camphene	946	945	-	-	0.6	-	-	-
sabinene	962	971	-	-	11.0	-	-	-
β-pinene	974	972	0.6	-	12.4	0.7	1.3	-
myrcene	988	988	-	-	5.4	-	-	-
α-phellandrene	1002	1003	-	-	1.0	-	-	-
δ-3-carene	1008	1005	-	-	-	3.4	-	-
α-terpinene	1014	1015	-	-	1.3	-	-	-
p-cymene	1020	1016	-	-	-	1.1	-	-
o-cymene	1022	1021	-	-	3.3	1.8	1.6	-
limonene	1024	1026	-	-	2.2	-	-	-
1,8-cineole	1026	1028	1.1	4.0	3.2	1.7	-	-
γ-terpinene	1054	1054	-	-	2.0	-	1.6	-
cis-sabinene hydrate	1065	1066	-	-	0.9	-	-	-
terpinolene	1086	1081	-	-	0.5	-	-	-
linalool	1095	1097	-	0.9	2.6	2.2	3.8	-
terpinen-4-ol	1174	1176	-	-	5.5	1.6	4.9	-
α-terpineol	1186	1190	0.5	-	0.9	0.7	-	-
(Z)-safrole	1285	1292	57.1	88.5	-	-	-	-
bicycloelemene	1336	1324	-	-	0.6	-	-	-
α-cubebene	1345	1341	-	-	-	0.7	-	-
eugenol	1356	1346	1.5	0.5	-	-	-	-
α-copaene	1374	1371	-	-	-	2.3	5.5	1.5
7-epi-sesquithujene	1390	1383	-	-	-	1.9	-	-
(Z)-caryophyllene	1408	1413	-	-	-	0.9	1.9	-
α-cis-bergamotene	1411	1428	-	-	-	3.2	-	-
β-funebrene	1413	1435	-	-	-	1.4	-	-
α-humulene	1452	1448	-	-	-	-	1.6	-
β-trans-farnesene	1454	1448	-	-	-	3.1	-	-
α-acoradiene	1464	1473	-	-	-	3.4	-	-
dauca-5,8-diene	1471	1474	-	-	0.6	-	-	-
ar-curcumene	1479	1476	-	-	-	1.9	-	-
amorpha-4,7(11)-diene	1479	1478	-	-	-	0.7	-	-
γ-himachalene	1481	1483	-	-	-	-	4.7	1.7
γ-curcumene	1481	1488	1.7	-	-	-	-	-
aristolochene	1487	1488	-	-	-	0.7	-	-
β-selinene	1489	1489	-	-	-	-	2.0	-
N.I.: M^+ 121 (100%), 73 (93%), 107 (52%), 91 (40%)		1489	-	-	3.5	-	-	-
2-tridecanone	1495	1491	-	-	-	1.6	-	-
γ-patchoulene	1502	1497	0.6	-	-	-	-	-
α-cuprenene	1505	1505	-	-	-	5.2	-	-
δ-amorphene	1511	1511	-	-	-	1.4	1.8	2.0
trans-calamenene	1521	1515	-	-	-	1.6	-	-
α-dehydro-ar-himachalene	1516	1534	-	-	-	-	1.8	1.7
(E)-iso-γ-bisabolene	1528	1538	-	-	-	2.4	-	-
hedycaryol	1546	1542	-	-	1.7	1.8	-	-

N.I.: M^+ 79 (100%), 91 (85%), 106 (84%), 93 (78%)		1544	-	-	-	-	1.2	-
N.I.: M^+ 138 (100%), 96 (78%), 109 (73%), 95 (66%)	-	1548	-	-	-	0.6	-	-
β-vetivenene	1554	1554	0.8	-	-	-	-	-
(E)-nerolidol	1561	1561	-	-	-	-	1.3	-
spathulenol	1577	1569	13.8	4.2	9.1	1.9	27.8	14.7
trans-sesquisabinene hydrate	1577	1574	-	-	-	3.3	-	-
N.I.: M^+ 105 (100%), 43 (95%), 91 (91%), 93 (88%)		1577	-	-	-	0.6	-	-
N.I.1: M^+ 91 (100%), 43 (97%), 105, 159 (82%)		1578	-	-	1.6	-	-	-
caryophyllene oxide	1582	1580	-	-	-	-	7.6	4.8
globulol	1590	1596	3.8	-	-	1.6	-	-
viridiflorol	1592	1596	-	-	-	0.9	-	2.6
humulene epoxide II	1608	1601	-	-	-	1.9	11.3	5.1
β-atlantol	1608	1606	0.9	-	-	-	-	-
N.I.: M^+ 93 (100%), 91 (94%), 69 (91%), 119 (82%)		1608	-	-	-	1.0	-	-
tetradecanal	1611	1609	-	-	-	-	-	3.5
isolongifolan-7-α-ol	1618	1613	0.5	-	-	-	-	-
epi-cedrol	1618	1616	-	-	-	-	-	2.1
1,10-di-epi-cubenol	1618	1619	-	-	-	0.9	-	2.9
N.I.: M^+ 119 (100%), 91 (73%), 105 (72%), 161 (70%)		1620	0.7	-	-	-	-	-
N.I.: M^+ 161 (100%), 59 (59%), 119 (57%), 93 (51%)		1622	-	-	-	2.1	-	-
isospathulenol[c]	1627	1625	1.4	-	0.8	-	2.2	3.7
γ-eudesmol	1630	1625	-	-	-	1.5	-	-
muurola-4,10(14)-dien-1-β-ol	1630	1627	-	-	-	-	2.9	-
α-acorenol	1632	1630	-	-	-	3.8	-	-
camphoric acid	1634	1631	-	-	-	-	1.5	-
cis-cadin-4-en-7-ol	1635	1635	-	-	-	0.7	-	-
N.I.: M^+ 159 (100%),105 (79%), 91, 131		1635	0.5	-	-	-	-	-
α-epi-muurolol	1640	1636	1.1	-	1.1	0.6	-	-
hinesol	1640	1639	-	-	-	1.2	-	-
selina-3,11-dien-6- α-ol	1642	1639	0.6	-	-	-	3.5	1.8
α-eudesmol	1652	1647	-	-	-	4.0	-	-
α-cadinol	1652	1648	-	-	-	-	-	4.9
N.I.: M^+ 79 (100%),43 (75%), 80 (64%), 67 (50%)		1653	-	-	-	1.9	-	-
selin-11-en-4-α-ol	1658	1655	0.6	-	-	-	-	-
N.I.: M^+ 95 (100%),69 (97%), 109 (75%), 93 (67%)		1659	-	-	1.1	-	-	-
(E)-10,11-dihydroaltantone	1668	1660	-	-	-	3.8	-	-
β-bisabolol	1674	1666	-	-	-	12.2	-	-
cadalene	1675	1666	-	-	-	-	7.1	3.5
mustakone	1676	1668	-	-	-	-	-	2.9
α-bisabolol	1685	1681	-	-	-	1.5	-	-
N.I.: M^+ 58 (100%),43 (88%), 159 (55%), 71 (47%)		1692	-	-	-	-	-	1.6
2-α-hydroxy-amorpha-4,7(11)-diene	1775	1813	-	-	-	-	1.1	-
N.I.: M^+ 93 (100%),43 (48%), 121 (45%), 80 (31%)		1829	-	-	-	0.9	-	-
N.I.: M^+ 73 (100%),60 (75%), 43 (72%), 41 (67%)		1859	-	-	-	-	-	1.7
phthalic acid, isobutyl octyl ester*	-	2045	-	-	-	-	-	1.7
N.I.: M^+ 73 (100%),60 (78%), 43 (69%), 57 (60%)		2057	-	-	-	-	-	2.5
methyl octadecanoate	2124	2174	-	-	-	-	-	23.7
tricosane	2300	2307	-	-	-	-	-	1.9
N.I.1: M^+ 67 (100%),81 (93%), 55 (92%), 82 (86%)		2323	-	-	-	-	-	1.4
octacosane	2800	2732	-	-	-	-	-	5.8
nonacosane	2900	2936	-	-	-	3.4	-	-
triacontane	3000	2975	9.6	-	11.6	-	-	-
Total identified (%)			*98.8*	*100.0*	*93.8*	*92.8*	*98.8*	*91.1*
Monoterpene hydrocarbons			1.6	-	53.8	8.9	4.5	-
Oxygenated monoterpenes			1.6	5.0	13.2	6.2	10.3	-
Sesquiterpene hydrocarbons			3.1	-	1.3	30.9	26.4	10.4
Oxygenated sesquiterpenes			22.7	4.2	12.7	41.8	57.6	45.6
Phenylpropanoids			58.6	89.1	-	-	-	-
Fatty-acid-derived compounds			-	-	-	1.6	-	27.3

Hydrocarbons			9.6	-	11.6	3.4	-	7.8
Other compounds			1.6	1.8	1.2	-	-	-

1. Ocotea odorifera (leaf); 2. O. odorifera (stem); 3. O. indecora (leaf); 4. O. indecora (stem); 5. Persea venosa (leaf); 6. P. venosa (stem).
*solvent artefact.
RI = Retention indices on DB-5 column: [a]RI literature (Adams, 2007; NIST); [b] Calculated RI.
N.I.: not identified.

b) Biological Assays

i. *DPPH Radical Scavenging Assay*

The free radical scavenging activity for DPPH radical expressed as IC_{50} ranged from 0.142 to 10 mg/mL is shown in the Table 4.The leaf (0.142 mg/mL) and stem (0.180 mg/mL) oils from *O. indecora* were the most active as compared to the other plant essential oilhere studied.

In previous studies evaluating the antioxidant activity of essential oils, using DPPH assay, it was observed that more expressive activities may be related to the presence of compounds containing phenolic groups (Miguel, 2010). Among the studied oils, *O. odorifera* (leaf and stem) presented eugenol (1.5 and 0.5%, respectively), as the only representative phenolic compound and the phenylpropanoid safrole (57.1 and 88.5%, respectively), that can also form stable radicals. However, the most active oil was *O. indecora* leaf oil, which was composed mostly by monoterpene hydrocarbons.

Table 4: Antioxidant activities of the essential oils from Lauraceae species collected in Atlantic Rainforest areas

Species	Part used	IC_{50} (mg/mL) (M±SD)
Ocotea odorifera	Leaf	0.730±0.048
	Stem	1.670±0.110
Ocotea indecora	Leaf	0.142±0.002
	Stem	0.180±0.003
Persea venosa	Leaf	>10
	Stem	N.D.
Quercetin		0.010±0.009

M: average; SD: standard deviation; N.D.: not determined

ii. *Cytotoxicity Assay*

The essential oils' cytotoxic activity was assayed at two concentrations, where their respective growth inhibition percentages against two human tumor lines HCT-116 (colon) and MCF-7 (breast).The results obtained can be seen in Table 5. All essential oils showed activity, varying for HCT-116 from 42.4% (*O. indecora* stem oil) to 100% (*O. indecora* leaf oil) and for MCF-7 from 42.6% (*O. indecora*stem oil) to 99.2% (*O. indecora* leaf oil).Among the tested oils, *O. indecora* stem at the lowest concentration (5 μg/mL) showed low cytotoxic activity against the two cells tested.

Comparing the results between the cytotoxic and antioxidant activities, the most active essential oil (*O. indecora* leaf oil) against the two cell lines also presented the highest DPPH free radical scavenging capacity. Many studies have reported different biological activities for essential oils and their isolated compounds, including cytotoxic activity against tumor cell lines. This activity might be associated with their antioxidant capacity. (Bayala et al., 2014).

For the *O. indecora* leaf oil, the observed cytotoxicity might be due to the presence of monoterpenes such as α-pinene and β-pinene, known for their synergistic association, regarding the cytotoxic effect (Zhang et al., 2015). Some other monoterpenes such as limonene, also present in small concentration in this oil, is pointed as capable to prevent the formation or progression of cancer cells, and it can also cause regression of existing malignant tumors (Crowell, 1999).

Table 5: Cytotoxic activity of essential oil from Lauraceae on human cell lines HCT-116 (colon adenocarcinoma), MCF-7 (breast cancer).

Species		Cell lines			
		HCT-116 (% mortality) (M±SD)		MCF-7 (% mortality) (M±SD)	
		At 50 μg/mL	*At 5 μg/mL*	*At 50 μg/mL*	*At 5 μg/mL*
Ocotea odorifera	Leaf	86.3±6.5	83.6±3.3	93.4±3.6	95.9±2.3
	Stem	95.7±1.9	88.1±1.0	86.1±1.4	89.8±1.3
Ocotea indecora	*Leaf*	*100*	*100*	*97.4±0.3*	*99.2±0.1*
	Stem	89.9±0.4	42.4±0.8	96.6±0.8	42.6±7.3
Persea venosa	Leaf	72.9±1.5	83.4±10.8	98.7±0.1	98.62±0.2
	Stem	86.5±1.1	95.3±0.4	63.2±36.2	98.1±0.1
Doxorubicin (IC_{50}, μg/mL) (C^+)		0.02 (0.02-0.03)		0.16 (0.09-0.29)	

HCT-166: colon tumor line; MCF-7: breast tumor line; M: average; SD: standard deviation; C^+: confidence interval

IV. Conclusions

Essential oils and their components often exhibit interesting bioactivities useful in the fields of cosmetics, food and pharmaceuticals. For the species evaluated in this work, the leaf and stem essential oils from *Persea venosa* and stems of *O. indecora* were evaluated for the first time, opening the field for further studies of biological activity. In addition, *O. indecora* oil was promising for antioxidant and cytotoxic activities. Further evaluation studies of other biological activities and further cytotoxicity studies should be performed to determine the active compounds of this oil and their mechanisms of action.

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Pharmacovigilance Programme of India: A Review

By Saurabh Nimesh, Surabhi Gupta & Kapil Dev Negi

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Abstract- In India, a proper adverse drug reaction monitoring system was started in 1986 with 12 regional centers. In 1997, India became the member of the World health organization Programme for International Drug watching managed by the Upsala Monitoring Centre, Sweden. At origination, 6 regional centers were created in Mumbai, New Delhi, Kolkata, Lucknow, Pondicherry, and Chandigarh for ADR watching within the country. Promoting safe use of drugs may be a priority of the Indian Pharmacopoeia Commission that functions as the National Coordination Centre for Pharmacovigilance Programme of India. Today, 179 adverse drug reactions monitoring centers presently report adverse events to the National coordinative centre in India.

Keywords: *vigiflow, UMC, death, thalidomide, reporting form, phocomelia.*

GJMR-B Classification: *NLMC Code: QV 20.5*

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Pharmacovigilance Programme of India: A Review

Saurabh Nimesh [α], Surabhi Gupta [σ] & Kapil Dev Negi [ρ]

Abstract- In India, a proper adverse drug reaction monitoring system was started in 1986 with 12 regional centers. In 1997, India became the member of the World health organization Programme for International Drug watching managed by the Upsala Monitoring Centre, Sweden. At origination, 6 regional centers were created in Mumbai, New Delhi, Kolkata, Lucknow, Pondicherry, and Chandigarh for ADR watching within the country. Promoting safe use of drugs may be a priority of the Indian Pharmacopoeia Commission that functions as the National Coordination Centre for Pharmacovigilance Programme of India. Today, 179 adverse drug reactions monitoring centers presently report adverse events to the National coordinative centre in India.

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I. Introduction

According to World Health Organisation (WHO), Pharmacovigilance (PV) as the pharmacological science and activities relating to the monitoring, detection, assessment, understanding, and prevention of adverse drug reactions (ADRs), or any long-term and short-term medicine-related problems (Figure 1&2). Variety of ADRs associated with medication prompted the event of the science of PV [1-4]. This prompted WHO for a systematic study of ADR of medicine, that is that the starting of PV. Thenceforth variety of ADRs was detected, a number of that square measure shows in (Table 1). ADR is taken into account to be the 6th leading reason behind death. India, with a current population of 1.27 billion, is that the 4th largest producers of prescription drugs within the world with quite 6000 licensed makers and over 60000 branded formulations within the market. In the United States of America, ADRs contribute 3-7% of hospital admissions. In England, 1% chronicles of the entire hospital admissions were due to ADRs throughout the year 1999-2008. ADRs square measure common in the Australian healthcare system additionally and that they contribute to a 1% of hospital admissions [5,6]. The percentage of hospital admissions due to ADRs in bound countries is 100% or additional. Drug attributed deaths square measure calculable to be 0.19% altogether medical inpatients. About 0.40% of ADRs known were directly joined to high costs. ADRs not solely increase the mortality and morbidity; however, additionally multiply the health care value [7]. The PV effort within India is coordinated bythe Indian Pharmacopoeia Commission (IPC) and conducted by the Central Drugs Standard Control Organization (CDSCO). The most responsibility of the IPC is to keep up and develop the PV database consisting of all suspected ADR to medicines observed. IPC is functioning as a National Coordination Centre (NCC) for the Pharmacovigilance Programme of India (PvPI). NCC is working underneath the direction of a committee that recommends procedures and guidelines for regulatory interventions [8].The main responsibility of NCC is to watch all the ADR of medicines being observed within the Indian population and to develop and maintain its PV information. The aim of the commission that acts just like the NCC for PvPI is for the safety of the patient, and the population with relevancy use of the drug. The Commission has become operational from 1st January 2009 an associate autonomous body, absolutely supported by the central government with specific fund allocations under the administrative control of the Ministry of Health and Family Welfare [9]. The Secretary, Ministry of Health and Family Welfare, is the Chairperson and therefore the Chairman-Scientific Body is that the Co-Chairman of the Commission. The Secretary-cum Scientific Director is that the Chief Scientific and Executive officer of the Commission. The CDSCO, Directorate General of Health Services underneath the aegis of Ministry of Health & Family Welfare, Government of India unitedly with IPC, Ghaziabad is initiating a nation-wide PV program for shielding the health of the patients by reassuring drug safety. The program shall be coordinated by the IPC, as an NCC. The center can operate underneath the superintendence of a steering committee. The PvPI was initiated by the government of India on 14th July 2010 with the All India Institute of Medical Sciences (AIIMS), New Delhi as the NCC for monitoring ADRs in the country for safe-guarding public health. Within the year 2010, 22 ADR monitoring center, as well as AIIMS, came upon underneath this program [10-13]. To confirm the implementation of this program in an exceedingly method, the NCC was shifted from the AIIMS to the IPC, Ghaziabad, Uttar Pradesh on 15th April 2011 (Figure 3).

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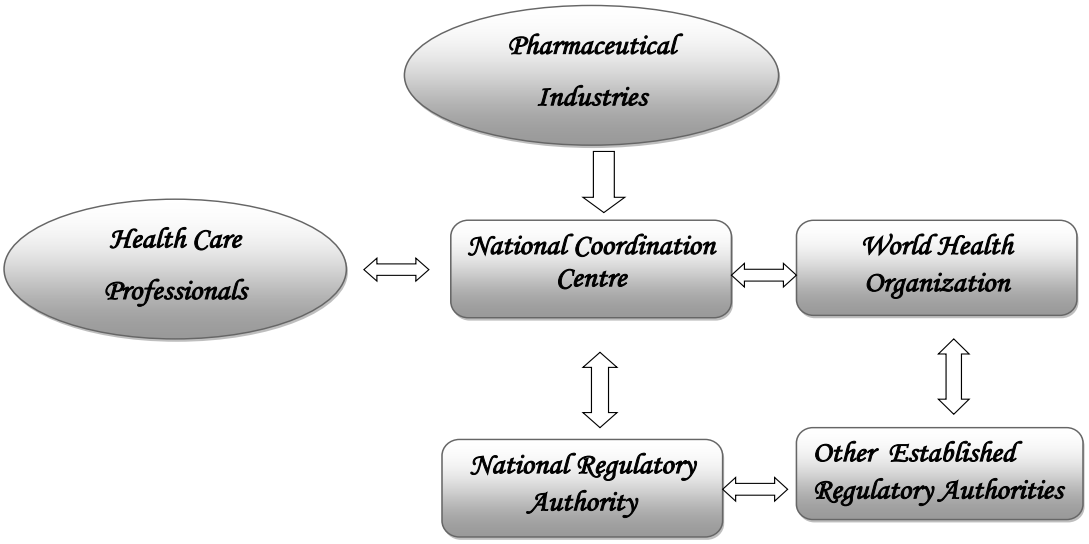

Figure 1: Diagrammatic representation of the Pharmacovigilance

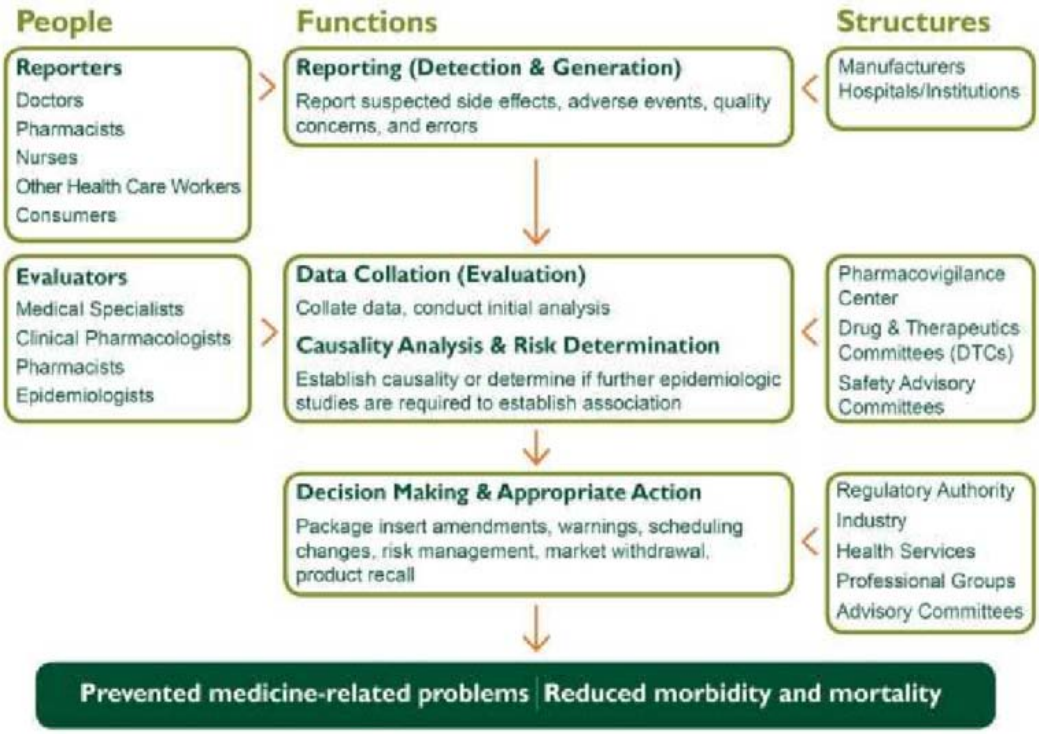

Figure 2: Pharmacovigilance framework

Table 1: Nine examples of serious & unexpected ADR cause to drugs [14]

Sr. No.	Drug	Year	Serious & unexpected adverse event
1	Chloroform (Anaesthetic)	1848	Episode of ventricular fibrillation & death
2	Sulphanilamide (Elixir)	1937	Death
3	Thalidomide	1961	Amelia, phocomelia &dysmelia
4	Clioquinol	1970	Subacute nephropathy
5	Practolol	1975	Sclerosing peritonitis
6	Benoxaprofen	1982	Nephrotoxicity&cholestatic jaundice
7	Terfenadine	1997	Torsade de pointes
8	Rofecoxib	2004	Cardiovascular effects
9	Veralipride	2007	Anxiety, depression & movement disorders

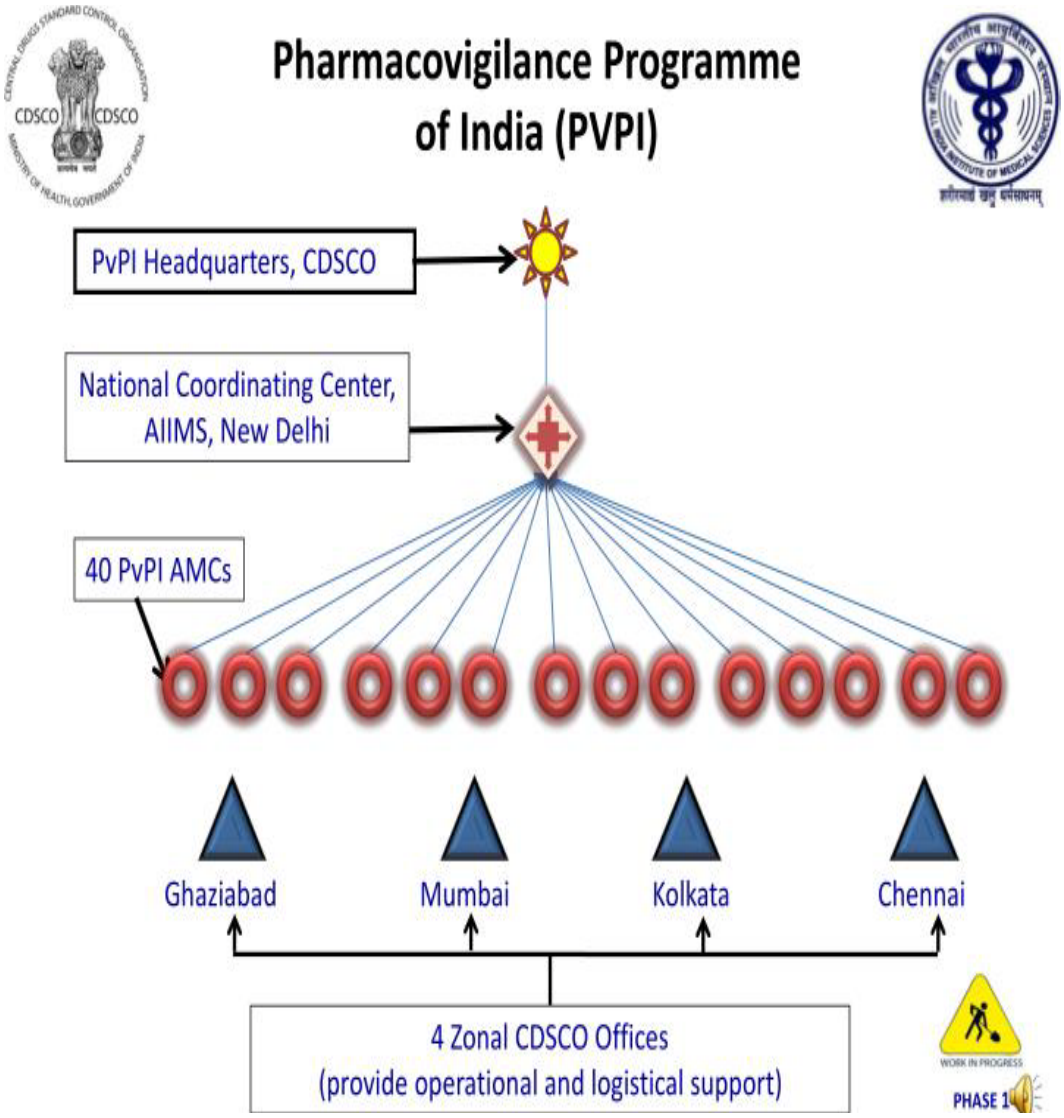

Figure 3: Pharmacovigilance Programme of India

II. History of the Pharmacovigilance Programme in India

The concept of PV is not new, because the time of Charak Samhita in 700 BC had cautioned that properly understood however improperly administered drug is Vagueness poison and Vagbhatta- a physician represented adverse events, reason, delayed ADRs to Ayurvedic Drugs' around 500 AD. After that, many reports of ADRs from India area unit found within the history of modern medicine, but there was no systematic effort of ADR monitoring since the primary try was created in 1989 [15,16].

III. Scope of the Pharmacovigilance Programme of India

Before registration and selling of drugs within the country, its safety and efficaciousness expertise area unit primarily based totally on the employment of the drugs in clinical trials. These trials in the notice common ADR. Some vital reactions, like those, that take a protracted time to develop, or those, that occur seldom, might not be detected in clinical trials. Additionally, the controlled conditions beneath that medicines area unit utilized in clinical trials don't essentially replicate the method they will be utilized in observe. For a drug to be thought-about safe, its expected advantages ought to be more than any associated risks of harmful reactions. So, to achieve a comprehensive safety profile of drugs, a continuous post-marketing monitoring system, i.e. PV is crucial. To monitor the security of drugs, information from several sources is employed for PV [17]. These embrace spontaneous ADRs coverage mechanism; medical literature published worldwide; action taken by regulative authorities in alternative countries. Since there exist substantial social and economic consequences of ADRs and therefore the positive benefit/cost magnitude relation of implementing applicable risk management -there may be a have to be

compelled to interact health care professionals and therefore the public at massive, during a well-structured program to make synergies for watching ADRs within the country. The PvPI aims is to collate data, method and analyze it and use the inferences to advocate regulative interventions, besides human action risks to health care professionals and therefore, the public [18].

IV. Management of the Pharmacovigilance Programme of India

This is headed by the Secretary cum scientific Director: Dr. Gyanendra Nath Singh, who is working with the help of Advisor and National Scientific Coordinator supported by the several committees like- Steering Committee, Working Group, Quality Review Panel, Core Training Panel, etc. involving experts from all over the country.Current Status of NCC-PvPI Presently the PvPI program has more than 200 Adverse Drug Monitoring Centres (AMCs) involving all states and Union Territories through-out India[19].

V. Reporting of Adverse Drug Reactions

Suspected ADR reporting forms for health care professionals (Figure 4) and consumers (Figure 5) a unit available on the website of IPC to report ADR. To get rid of barrier in ADR reporting, the consumer reporting form is available in 10 vernacular languages (Hindi, Tamil, Telugu, Kannada, Bengali, Gujarati, Assamese, Marathi, Oriya, and Malayalam). ADRs will be conjointly reportable via PvPI helpline number (18001803024) on week days from 9:00 am to 5:30 pm. The mobile Android application for ADR reporting has conjointly been created available to the general public [20].

Version-1.2

SUSPECTED ADVERSE DRUG REACTION REPORTING FORM

For VOLUNTARY reporting of Adverse Drug Reactions by Healthcare Professionals

INDIAN PHARMACOPOEIA COMMISSION
(National Coordination Centre-Pharmacovigilance Programme of India)
Ministry of Health & Family Welfare, Government of India
Sector-23, Raj Nagar, Ghaziabad-201002

FOR AMC/NCC USE ONLY

AMC Report No. :

Report Type ☐ Initial ☐ Follow up

Worldwide Unique No. :

A. PATIENT INFORMATION

1. Patient Initials _____

2. Age at time of Event or Date of Birth _____

3. M ☐ F ☐ Other ☐

4. Weight_____Kgs

12. Relevant tests/ laboratory data with dates

B. SUSPECTED ADVERSE REACTION

5. Date of reaction started (dd/mm/yyyy)

6. Date of recovery (dd/mm/yyyy)

7. Describe reaction or problem

13. Relevant medical/ medication history (e.g. allergies, race, pregnancy, smoking, alcohol use, hepatic/renal dysfunction etc.)

14. Seriousness of the reaction: No ☐ if Yes ☐ (please tick anyone)

☐ Death (dd/mm/yyyy) ☐ Congenital-anomaly
☐ Life threatening ☐ Required intervention to Prevent permanent impairment/damage
☐ Hospitalization/Prolonged
☐ Disability ☐ Other (specify)

15. Outcomes

☐ Recovered ☐ Recovering ☐ Not recovered
☐ Fatal ☐ Recovered with sequelae ☐ Unknown

C. SUSPECTED MEDICATION(S)

S.No	8. Name (Brand/Generic)	Manufacturer (if known)	Batch No. / Lot No.	Exp. Date (if known)	Dose used	Route used	Frequency (OD, BD etc.)	Therapy dates		Indication	Causality Assessment
								Date started	Date stopped		
i											
ii											
iii											
iv											

S.No as per C	9. Action Taken (please tick)						10. Reaction reappeared after reintroduction (please tick)			
	Drug withdrawn	Dose increased	Dose reduced	Dose not changed	Not applicable	Unknown	Yes	No	Effect unknown	Dose (if reintroduced)
i										
ii										
iii										
iv										

11. Concomitant medical product including self-medication and herbal remedies with therapy dates (Exclude those used to treat reaction)

S.No	Name (Brand/Generic)	Dose used	Route used	Frequency (OD, BD, etc.)	Therapy dates		Indication
					Date started	Date stopped	
i							
ii							
iii							

Additional Information:

D. REPORTER DETAILS

16. Name and Professional Address:_____
_____
Pin:_____E-mail_____
Tel. No. (with STD code)_____
Occupation:_____ Signature:_____

17. Date of this report (dd/mm/yyyy):

Confidentiality: The patient's identity is held in strict confidence and protected to the fullest extent. Programme staff is not expected to and will not disclose the reporter's identity in response to a request from the public. Submission of a report does not constitute an admission that medical personnel or manufacturer or the product caused or contributed to the reaction.

Figure 4: Suspected ADR reporting form for Healthcare professionals

Version 1.0

MEDICINES SIDE EFFECT REPORTING FORM (FOR CONSUMERS)

Indian Pharmacopoeia Commission, National Coordination Centre- Pharmacovigilance Programme of India,
Ministry of Health & Family Welfare, Government of India.

1.Patient Details

Patient Initials: ☐☐	Gender (√): Male ☐ Female ☐ Other ☐	Age (Year or Month) :

2. Health Information

a. Reason(s) for taking medicine(s)(Disease/Symptoms):

b. Medicines Advised by (√): Doctor ☐ Pharmacist ☐ Friends/Relatives ☐ Self (Past disease experienced/No past disease experienced) ☐

3. Details of Person Reporting the Side Effect

Name (Optional):

Address:

Telephone No:	Email:

4. Details of Medicine Taking/Taken

Name of Medicines	Quantity of Medicines taken (e.g. 250 mg, Two times a day)	Expiry Date of Medicines	Date of Start of Medicines	Date of Stop of Medicines
			dd/mm/yy	dd/mm/yy
			dd/mm/yy	dd/mm/yy
			dd/mm/yy	dd/mm/yy

Dosage form (√) : Tablet ☐ Capsule ☐ Injection ☐ Oral Liquids ☐ If Others (Please Specify.....)

5. About the Side Effect

When did the side effect start? dd/mm/yy Side Effect is still Continuing (Yes/No): ☐

When did the side effect stop? dd/mm/yy

6.How bad was the Side Effect? (Please √ the boxes that Apply)

☐ Did not affect daily activities	☐ Affect daily activities
☐ Admitted to hospital	☐ Death
☐ Others	

7.Describe the Side Effect (What did you do to manage the side effect?)

This reporting is voluntary, has no legal implication and aims to improve patient safety. Your active participation is valuable. The information provided in this form will be forwarded to ADR Monitoring Centre for follow-up. You are requested to cooperate with the programme officials when they contact you for more details. Please do report even if you do not have all the information.

Figure 5: ADRs reporting form for consumers

VI. World Health Organization-Uppsala Monitoring Centre & India

The WHO Program for International Drug Monitoring provides a forum for WHO member states that has India to collaborate within the monitoring of drug safety. At intervals the Program, individual case reports of suspected ADRs are collected and keep in an exceedingly common information, presently containing over 3.7 million case reports. Since 1978, the Uppsala Monitoring Centre (UMC) in Sweden has dispensed the Program. The UMC is accountable for the gathering of knowledge concerning ADRs from around the world, particularly from countries that are members of the WHO together with India. Member countries send their reports to the UMC wherever they are processed, evaluated, and entered into the WHO International information. When there are several reports of adverse reactions to a particular drug, this process may lead to the detection of a signal- an alert about a possible hazard communicated to member countries. This happens solely once elaborated analysis and expert review. These ADR reports are assessed regionally and will cause the action at intervals in the country. Through membership of the WHO International Drug Monitoring Program, a rustic will recognize if similar reports are being created elsewhere. India is a country with a large patient pool and healthcare professionals, yet ADR reporting is in its infancy (Table 2) [21-23].

Table 2: Responsibilities & functions of the stakeholders in the program

Centre	Role
ADR monitoring centre	Collection of ADR reports, perform follow up with the complainant to check completeness as per standard operating procedure (SOPs), data entry into Vigiflow, reporting to PvPI-NCC through Vigiflow with the source data (original) attached with each ADR caseTraining/ sensitization/ feedback to physicians through newsletters circulated by the PvPI-NCC.
PvPI AMC other than medical colleges [Corporate hospitals, autonomous institutes, Pharmaceutical industry and public health Programmers]	Collection of ADR reports, perform follow up with the complainant to check, completeness as per SOPs, report the data to CDSCO- Headquarter (HQ).
Pharmacovigilance programme of India, National coordinating centre, Indian pharmacopoeia commission(Ghaziabad)	Preparation of SOPs, guidance documents & training manuals, data collation, Cross-check completeness, Causality Assessment etc as per SOPs, conduct Training workshops of all enrolled centres, publication of medicines safety newsletter, reporting to CDSCO-HQ, Analysis of the Performance measurement system, Periodic safety update report, Adverse event following immunization data received from CDSCO-HQ.
Zonal/Sub-zonal CDSCO Offices	Provide procurement, financial and administrative support to ADR monitoring centres, report to CDSCO-HQ.
Central drugs standard control organization-Headquarter (New Delhi)	Take appropriate regulatory decision & actions on the basis of recommendations of PvPI NCC at IPC, propagation of medicine safety related decisions to stakeholders, collaboration with WHO-UMC, provide for budgetary provisions &administrative support to run PvPI.

VII. Aim of the Pharmacovigilance Programme of India

Pharmacovigilance has specific aims as follows:

1. Improve patient care and safety in about the use of medicines and all medical and paramedical interventions.
2. Improve public health and safety in about the use of medicines.
3. Contribute to the assessment of benefit, harm, effectiveness, and risk of medicines, encouraging their safe, rational and more effective (including cost-effective) use.
4. Promote understanding, education, and clinical training in PV and its effective communication to the public[24].

VIII. Objectives of the Pharmaco-vigilance Programme of India

1. To create a nation-wide system for patient safety reporting.
2. To identify and analyze the new signal ADR from the reported cases.
3. To analyze the benefit-risk ratio of marketed medications.

4. To generate the evidence-based information on the safety of medicines.
5. To support regulatory agencies in the decision-making process on the use of medications.
6. To communicate the safety information on the use of medicines to various stakeholders to minimize the risk.
7. To emerge as a national center of excellence for PV activities.
8. To collaborate with other national centers for the exchange of information and data management.
9. To provide training and consultancy support to other national PV centers located across the globe[25,26].

IX. Conclusion

The adverse drug reaction observation and reporting programs or pharmacovigilance program of India is aiming to identify the risks related to the utilization of the drugs. The current analysis has disclosed opportunities or interventions particularly or avertible adverse events, which arecan to facilitate in promoting safer drug use, data to the health care professionals. Improve the standard of patient care and educate to extend awareness. Therefore, currently, this point has returned to aware the general public too for the reporting the adverse drug reaction to the nearest hospital or ADR monitoring center or the health care professionals. They will directly report the adverse drug reaction through the government. Toll-free number 18001803024, adverse drug reaction application, email, and alternative methodology like social media.

Acknowledgement

None

Conflict of Interest

The Authors declare that there is no conflict of interest.

Abbreviations: WHO: World health organization, CDSCO: Central drugs standard control organization, PvPI: Pharmacovigilance programme of India, NCC: National coordinating centre, AIIMS: All India institute of medical sciences, IPC: Indian pharmacopoeia commission, PV: Pharmacovigilance, ADR: Adverse drug reaction, AMC: ADR monitoring centre, UMC: Uppsala monitoring centre.

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Approaching Treatment for Psychodermatology

By Patricia Karen Paucar Lescano

Abstract- The interaction between the mind and skin diseases has been the focus of study of many researchers around the world; Psychodermatology is the result of the fusion of medical specialties: psychology, psychiatry and dermatology. Dermatologists are aware of the potential for significant improvement of dermatological pathology when addressing the psychological-psychiatric dimension and vice versa, the bidirectional relationship has already been described, we know that it is necessary to break this cycle, but we still have to define the treatment, for which we must know the different therapies.

Keywords: psychosomatic medicine, medicine traditional, complementary therapies, sychopharmacology, psychotropic drugs.

GJMR-B Classification: NLMC Code: WR 1

Strictly as per the compliance and regulations of:

Approaching Treatment for Psychodermatology

Patricia Karen Paucar Lescano

Abstract- **The interaction between the mind and skin diseases has been the focus of study of many researchers around the world; Psychodermatology is the result of the fusion of medical specialties: psychology, psychiatry and dermatology. Dermatologists are aware of the potential for significant improvement of dermatological pathology when addressing the psychological-psychiatric dimension and vice versa, the bi-directional relationship has already been described, we know that it is necessary to break this cycle, but we still have to define the treatment, for which we must know the different therapies.**

Keywords: psychosomatic medicine, medicine traditional, complementary therapies, psychopharmacology, psychotropic drugs.

I. Introduction

Psychodermatology is a sub-specialty of dermatology, where patients present: 1) primary psychiatric condition, which they go to dermatologists; 2) primary dermatological disease with psychological or psychiatric comorbidities; 3) dermatoses that influence the psychological state, maintaining or aggravating it[1]. The relationship of mental pathologies and dermatological diseases: it is bidirectional, being necessary to break this cycle to treat patients[2]. Because it involves the skin, the nervous system and the mind, psychodermatology needs the collaboration and integration of the dermatologist, with the psychologist and the psychiatrist; otherwise, psychodermatosis will not be treated in its complexity[1]. Dermatologists should be able to know several non-pharmacological treatments, initiate basic pharmacotherapy and recognize the correct time to refer patients to the psychiatrist[3]. They also need to approach the patient, which is obtained when considering dermatosis from the perspective of those who experience the disease[1], so the dermatologist, in the consultation must be empathic, meet the patient's expectations and be optimistic, aspects of the encounter clinical conditions that can foster a positive therapeutic relationship[2]. For treatment, you should always start using stress reduction techniques, the main causative agent of diseases[1].

The main objective is to know the different therapies we have to treat psychocutaneous pathologies.

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II. Development of the Topic

Most diseases are multifactorial: Biological, psychological, emotional, social and spiritual factors, add to previous situations, from conception, pregnancy, birth to the presentation of the disease, whose effects accumulate in the body: these circumstances exert a unique role for each person, which will trigger a disease, whose presentation will be particular for each patient[1].

Established as a subspecialty of dermatology, Psychodermatology studies the bidirectional relationship, in which psycho-psychiatric disorders cause skin diseases and skin diseases cause psychiatric disorders[2]. In dermatology, what affects the skin is visible to both people and the same patient, damaging the physical appearance, compromising the patient's image and achieving self-esteem, producing unpleasant physical sensations that unbalance the person, creating discomfort, irritation and impatience; sometimes triggered, various mental states, such as depression, anxiety and distortion of body image[1]. In this scenario, we emphasize the idea that a dermatologist should be prepared to diagnose, provide appropriate psychological support and treat his patients[3]. A good doctor-patient relationship is the key to success[4].

III. Stress

The skin is particularly affected by stress and it is important to take into account the role it plays in the generation, maintenance or aggravation of dermatosis[1]. Stress is defined as the set of physiological responses and adaptations that occur in the body every time a threat is perceived, real or imaginary, affecting physical, mental and emotional balance[5]. Stressful thoughts are varied, because they depend on the interpretation that each person gives to what happens in their mind; many can be imagined or happening, so fantasy and reality produce the same biochemical states and emotions in the body; thoughts, therefore, affect the skin by chemical mediators brought to the skin[1]. The hypothalamic pituitary axis (HPA) responds to psychological stress, with increased stress hormones (releasing corticotropin hormone, adrenocorticotropin, cortisol and prolactin); activating the sympathetic nervous system, which raises the levels of catecholamines and increases neuropeptides and neuromediators, such as, substance P and calcitonin gene structure peptide (CGRP); mastocytic skin cells are an important target of stress hormones and

mediators and their activation leads to immune dysregulation, neurogenic inflammation, proinflammatory response and vasodilation; producing various skin diseases, inflammatory, autoimmune and allergic[4], in addition to aging[6,7].

IV. Psychodermatosis

Psychodermatoses are changes in the skin that: 1. Are caused by psychiatric problems; 2. Cause psychiatric or psychological disorders due to their clinical manifestation; 3. They influence the psychological state and are maintained or aggravated by this[1].

They are divided into four types: [1]

- *Self-inflicted dermatoses:* artificial dermatitis, epidermotilomania, excoriated acne, artificial cheilitis, onychophagy, Gardner-Diamond syndrome.
- *Dermatosis due to illusions and hallucinations:* delusions of parasitosis; olfactory, tactile and body hallucination; hypochondriac illusions; body dysmorphic disorder
- *Somatomorphic disorders:* pruritus, allergies, glossloss, vulvodynia, trichodynia, paraesthesia.
- *Dermatosis by compulsion:* eczema of hands by repeated washing, chronic lichen simplex, trichotillomania, psychogenic excoriations, cutaneous hypochondria, dysmorphic disorder of the body.

The manifestations of group 1 are the mirror of what happens in the patient's mind, it is not possible to effectively attend to patients, without acting on mental disorders[1].

In the dermatoses of the second group, the mind is secondarily affected by skin diseases; they are stigmatizing, anti-aesthetic, diseases that cause intense or prolonged symptoms of stress, irritability, fear, shame, catastrophic prediction, anxiety, depression, anger, self rejection, isolation, discouragement and fatigue[1]. In this group, psoriasis, vitiligo, atopic dermatitis, alopecia areata and hydradenitis are the most common[2]; other dermatoses with less intense effects are acne, dyshidrosis, hyperhidrosis of the hands, feet and armpits, leprosy, herpes simplex, hypertrichosis, lichen planus, perioral dermatitis, rosacea, seborrheic dermatitis, scleroderma, lupus erythematosus, pemphigus, leg ulcers and dermatoses with a devastating effect on the psyche are ichthyosis, epidermolysis, hemangiomas and any other dermatosis interpreted as disastrous or harmful for a particular patient[1].

In the third group, dermatoses that affect the psyche as they affect the clinical picture, cause its maintenance or aggravation and facilitation of the cure or resistance to treatment; many are part of the second group to which allergies and serious diseases such as neoplasms are added[1], some of them when treating psychological imbalance or psychiatric illness and remitting them, are potentially cured, because the origin of the disease is being treated.

V. Interdisciplinary Care

Given the permanent interaction of the mind and the skin, it is necessary that the patient be treated as a unit consisting of several levels, which correspond to cutaneous, emotional and mental aspects; it is necessary to use the resources available by Dermatology, as well as those that are in the domain of other areas that participate in these diseases, how, psychology and psychiatry[2]. First, it is necessary for the dermatologist to acquire skills that go beyond the diagnosis and management of skin diseases; specialist who understands that the patient's emotional complaints are part of the clinical picture and has the ability to explore them and provide some type of support to the affected patients may be more effective in their treatment[1]. In many cases, dermatoses are followed by an inability to control emotions that require a systematic and specialized correction for which the dermatologist would have no preparation or time; thus the dermatologist can obtain the basic preparation to explore and attend to the emotional states of the patient, giving him/her, through amical language and appropriate questions, conditions to see the real dimension of the problem and provide management options[1]. Often, a welcoming attitude of the doctor is the first step, to treat and facilitate the cure of the patient[2]. However, a complete systematic work requires the participation of a psychologist, preferably someone interested in dermatological problems[1]. And in the psychodermatoses of the first group, which involve psychopathology, the participation of a psychiatrist is essential for the precise diagnosis of the underlying disorder and follow-up with specific medications, which require depth of knowledge, daily experience in the control of the underlying pathology and possible undesirable adverse events; despite this, it is necessary for the dermatologist to have basic knowledge of psychopharmacology and psychoactive drug management to attend to simpler cases[8] or those in which the patient takes a while to accept that he or she needs help from the psychiatrist.

VI. Integrated Therapeutic Resources

Considering the participation of emotions and the mind, it is necessary to integrate all the resources that these areas may involve[1]. Basically, the dermatologist will pay attention to the cutaneous condition, seeking to correct the dermal pathology; however, it is important to master the mind-body anti-stress techniques; they are natural attitudes that,

surprisingly, are not taught and therefore are not followed by patients or doctors and can keep stress at a non-harmful level at no cost; these techniques can be applied during the consultation and the doctor must instruct the patients to practice them routinely to maintain their physical and mental balance, the fundamental ones are four: upright posture, change of respiratory pattern, muscle relaxation and meditation; All have proven efficacy to reduce stress and promote body balance[1]. Simple conscious breathing is changed from the thoracic to the abdominal pattern, producing important changes in the organism in the sense of physiological and psychological balance[9]. Remembering or teaching patients to say what we think and feel, properly, helps us to let off steam and relax; The concept of Alexithymia, is characterized by the inability to identify and express their emotions, several studies have reported a high incidence of alexithymia in patients with alopecia areata (58%)[10], psoriasis (35%)[11], chronic urticaria (50%)[12] and vitiligo (35.5%)[13]. It would also be necessary to perform an activity that the patient enjoys, which will contribute to his muscle relaxation; how to dance, paint, exercise, travel, play a musical organ, learn a language, etc: an activity that brings a smile to the patient; we must consider that each person is unique, so the activity you choose will also be special for each patient. Transcendental meditation is a meditation technique, associated with yoga, tantra, Tibetan Buddhism and Zen Buddhism; produces neurochemical, neurophysiological and cognitive behavioral effects in its practitioners, significant and positive; Among the main effects is the decrease in anxiety and stress (due to the decrease in cortisol and norepinephrine levels), increasing the feeling of pleasure and well-being (due to an increase in the synthesis and release of dopamine and serotonin) [14].

Intervention with psychoactive drugs is used in cases with marked mental changes such as personality disorder, bipolar disorder, narcissistic personality disorder, depressive disorders, anxiety disorders, posttraumatic stress disorder, schizophrenia, obsessive compulsive disorder, should be performed by a psychiatrist in consultation with Dermatology because it involves another area of Medicine[15].

Almost all skin diseases are capable, to a lesser or greater degree, of emotionally affecting patients; Today there is the concept that in certain diseases, the involvement may be minimal and we should refer the patient to a psychologist, as if the mind were the exclusive cause of the disease, which, once treated, will lead to a cure; This aspect deserves careful consideration by the specialist because the patient often treats the problem perfectly well without emotionally affecting it; but for some people, the degree of emotional deterioration is so complex that it becomes imperative to refer the patient to psychotherapy[1]. So in some cases, a prudent period must be expected, for the patient to use their own stress management techniques and not to balance, refer to the psychologist or psychiatrist, as appropriate. With regard to psychotherapies, there are several types and each person adapts to one, the most commonly used is cognitive behavioral therapy, which is recognized as effective in many cases[16]. Other techniques include transactional analysis, bioenergetic analysis, gestalt therapy, psychodrama and reprogramming techniques such as neurolinguistic programming, timeline therapy, EMDR (desensitization and reprocessing of eye movement) and energy techniques such as TFT (Field of thought therapy) and EFT (Emotional Freedom Techniques); although the mode of action of some of them is not perfectly clear, it is necessary to maintain the integrative concept; There are reports of positive effects with these techniques in individual cases or in large numbers of people[1]. Other resources, well known and used, are biofeedback, guided imagery, visualization and support groups[17,18]. Hypnosis is a technique with proven effects on the brain and capable of producing unexpected results, in addition to being usable in a large number of dermatoses[19], such as trichotillomania, where hypnotic suggestions are used that cause pain when touching the scalp or tearing the hair[20]. There is also self hypnosis and self massage, which are techniques of self application, as well as yoga and tai chi chuan, originally from India and China, the first being a philosophy of life, which integrates mind and body, and the second originated in the martial arts, producing energizing effects on the body[1].

It is important to remember that, rarely, dermatologists relate the ability to react the skin with touch and its influence on the nervous system and immune system; touching the skin and stimulating it in the form of massages, it has the power to facilitate the recovery of burns[21], reduce levels of stress and anxiety hormones, increase the delta waves that indicate relaxation and decrease of the alpha and beta waves in the electroencephalogram, reducing the cortisol and raising the cytotoxic capacity, increasing the number of natural killer cells; This resource is available to specialists and can be of great value in the treatment of psychodermatosis, using the assistance of massage therapists[1].

VII. Discussion

Multidisciplinary services have been developed within specialties and subspecialties such as dermatology, which can be operated by several specialists (group approaches) or by a single specialist with a multidisciplinary approach[22]. Intervention levels may vary from providing tranquility and effective communication (either in primary care or in medical specialties) to specific psychotherapies and psychopharmacological treatments[23]. Psychotherapeutic interventions, we have: psychoeducational

interventions, stress management procedures, cognitive behavioral therapy, brief dynamic therapy, family therapy and group interventions; they have been applied to patients in controlled research[24,25]. The prescription of psychotropic drugs is applied, individually, to a careful balance between potential benefits and adverse effects[26]. A macroanalysis[22] recommends it in specific clinical situations: (1) presence of psychological disorders (for example, demoralization, irritable mood) or psychiatric illness (for example, major depression, panic disorder); (2) refractoriness of lifestyle modifications guided by primary care or other non-psychiatrists; (3) the presence of abnormal disease behavior (from hypochondria to disease denial) that interferes with the treatment or that leads to frequent use of medical care, and (4) impaired quality of life and functioning, not all justified by the medical condition.

A review on psychiatric comorbidity in patients with dermatological disease, indicates that most dermatologists are not mental health professionals with extensive training in psychotherapy and psychopharmacology, but have mental abilities to acquire basic principles of these fields and apply them in the improvement of their patients; dermatologists should implement screening tools, diagnose psychiatric comorbidities and refer to psychiatry is an excellent option for management, if the patient agrees, but if you do not want to go, start the treatment falls into the hands of the dermatologist; i describe psychiatric comorbidities and some common psychotropic agents[27].

a) Anxiety

Either a secondary psychiatric disorder in response to severe psoriasis, an exacerbation factor in cutaneous pathology such as eczema, or primary psychiatric disorder such as neurotic excoriations; a class of anxiolytics are benzodiazepines, they have a rapid onset of action and an effect that goes from short to long-acting; quick start, gratification is immediate and this kind of medication can be very addictive, particularly if used for extended periods of time; risks: sedation and respiratory depression, and withdrawal seizures are dangerous with a life-threatening risk of abrupt discontinuation after long-term use; alprazolam is one of the most used benzodiazepines and confers a unique antidepressant effect, unlike other benzodiazepines, therefore, for an individual with a mixture of depressive symptoms and anxiety, alprazolam; It may be a good choice; starting with a low dose is always a good option and climbing slowly until you reach the minimum effective dose is important to avoid excessive sedation and limit the risks; the typical starting dose is 0.25 mg three times a day (TID), with the ability to increase the dose every 3 to 4 days to a maximum of 4 mg/day and it is recommended to start even lower, at 0.125 mg (half of a 0.25 mg tablet) TID and holder up to a maximum of 2 mg/day; An additional recommendation is to use benzodiazepine for 2–3 weeks and to process a psychiatric referral, for providers who are not accustomed to administering this medication in the long term, note that alprazolam is particularly addictive due to its rapid onset and short duration of action[27]. A long-acting benzodiazepine, such as clonazepam, is suggested as a reasonable alternative[28]. Although clonazepam does not confer the same antidepressant effect, it is less addictive and may be more suitable for patients with strict anxiety conditions, without signs of depression, this medication is started with 0.25 mg twice daily (BID) and increases every 2 days up to 0.5 mg TID, with a maximum dose of 4 mg/day, although as with alprazolam, a maximum of 2 mg/day may be more practical for dermatologist prescribers[27]. Once again, benzodiazepines are the most suitable for short-term use, and as such, they are commonly used to control anxiety while safer, long-term but slower-acting treatments (as discussed later in the text) are taking time to produce the physiological changes necessary for therapeutic benefit[28].

Another type of anxiolytic is buspirone, which is classified as a non-benzodiazepine anxiolytic, which means that it does not carry the same risks of addiction, withdrawal and sedation: this medication is typically prescribed to treat generalized anxiety disorder and its effects may appear at less 2 weeks after taking it; initial dose of buspirone is 5 mg TID or 7.5 mg BID; due to its linear pharmacokinetics and short half-life, it is possible to increase the dose by 5 mg/day every 2-3 days to a goal of 20-30 mg/day, divided into two or three daily doses; if, after several weeks, an adequate clinical improvement has not been obtained, it is possible to assess a maximum dose of 60 mg/day; side effect profile of buspirone is relatively mild, with common symptoms such as gastrointestinal (GI) disorders (nausea, vomiting and diarrhea), drowsiness, fatigue, lightheadedness/dizziness, and headache[27].

As another alternative, selective serotonin reuptake inhibitors (SSRIs), escitalopram and paroxetine are also approved for the treatment of generalized anxiety disorder[29]. SSRIs are first-line antidepressant medications that have been used safely for years, with common adverse effects that include GI disorders, sexual dysfunction and drowsiness, and possible serious reactions such as serotonin syndrome, paradoxical increased suicidality and inappropriate secretion syndrome of antidiuretic hormone (SIADH) [27]. In comparison, escitalopram has demonstrated superiority over paroxetine and has demonstrated long-term efficacy and safety in the treatment of generalized anxiety disorder[30-32]. Escitalopram can be started at 10 mg/day and increase after 1 week to a maximum of 20 mg/day; both doses have demonstrated efficacy and good tolerability; as escitalopram is an antidepressant, unlike benzodiazepines and buspirone, an additional potential risk is to trigger a manic episode in a patient

with bipolar disorder, it is important to ensure that there is no history of mania in patients before starting escitalopram or any other antidepressant[27].

b) Depression

For some people with depression, irritability and psychomotor skills, agitation can be a prominent feature, and this can contribute to the development of primary psychiatric conditions such as neurotic excoriations, factitious dermatitis and excoriated acne; doxepin is a tricyclic antidepressant drug (TCA) that has proven very useful in the treatment of this type of patients, the reason why doxepine is unique among other antidepressants is that it demonstrates potent antihistamine effects, reducing itching, antihistamine effects as well they can cause drowsiness, so it is recommended to take it while sleeping; Doxepin can be started at 25 mg/day and increased by 25 mg every 5-7 days until the ideal therapeutic dose is reached, typically between 100 and 300 mg/day; like TCA, doxepin comes with all the classic side effects and risks that this type of medication entails, including anticholinergic symptoms (dry mouth, urinary retention, blurred vision, tachycardia, etc.), cardiac conduction problems and orthostatic hypotension; TCAs are potentially lethal in overdoses, so be sure to ask directly and explicitly about suicidal thoughts or self-harm, any suspicion of suicide in a patient should cause caution when prescribing, making sure to avoid providing an excessive amount of tablets beyond what is necessary until your next appointment, closer follow-up (more frequent visits) may also be justified[27]. Fortunately, it is possible to verify the serum levels of doxepine, and this can be useful not only in the investigation of possible cases of overdose, but also to confirm the patient's compliance with the treatment and determine if the therapeutic levels have been reached[33].

For other variants of depression, SSRIs are typical first-line medications, due to their proven effectiveness, better safety and tolerability compared to alternative antidepressants such as TCA (tricyclic antidepressants) and monoamine oxidase inhibitors[27]. Serotonin-noradrenaline reuptake inhibitors (SNRIs) are also a first-line option, and some studies have shown SNRI, venlafaxine, is particularly effective in melancholic depression and patients with significant psychomotor retardation[34]. SSRIs fluoxetine and sertraline are considered "activation" medications, they are also good for melancholic depression; sertraline demonstrates better effectiveness and better tolerability[35,36]. In fact, a large meta-analysis concluded that sertraline is the best option for initial treatment in patients with moderate to severe depression, since it has the best balance of effectiveness, tolerability and cost36. Sertraline can be started at 50 mg/day and increase every week by 25 mg/day to a maximum of 200 mg/day, if necessary, some psychiatrists start with an even lower dose (12.5 or 25 mg/day) and wait for see the benefits at 100 mg/day, in most cases[27].

SSRIs and SNRIs are widely prescribed and are generally safe options, which dermatologists can prescribe[27].

c) Psychosis

Psychosis is the main psychopathology underlying psychodermatology, disorders such as delusions of parasitosis, where patients maintain fixed and false ideas (delusion) that parasites reside within their skin; such delusional conditions are part of a subset of psychosis, called monosymptomatic hypochondriacal psychosis (MHP), in which delusions are confined and much less penetrating and harmful than the psychotic symptoms of conditions such as schizophrenia[37]. When dealing with patients suffering from delusions, it is important to accept and not argue to establish a good relationship[27,38]; willingness to examine the evidence, keep an open mind and the clinician, at the same time, should avoid validating or reinforcing the patient's false beliefs[27].

Before prescribing psychotropic medications, it is imperative that the clinician determine if the patient's symptoms come from real organic origins; a patient with suspected DI (delusional infestation), for example, may have an infestation with scabies or lice (careful examination and skin scraping, are vital), or they may experience training (tingling sensation in the skin) as a result of abuse of recreational drugs such as amphetamines, cocaine, alcohol or other illegal substances27. Other causes include vitamin B12 deficiency, cerebrovascular disease, multiple sclerosis, Parkinson's disease, syphilis, hypothyroidism, diabetes, cancer and iatrogenic[39,40]. Dopamine medications prescribed for Parkinson's disease, including ropinirole and pyribedil, have been identified as causes of DI in several cases[41]. Discarding these triggers is important, since only primary DI (caused by true delirium/psychosis) is treated with antipsychotics, while secondary DI (which has an organic basis) is treated by addressing the underlying problem[27]. Discarding substance abuse may require more than simply asking the patient if they use drugs, since substance abuse seems to be quite frequent in this patient population, and they do not always openly reveal the habit[42]. As result, routine urine drug tests may be recommended for new patients with ID, even if they deny drug use[27].

Pimozide is a typical first-generation antipsychotic, it has demonstrated effectiveness in the treatment of MHP in dermatological patients, particularly delusions of parasitosis[37,43]. The initial dose is 1 mg/day and can be increased by 1 mg every week, the maximum dose is 10 mg/day, but patients with MHP generally show a good response at doses of 4 mg/day or less; extrapyramidal symptoms, such as dystonia and parkinsonism, are possible and can be combated with benztropine mesylate, taking 1-2 mg BID or diphenhydramine by taking 25 mg 3-4 times a day;

cardiac conduction abnormalities have also been detected, reporting electrocardiographic changes such as T-wave abnormalities and prolongation of the QT interval, so an electrocardiogram is recommended before starting to take pimozide and after treatment has begun; if there is prolongation of the QT interval, the medication should not be started or should be discontinued[27]; pharmacological interactions are also possible, particularly with drugs that are metabolized by cytochrome P-450 isoenzyme 3A4[44].

Although pimozide has historically been the best option for ID, the development of second-generation atypical antipsychotics, new and safe (SGAs), cause less extrapyramidal and anticholinergic side effects[44,45], a recent and thorough investigation into the effectiveness of SGA identified 63 published cases of DI in which SGAs were used, demonstrating partial or total remission obtained in 75% of patients44. Olanzapine and risperidone were the most used agents[27]. Other atypical antipsychotics recommended for the treatment of ID include quetiapine, amisulpride and a third generation antipsychotic, aripiprazole[46,47]. Dosage of these medications (risperidone 0.5–1 mg daily; olanzapine 5 mg daily; quetiapine 50 mg daily; amisulpride 50 mg daily; and aripiprazole 5 mg daily) are low doses for DI than for more generalized psychotic conditions such as schizophrenia, and routine laboratory monitoring is not usually necessary[27]. Due to the risks of cardiotoxicity and pharmacological interactions with pimozide, these agents have now replaced pimozide as a first-line treatment for DI[44]. It should be noted that SGA clozapine was not included in this list, since this medication requires frequent monitoring of blood count due to the risk of agranulocytosis[27]. In addition, it is important to recognize that almost all antipsychotic agents can cause weight gain and/or metabolic syndrome, presenting a greater risk with olanzapine and clozapine, and little or nothing with amisulpride and aripiprazole[48]. It has been determined that this weight gain is mediated by an antagonistic effect on H1 histamine receptors (H1R), and the commonly prescribed H1R agonist and anti-vertigo drug, betahistine, is able to safely and effectively mitigate weight gain associated with antipsychotics[49-51].

Although it may be difficult to convince a patient to try an antipsychotic medication, present the medication as capable of diminishing uncomfortable sensations (instead of explicitly stating that it will treat psychosis or improve the patient's skin), it is recommended; also explain to the patient the importance of treating their condition from the outside in (with topical medications and creams, such as mupirocin and moisturizers) as well as from the inside out (with oral medications), a successful treatment requires both attack routes, to support the patient compliance; It is useful to keep in mind that these medications take 6 weeks to start working and their maximum effect is expected up to 6 months after starting, if the treatment is effective and the patient experiences remission of ID, it is reasonable to try to start weaningm antipsychotic 3 months after obtaining remission, with a plan to restart if a relapse occurs[27]. The greatest risk of recurrence is within the first 3 to 4 months after discontinuation of the antipsychotic, 25% of patients experience the return of symptoms requiring longer courses of treatment or possibly long-term maintenance therapy[52].

Although the prescription of antipsychotics is not a typical activity for dermatologists, some have argued that patients with MHP differ dramatically from more affected individuals seen by psychiatrists; antipsychotic treatment can improve the patient, since they are "difficult" patients who continuously rotate in the offices without any sign of improvement despite extensive and repetitive advice, represent an opportunity for dermatologists, take care of the health of their patients and focus your efforts on the cause of the skin condition[27].

d) Obsessive compulsive

The last conditions to discuss are those based on obsessive behavior: compulsive, although referral for psychological counseling, such as cognitive behavioral therapy, exposure and response prevention or other behavior modification therapies, can be extremely effective and should be considered first-line, patients may be resistant to these options or may not respond, in which case psychopharmacological interventions are necessary[53-55].

Clomipramine is a TCA that has demonstrated superiority in its class for the treatment of OCD and related conditions, such as trichotillomania and onychophagy; Clomipramine starts at 25 mg/day and can be increased to 250 mg/day if necessary; for children, the maximum dose is 3 mg/kg /day; side effects are similar to other eating disorders as previously discussed, with a little more seizure onset (seizure threshold decreases) and sexual dysfunction[27].

Fluoxetine is an SSRI alternative for OCD, which showed similar efficacy and has been successful in treating dermatological conditions such as habit-tic nail deformity; it is prescribed at 20 mg/day and can be increased up to 80 mg/day maximum if necessary, although 20–40 mg/day is typically effective; as with other SSRIs, the effects may not be noticed for a few weeks, and the maximum benefit may take 6 to 8 weeks; it should be noted that fluoxetine is approved by the FDA for depression, but not OCD (obsessive compulsive disorder), so its use in this condition would be off-label[27].

A more exclusive treatment option for this class of conditions, it´s N-acetylcysteine (NAC), which has shown promise in the treatment of trichotillomania[56-59].

Unlike other impulse control disorders, trichotillomania is often resistant to SSRIs, but a Cochrane review by Rothbart et al. determined that NAC, as well as clomipramine and olanzapine (an antipsychotic), can be effective[60]. NAC is an amino acid that acts as a glutamate modulator and can exert its effect by normalizing dysregulated extracellular glutamate in the nucleus accumbens: an area of the brain that plays a key role in motivation and reward[57]. The dose of NAC for trichotillomania is 1,200 mg/day, with few adverse effects reported by patients[27]. Some argue that the apparent efficacy of NAC in trichotillomania suggests could and should be tested for other impulse control disorders that involve scratching or pulling[59].

For the habit-tic deformity of the nail, a clinician discovered an economical and safe treatment that was effective in normalizing the nails of two patients after 3 to 6 months of use, made the patients apply a cyanoacrylate adhesive (instant glue) to the proximal nail fold of the affected nails 1 to 2 times per week, effectively forming a physical barrier to external trauma, although it is creative, this method does not necessarily cure the underlying motivation of patients to scratch their cuticles, and a relapse can be expected and, in fact, was seen in some patients, interestingly, this reconstituted treatment achieved normalization of the nail, and after the subsequent interruption of therapy, he was able to maintain normal nails[61]. It is also important to note the possibility of developing contact dermatitis in response to cyanoacrylate[62-64].

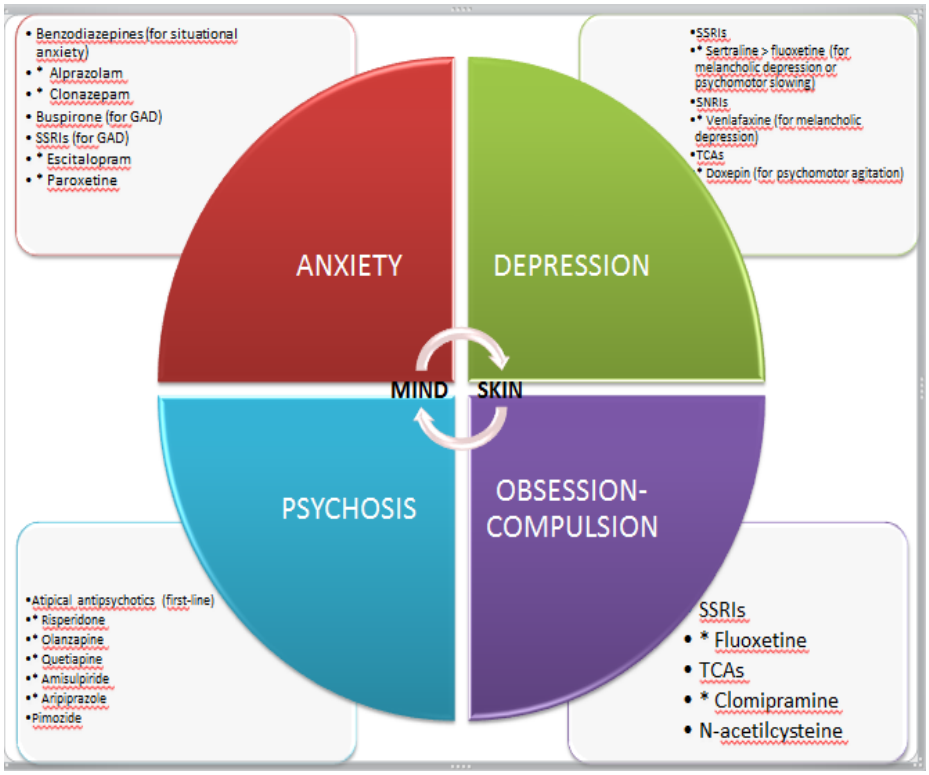

Figure 1: Use of psychotropic medications to treat dermatological conditions[65].

It is known that certain psychotropic agents are useful in the treatment of dermatological conditions; if pruritus is the main problem, doxepine is the preferred agent; on the other hand, if pain predominates, such as burning, itching or irritation, amitriptyline is the preferred agent[65].

Doxepine: Is often used to treat pruritus when more conventional antipruritic agents, such as diphenhydramine or hydroxyzine, are inadequate; there are several advantages of the use of doxepine for the control of pruritus compared to conventional antipruritic agents; first, doxepine has a much greater affinity for histamine receptors. than traditional antihistamines and therefore it can exert much more potent antipruritic effect, the affinity of doxepine for the histamine (H1) receptor in vitro is approximately 56 times hydroxyzine and 775 times greater than diphenhydramine; second, the therapeutic effect of doxepine is much longer and longer lasting than any of these antihistamine medications because of its long half-life, doxepine is taken once a day, usually at bedtime to provide a therapeutic benefit for 24 hours; therefore, patients with conditions that present with severe pruritus, such as atopic dermatitis, who complain of waking up in the middle of the night, even if they are taking hydroxyzine or diphenhydramine before bedtime, usually find calm when they switch to doxepin and can sleep all the time night; third, doxepine normalizes the architecture of

sleep, when the patient spends more time in a deep state of sleep, the excoriations decrease dramatically; doxepine may also be useful in the treatment of patients with chronic urticaria or other histamine-mediated disorders who have failed traditional antihistamine treatment; there is no good data on the optimal therapeutic blood level of doxepine for the treatment of conditions such as pruritus or hives, a wide range of doses may be possible depending on each patient, for example, dose of doxepine sufficient to control pruritus can vary from only 10 mg at bedtime (often used in liquid preparation doxepine 10 mg/cc) up to the maximum dose for the treatment of depression 300 mg at bedtime and if a patient does not show an initial therapeutic response, the The physician should consider gradually increasing the dose of doxepine according to tolerance to the desired therapeutic response[65].

Amitriptyline: For various manifestations of pain sensations such as burning, itching or irritation, amitriptyline is a preferred agent over doxepine due to better documentation of its effectiveness as an analgesic agent; when eating disorders are used as analgesics, the required dose tends to be much lower than the dose required for its antidepressant effect; the patient can start with 25 mg at bedtime and start the maximum effective dose to use as an analgesic, a dose of 50 mg/day or less should generally be sufficient; the side effects of amitriptyline are similar to those of doxepine, namely sedatives, cardiac, anticholinergics and α-adrenergic side effects, including orthostatic hypotension, which can be problematic in elderly patients; adverse effects can be minimized by using the lowest effective dose possible; if the patient is unable to tolerate amitriptyline, other eating disorders, such as imipramine or desipramine, may be used; dosage range for these medications are similar to those of amitriptyline; if these new TCAs are not tolerated, SSRIs can be tried, there are some useful SSRI reports as analgesics; additionally, duloxetine, an SNRI, has an FDA indication for the treatment of chronic pain and can be considered in these cases[65].

VIII. Search Methodology

A computerized bibliographic investigation was conducted in the Pubmed search engine https://www.ncbi.nlm.nih.gov/pubmed/, during the period from January 2019 to August 2019; using the following keywords in English: Psychosomatic medicine, traditional medicine, complementary therapies, psychopharmacology, psychotropic drugs; found 4,255 articles; articles with a level of evidence I, II and III were selected; with a period of seniority of 20 years and for its content of scientific interest and originality a total of 4,190 articles were excluded from the analysis: studies without specific description of the treatment of Psychosomatic medicine that did not describe the relationship between psychopharmacology, psychotropic drugs and complementary therapies ; so 65 articles were used. Microsoft Windows, version 6.3 (build 9600), from 2013 was used.

IX. Conclusion

In the clinical practice of dermatology, one in four patients who go to consultation with an acute or chronic dermatological disease is affected by a psychological/psychic disorder or a psychological/psychiatric pathology triggers or aggravates a dermatological disease. They do not know it, and it is the doctor who must suspect that behind a dermatosis a psychiatric disorder can be hidden or vice versa. This must be confirmed by a specific systematic interrogation, and if it exists, it must be treated properly, thus contributing to cure the dermatosis consulted and the associated pathology. The most frequent psychiatric disorders in dermatological patients are anxiety, depression, psychosis and obsessive-compulsive disorders. But, while the patient with anxiety may be more or less aware of his problem, depression, psychosis or obsessive-compulsive disorders usually present themselves in a masked way or not recognized. Dermatologists must be aware of the potential for significant improvement in the quality of life when addressing the psychic dimension of skin disease.

The relationship of mental pathologies and dermatological diseases: generally, it is bidirectional, it is necessary to analyze the impact that dermatological pathologies have on psychic disorders or vice versa and cut this cycle. Depression is most often observed in patients with psoriasis; anxiety and depression, in patients with vitiligo, pruritus, acne, alopecia areata and urticaria, anxiety more frequently in patients with rosacea and chronic chronic lichen, psychosis in patients with delusional infestation and obsessive-compulsive disorder in trichotillomania and onychophagy; finding numerous evidence for these pathologies. Thus, treating patients with mental processes that triggered some dermatological pathology, remitting the cause we can control the skin disease and vice versa, in the event that the dermatological disease triggers or exacerbates the psychic pathology, treating the skin component will relieve the psychic pathology ; the dermatologist, in the consultation must be empathetic, meet the expectations of the patient and be optimistic, aspects of the clinical encounter that can foster a positive therapeutic relationship; in addition, you must master the basic anti-stress mind-body techniques; that they can keep stress at a non-harmful level at no cost; encouraging them to perform them in leisure time and to practice them routinely to maintain their physical and mental balance, namely: upright posture, change of respiratory pattern from thoracic to abdominal, muscle relaxation and

meditation; You should also know and recommend, as appropriate, various complementary therapies. Multidisciplinary services have been developed within specialties and subspecialties such as dermatology, which can be operated by several specialists (group approaches) or by a single specialist with a multidisciplinary approach, the levels of intervention can vary from providing tranquility and effective communication to specific psychotherapies and Psychopharmacological treatments, which are applied, individually, to a careful balance between potential benefits and adverse effects.

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Moringa Oleifera Seed Protein Hydrolysates Inhibit Haemoglobin Glycosylation and α-Glucosidase Activity in-vitro

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Abstract- In recent times, the biological activities of enzymatic digests of plant and animal proteins have been investigated and have been shown to exhibit multidirectional effects against key enzymes involved in the pathophysiology of a number of diseases. The present study evaluated the inhibitory effects of M.oleifera seed protein hydrolysates on haemoglobin glycosylation and α-glucosidase. Proteins were hydrolyzed using the enzymes pepsin, trypsin, papain and chymotrypsin. The resulting hydrolysates were evaluated for inhibitory activities against non-enzymatic haemoglobin glycosylation as well as α-glucosidase. Peptic and chymotrypsin hydrolysates demonstrated the best inhibitory effects against hemoglobin glycosylation, while chymotryptic and tryptic hydrolysates had better α-glucosidase inhibitory activities. Kinetic data showed that the hydrolysates inhibited α-glucosidase inhibitory effects by different mechanisms, such tryptic and chymotrypsin hydrolysates indicated a competitive mode of inhibition while papain and pepsin hydrolysates displayed mixed inhibition of α-glucosidase. These results suggest that M.oleifera seed proteins contain peptides that can be harnessed to formulate peptides which could serve as novel alternatives to current therapies in the management of diabetes mellitus.

Keywords: M.oleifera, hydrolysates, pepsin, trypsin, papain, chymotrypsin, hemoglobin, α-glucosidase, diabetes mellitus.

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Augustine Olusegun Olusola [α] & Oluwafemi Emmanuel Ekun [σ]

Abstract- In recent times, the biological activities of enzymatic digests of plant and animal proteins have been investigated and have been shown to exhibit multidirectional effects against key enzymes involved in the pathophysiology of a number of diseases. The present study evaluated the inhibitory effects of *M.oleifera* seed protein hydrolysates on haemoglobin glycosylation and α-glucosidase. Proteins were hydrolyzed using the enzymes pepsin, trypsin, papain and chymotrypsin. The resulting hydrolysates were evaluated for inhibitory activities against non-enzymatic haemoglobin glycosylation as well as α-glucosidase. Peptic and chymotrypsin hydrolysates demonstrated the best inhibitory effects against hemoglobin glycosylation, while chymotryptic and tryptic hydrolysates had better α-glucosidase inhibitory activities. Kinetic data showed that the hydrolysates inhibited α-glucosidase inhibitory effects by different mechanisms, such tryptic and chymotrypsin hydrolysates indicated a competitive mode of inhibition while papain and pepsin hydrolysates displayed mixed inhibition of α-glucosidase. These results suggest that *M.oleifera* seed proteins contain peptides that can be harnessed to formulate peptides which could serve as novel alternatives to current therapies in the management of diabetes mellitus.

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I. Introduction

Peptide products of plant and animal proteins have in recent times have been exploited for therapeutic purposes (Olusola *et al.,* 2018). Peptides of therapeutic value have been utilized in the treatment and management of a variety of disorders (Lien and Lowman, 2004). For the most part, emphasis has been placed on the use of peptides and protein hydrolysate preparations as possible alternatives in the management of cardiovascular diseases such as hypertension (Arise *et al.,* 2016[a]) and diabetes mellitus (Arise *et al.,* 2016[b], Olusola and Ekun, 2019). One plant whose proteins encode potentially bioactive peptides is *M. oleifera*.

Moringa oleifera is naturalized in India, especially in the Western & Himalayan regions. It is also found in the tropical regions of Africa as well as the Middle East (Madubuike *et al.,* 2015). Mune- Mune *et al.,* (2016) reported that *M. oleifera* seeds have a relatively high protein content, over 30%, making it an excellent source of potentially therapeutic peptide products when subjected to enzymatic hydrolysis. Globulins and albumins constitute the major portion of *Moringa oleifera* seeds (Baptista *et al.,* 2017), and amino acid analysis reveals that it contains high proportion of basic and acidic amino acids, moderate amount of most hydrophobic amino acids, but limiting in sulfur-containing amino acids such as methionine and cysteine (Okereke and Akaninwor, 2013). Parts of the plant such as its leaves, seeds and roots have been evaluated for their health promoting benefits which include hypoglycemic effects (Villarruel-López *et al.,* 2018) antimicrobial activity (Bukar *et al.,* 2010), antioxidative potentials (Wright *et al.,* 2017) among other properties.

Diabetes mellitus is a metabolic disorder occurring as a result of disturbances in insulin function. It is characterized by severe hyperglycemia and leads to a plethora of derangements in the metabolism of carbohydrate, proteins and lipids (Arise *et al.,* 2016[b]). These ultimately cause damage to organs, such as liver, kidney, retina, as the disease progresses. One of the adverse effects of elevated blood glucose is the formation of advanced glycated end products, and this occurs when blood glucose forms non-enzymatic covalent adducts with protein and lipids in plasma. (Ramasamy *et al*., 2005). These glycated products may set the stage for the onset of generation of reactive oxygen species; activating a number of pro-inflammatory pathways causing impaired cellular function, and these are thought to occur by signal transduction processes mediated by the receptor for advanced glycated end-products (RAGE) play key roles in pathogenesis the diabetic cataracts, diabetic neuropathy and nephropathy(Singh *et al.,* 2014).

Current therapeutic approaches are aimed at controlling glucose levels by slowing its rate of release into the blood stream by inhibiting glucoside cleavage enzymes such as α-amylase and α-glucosidase; increasing insulin sensitivity by altering the activities of incretin degrading enzymes such as dipeptidylpeptidase (iv) in combination with lifestyle changes (Katzung *et al*., 2012). However, these chemotherapeutic approaches give rise to certain untoward side effects such as

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gastrointestinal discomfort and renal damage. Also, many of these drugs, owing to their high cost of procurement, increase economic burden on patients as well as their relatives (Arise *et al.,* 2016). Hence, the search for newer, safe and cost-effective alternatives in the management of diabetes mellitus cannot be over-emphasized, and recently attention has turned to newer sources such as peptides and hydrolysate preparations (Olusola and Ekun, 2019).

There have been reports about several biofunctional properties of various parts of the *M. oleifera* plants (Anwar *et al.,* 2007). Previous work had demonstrated that hydrolysates obtained from its seed proteins possess α-amylase inhibitory activities (Garza *et al.,* 2017, Olusola *et al.,* 2018). Hence, this study aims to evaluate the inhibitory activities of *M. oleifera* seed protein hydrolysates on hemoglobin glycosylation and α-glucosidase to further justify their anti-diabetic potentials.

II. Materials and Methods

a) Materials

i. Collection of Moringa oleifera seeds

Moringa oleifera seeds were bought from stores in Ikere-Ekiti, Ekiti State, Nigeria, and authenticated by the Department of Plant Science and Biotechnology, Adekunle Ajasin University Akungba Akoko.

ii. Chemicals and Reagents

Enzymes: pepsin (from porcine stomach), trypsin (from bovine pancreas), papain (from *Carica papaya*), chymotrypsin (human), and alpha-amylase (from saccharomyces cerevisiae) were products of Kem Light Laboratories, India. alpha-glucosidase (*Saccharomyces cerevisiae*) and other reagents used were of analytical grade and were purchased from Sigma Aldrich (USA).

b) Methods

i. Isolation of Moringa oleifera seed proteins

The *Moringa oleifera* seeds were dried and pulverized before being kept in an air-tight container at 4^{0}C.This were defatted using n-hexane as described by Wani *et al.,* (2011).The meal was extracted four times with n-hexane(60-80^{o}C) using a meal/solvent ratio of 1:10(w/v). The meal was dried at 40^{0}C in a vacuum oven and ground again to obtain a fine powder, termed defatted seed meal, which was then stored at -20^{0}C.The protein component of the defatted meal was extracted using the method described by Alashi *et al.,* (2014) with modifications. Defatted Moringa seed meal was suspended in 0.5M NaOH pH 12.0 at a ratio of 1:10 and stirred for one hour to facilitate alkaline solubilisation. This was then centrifuged at 18^{0}C and 3000g for 10min. Two additional extractions of the residue from the centrifugation process were carried out with the same volume of 0.5M NaOH and the supernatants were pooled. The pH of the supernatant was adjusted to pH 4.0 to facilitate acid-induced protein precipitation using 5M HCl solution; the precipitate formed was recovered by centrifugation as described above. Also the pH of the supernatant formed was further adjusted to a pH of 5.5 using 0.1M NaOH. The precipitates formed were recovered by centrifugation. They were then washed with distilled water, adjusted to pH using 0.1M NaOH, freeze-dried and the protein isolate was stored at -20^{0}C until required for further analysis.

ii. Preparation of Moringa oleifera seed protein Hydrolysates

The protein isolate was hydrolysed using the methods described by Onuh *et al.,*(2015) with slight modifications. The conditions for hydrolysis were tailored for each enzyme in order to optimize its activity. Hydrolysis were done using each of pepsin (pH 2.2, 37^{0}C), trypsin (pH 8.0, 37^{0}C), papain (pH 6.0, 50^{0}C) and chymotrypsin (pH 8.0, 37^{0}C). The protein isolate (5% w/v, based on the protein content of the isolate) was dissolved in the appropriate buffer (phosphate buffer, pH 8.0 for trypsin and chymotrypsin, glycine buffer, pH 2.2 for pepsin, phosphate buffer, pH 6.0 for papain). The enzyme was added to the slurry at an enzyme-substrate ratio (E: S) of 2:100. Digestion was performed at the specified conditions for 24 hours with continuous stirring. The enzyme was inactivated by boiling in water bath (95-100^{0}C) for 15 minutes followed by centrifugation at 700g for 30 minutes. The supernatant containing target peptides were then collected. Protein content of samples were determined using biuret assay method with bovine serum albumin (BSA) as standard.

iii. Inhibition of Hemoglobin Glycosylation

This was investigated by estimating the degree of non-enzymatic hemoglobin glycosylation according to the method described by Venu, *et al.,* (2016) with modifications. Glucose solution (2%), 0.06% hemoglobin and Gentamycin (0.02%) solution were prepared in phosphate buffer 0.1M, pH 7.4. 1ml each of above solution was mixed. 0.25ml, 0.50ml, 0.75ml and 1ml of hydrolysate was added to above mixture. Gallic acid was used as standard. The mixture was kept in dark at room temperature for incubation for 72hours. At 520nm, haemoglobin glycosylation was measured with a spectrophotometer and % inhibition was calculated thus:

Percentage of hemoglobin glycosylation =
Abs(sample) - Abs (control)/ Abs (sample) x 100%

iv. Determination of α-glucosidase Inhibition

The effect of the hydrolysates on α-glucosidase activity were determined according to the method described by (Kim *et al.,* 2005) with slight modifications, using α-glucosidase from *Saccharomyces cerevisiae.* The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 200μL of α glucosidase (1.0 U/mL) was

pre-incubated with 100μL of the different concentrations of the hydrolysates for 10 min. Then 50μL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) will be added to start the reaction. The reaction mixture were incubated at 37◦C for 20 min and stopped by adding 2mL of 0.1 M Na_2CO_3 solution. The α-glucosidase activity was determined by measuring the yellow-colored para-nitrophenol released from pNPG at 405 nm. The results were expressed as percentage of the blank control. Percentage inhibition were calculated as:

$$\% \text{ Inhibition} = (\text{Acontrol} - \text{Asample}) / \text{Acontrol} \times 100$$

v. *Determination of Kinetic Parameters of α-glucosidase Inhibition*

The kinetic parameters of α-glucosidase by the hydrolysates were determined according to the modified method described by Ali *et al., (*2006). Briefly, 50μL of the (5 mg/mL) hydrolysate was pre-incubated with 100μLof α-glucosidase solution for 10 min at 25◦C in one set of tubes. In another set of tubes, α-glucosidase was pre-incubated with 50μL of phosphate buffer (pH 6.9). 50μL of pNPG at increasing concentrations (0.63–2.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixtures were then incubated for 10 min at 25°C, and 500μL of Na_2CO_3 solution was added to stop the reaction. The amount of reducing sugars released were determined spectrophoto- metrically at 405nm using a paranitrophenol standard curve and converted to reaction velocities. A double reciprocal plot (1/V versus 1/[S]) where V is reaction velocity and [S] is substrate concentration was then plotted. The mode of inhibition of the hydrolysates on α-glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis Menten kinetics.

vi. *Statistical Analysis*

Results were expressed as mean of replicates ± standard error of mean (SEM). The data were statistically analyzed using One Way Analysis of Variance (ANOVA) and Duncan's multiple range tests. Differences were considered statistically significant at $p<0.05$ using Microsoft Excel and GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA).

III. Results

a) Inhibition of Haemoglobin Glycosylation

The effects of *Moringa oleifera* seed protein hydrolysates on non-enzymatic haemoglobin glycosylation at a concentration range of 0.25mg/ml to 1.00mg/ml are illustrated in Figure 1. The hydrolysates showed a concentration-dependent reduction in the inhibition of hemoglobin glycosylation. Also, they demonstrated significantly lower ($p<0.05$) inhibitory effects when compared to gallic acid. Peptic hydrolysates exhibited inhibitory effect of 62.583±0.621% at a final concentration of 1.00mg/ml, which was significantly ($p<0.05$) higher when compared to other hydrolysates, at the same concentration. Chymotrypsin hydrolysates, with a inhibitory extent of 53.513±0.361% had higher inhibitory effects than tryptic and papain hydrolysates (38.360±0.439% and 46.540±0.323% respectively) while tryptic hydrolysates had lowest inhibitory activity.

Figure 3 depicts the IC_{50} Values of *Moringa oleifera* seed protein hydrolysates in inhibiting hemoglobin glycosylation as compared to gallic acid. Peptic hydrolysates inhibited hemoglobin glycosylation to a 50% extent at a concentration of 0.533±0.392mg/ml, while tryptic, papain and chymotrypsin hydrolysates exhibited 50% inhibition at 0.113±0.027mg/ml, 0.599±0.026mg/ml and 0.765±0.046mg/ml respectively. With the exception of tryptic hydrolysates, all the other hydrolysates had significantly ($p<0.05$) higher IC_{50} values when compared to gallic acid. Hydrolysates derived from tryptic digestion had significantly lower ($p<0.05$) IC_{50} values than other hydolysates, just as values obtained for peptic and papain hydrolysates were not significantly ($p<0.05$) different from each other, but were lower than those of chymotrypsin hydrolysates.

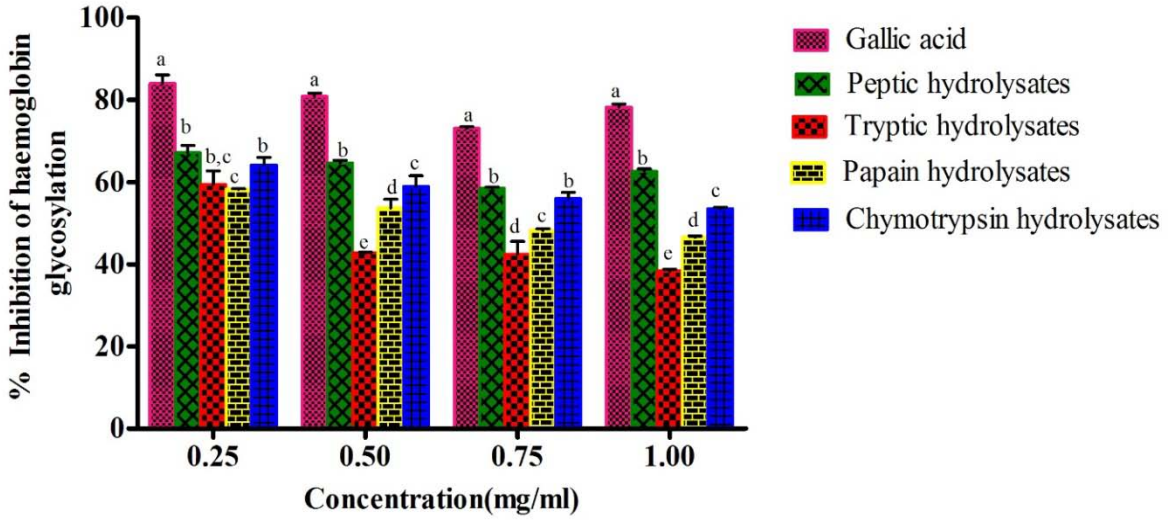

Figure 1: Percentage Haemoglobin Glycosylation Inhibition by *Moringa oleifera* Seed Protein Hydrolysates

Bars are expressed as means ± standard error of mean (SEM) of triplicate determinations (n=3). Bars with the same letters do not differ significantly while bars with different letters are significantly different ($P<0.05$) from one another.

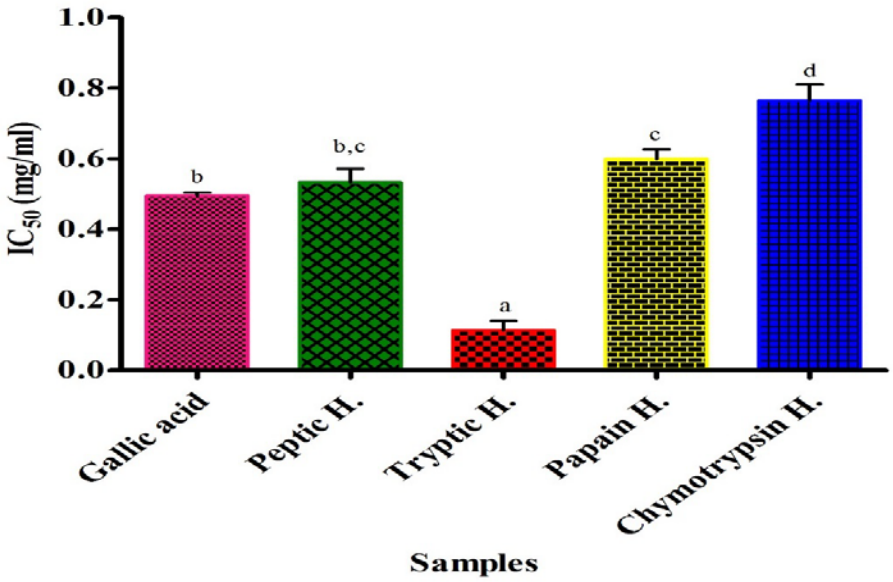

Figure 2: Values of 50% haemoglobin glycosylation inhibitory concentration (IC_{50}) of *Moringa oleifera* seed protein hydrolysates

Each bar represents the mean of triplicate determinations ± SEM. Bars with same letters are not significantly different at ($p < 0.05$), while bars with different letters are significantly different from one another.

b) α-Glucosidase Inhibitory Activity

The inhibitory activities of the hydrolysates on α-glucosidase – catalyzed hydrolysis of p-nitrophenyl glucopyranoside at varying concentrations are presented in Figure 3. The hydrolysates displayed increasing inhibitory activity with increasing concentration, with peptic, tryptic, papain and chymotryptic hydrolysates attaining 71.040±6.322%, 80.620±2.308%, 74.06±0.081% and 72.39±0.450% inhibition respectively at a final concentration of 1.0mg/ml. Tryptic hydrolysates demonstrated the highest inhibitory activity at all concentrations in this study($p<0.05$). At lower concentrations however, papain hydrolysates had significantly higher ($p<0.05$) inhibitory activity than peptic and chymotrypsin hydrolysates.

Figure 4 shows the IC_{50} values of the four hydrolysates in inhibiting the reaction catalyzed by α-glucosidase. Peptic and tryptic hydrolysates inhibited the reaction to a 50% extent at concentrations of 0.465±0.394mg/ml and 0.151±0.027mg/ml respectively, while papain and chymotrypsin hydrolysates had IC_{50} values of 3.348±0.028mg/ml and 0.085±0.013mg/ml respectively. The IC_{50} values of tryptic and chymotrypsin hydrolysates were not significantly ($p<0.05$) different from each other, but they were lower than those obtained by peptic and papain hydrolysates. Also, peptic hydrolysates had a significantly ($p<0.05$) lower IC_{50} value than papain hydrolysates.

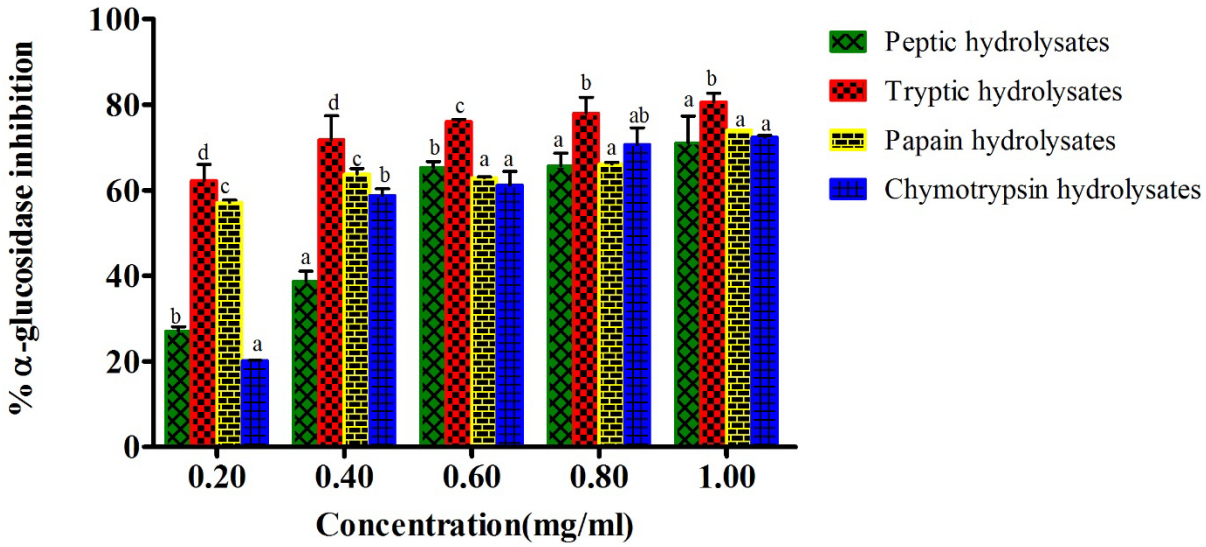

Figure 3: Percentage α-glucosidase Inhibition by *Moringa oleifera* Seed Protein Hydrolysates

Each bar represents the mean of triplicate determinations ± SEM. Bars with same letters are not significantly different at ($p < 0.05$), while bars with different letters are significantly different.

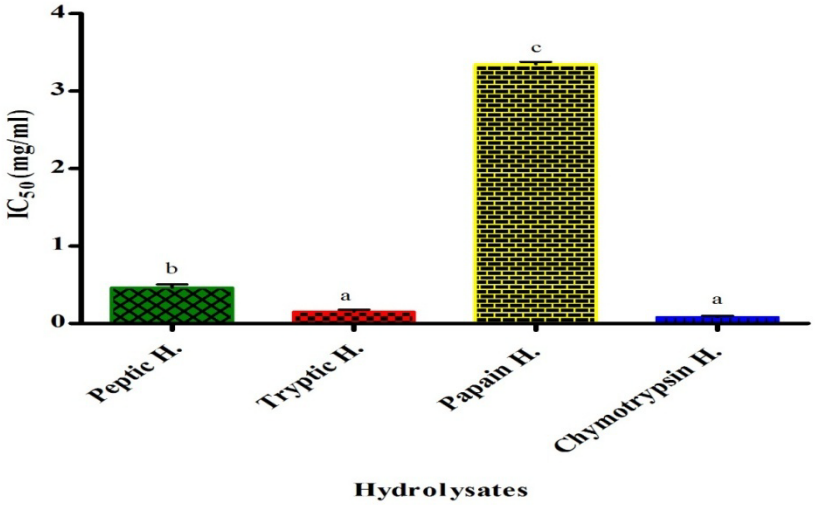

Figure 4: Values of 50% α-glucosidase inhibitory concentration (IC_{50}) of *Moringa oleifera* seed protein hydrolysates

Each bar represents the mean of triplicate determinations ± SEM. Bars with same letters are not significantly different at ($p < 0.05$), while bars with different letters are significantly different.

c) Kinetics of α-glucosidase inhibition

The effects of *M. oleifera* seed protein hydrolysates on the kinetics of α-glucosidase– catalyzed hydrolysis of p-nitrophenyl glucopyranoside, p-NPG, to p-nitrophenol are illustrated in figures 5-8. The kinetic parameters from the resulting Line-weaver Burk plots are summarized in Table 1. In the absence of inhibitory hydrolysates, the Michaelis constant, k_m of α-glucosidase for its substrate was determined to be 0.297 p-NPG, while maximum velocity, Vmax, was 270.27mM/mg/min. All hydrolysates except papain hydrolysates, caused a concentration dependent increase in the apparent k_m of the enzyme. Also, all

hydrolysates reduced the maximum velocity, Vmax as well as the catalytic efficiency, CE, of the α-glucosidase reaction. Hydrolysates from chymotrypsin digestion exhibited the most reduced CE, while peptic hydrolysates displayed the most reduced Vmax, when compared to other hydrolysates.

The enzyme-inhibitor dissociation constant, k_i, was lowest with chymotryps in hydrolysates (0.193mg/ml), although it was only slightly lower than 0.203mg/ml obtained for tryptic hydrolysates. Papain hydrolysates had the highest k_i value of 1.278mg/ml. The mode of inhibition of peptic, tryptic and papain hydrolysates was the mixed type, while chymotrypsin hydrolysates displayed a competitive inhibition of α-glucosidase.

Table 1: Kinetics of α-glucosidase-catalysed Reactions in the Presence and Absence of *Moringa oleifera* Seed Protein Hydrolysates

Kinetic Parameters	No inhibitor	Peptic hydrolysates (mg/ml)		Tryptic hydrolysates (mg/ml)		Papain hydrolysates (mg/ml)		Chymotrypsin hydrolysates (mg/ml)	
		0.5	1.0	0.5	1.0	0.5	1.0	0.5	1.0
k_m or k'_m (mg/ml)	0.297	0.789	0.331	4.269	3.400	0.667	0.672	5.189	4.192
V_{max} or V'_{max} (mM/mg/min)	270.270	192.308	78.740	149.254	200.000	196.078	149.254	270.270	192.308
CE (mmol/ml/min)	910.001	243.891	238.102	34.965	58.824	294.103	222.236	52.083	45.872
k_i (mg/ml)	-	0.305		0.203		1.278		0.193	

k_m/k'_m: Michaelis constant in the absence or presence of inhibitory hydrolysates; Vmax/V' max: Maximum velocity in the absence/presence of inhibitory hydrolysates; CE: Catalytic Efficiency; k_i: Enzyme-Inhibitor dissociation constant.

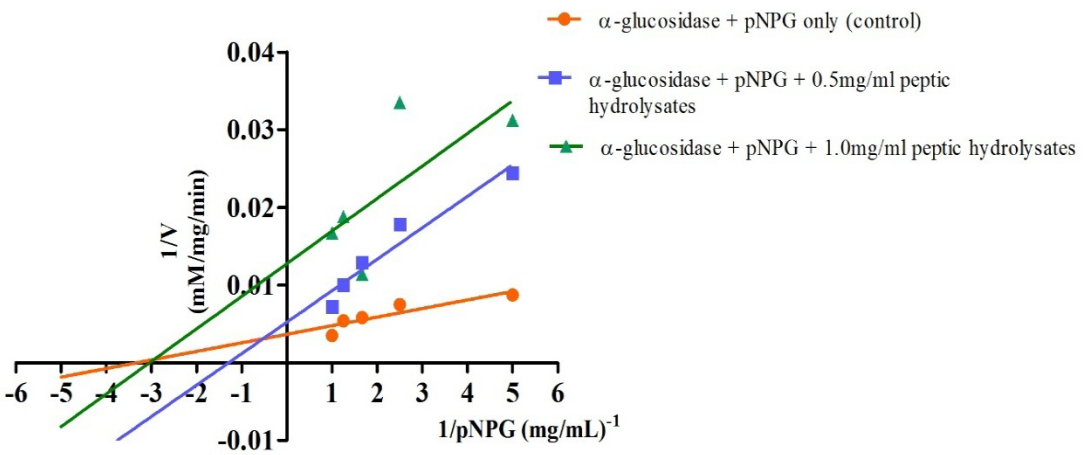

Figure 5: Lineweaver-Burk Plot of α-glucosidase inhibition by hydrolysates obtained from peptic proteolysis of *M. oleifera* seed proteins

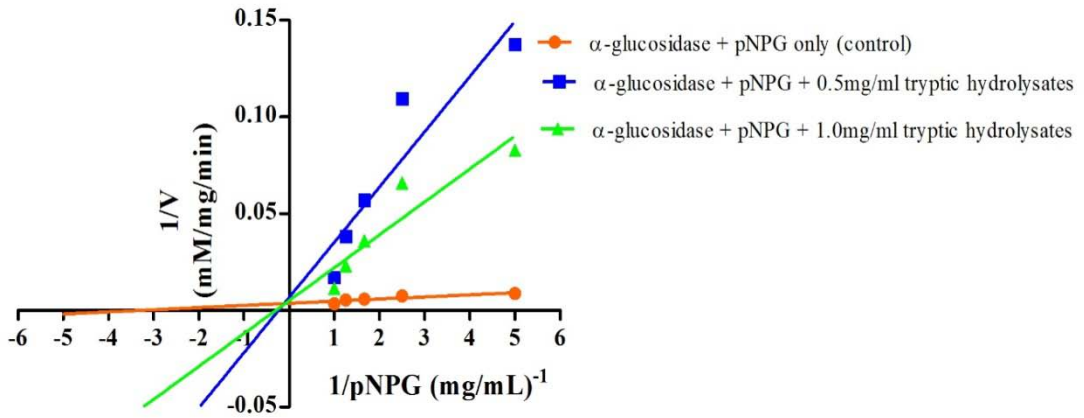

Figure 6: Lineweaver-Burk Plot of α-glucosidase inhibition by hydrolysates obtained from tryptic digestion of *M. oleifera* seed proteins

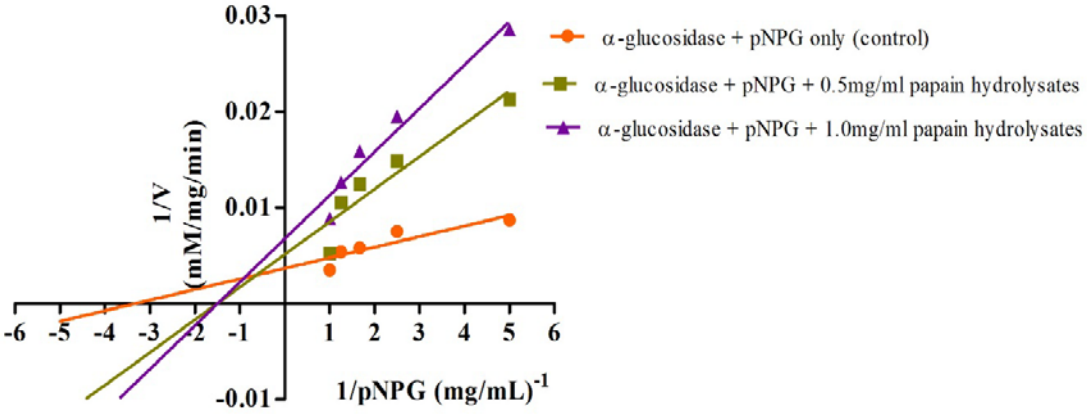

Figure 7: Lineweaver-Burk Plot of α-glucosidase Inhibition by hydrolysates obtained from papain hydrolysis

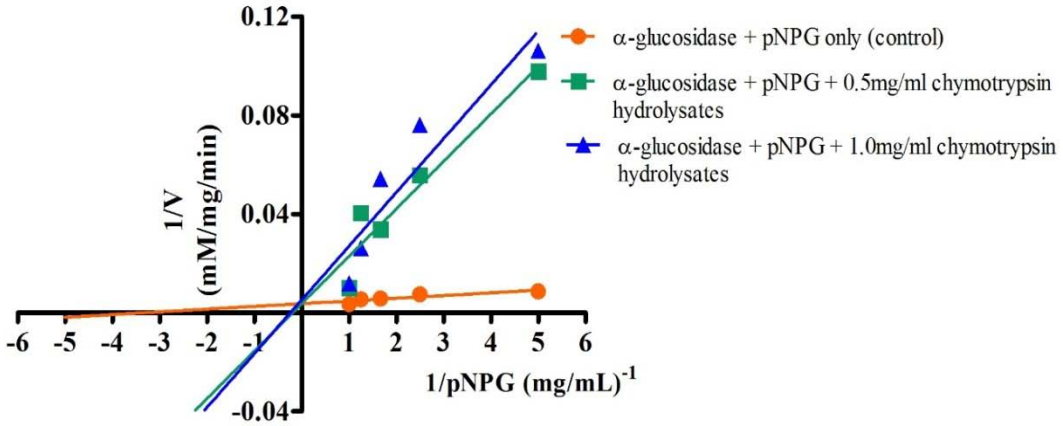

Figure 8: Lineweaver-Burk Plot of α-glucosidase Inhibition by hydrolysates obtained from chymotrypsin proteolysis

IV. Discussion

a) *Inhibition of Hemoglobin Glycosylation*

The formation of advanced glycation end products (AGEs) as a result of poorly controlled hyperglycemia in diabetes mellitus leads to a plethora of complications such as retinopathy, renal dysfunction atherosclerosis, among other devastating conditions (Ramasamy *et al.,* 2005, Singh *et al.,* 2014). These AGEs cause deleterious effects by promoting the generation of reactive oxygen species which activate a cascade of signaling pathways, leading to an increase in the production of pro-inflammatory mediators, leading to other complications such as the formation of atherosclerotic plaques and culminating in cardiovascular disease in diabetic patients (Han *et al.,* 2018). Certain plant extracts have been reported to inhibit haemoglobin glycosylation in vitro (Hosseini *et al.,* 2015), but information has been scarce on the abilities of peptides and protein hydrolysates to inhibit haemoglobin glycosylation. In this study, the hydrolysates displayed a concentration dependent reduction in their abilities to inhibit haemoglobin glycosylation in vitro, such that peptic and chymotrypsin hydrolysates had >50% inhibition at a final concentration of 1.00mg/ml, which was higher than those of other hydrolysates. This may be due to the possible influence of the nature of the peptides in the hydrolysate preparations. Chymotrypsin cleaves proteins specifically at C-terminals of aromatic amino acid residues, while pepsin being relatively non-specific, hydrolyzes proteins at C-terminals of aminoacyl residues having hydrophobic and aromatic side chains (Voet and Voet, 2011). This may give rise to residues such as Trp, Tyr, Leu, Phe, Ile found at these positions, and as such could be responsible for anti-AGE formation. This is evidenced by the recent report by Han *et al.,* (2014) that Asn-Trp dipeptides inhibited the formation of AGEs in mice models.

b) *α-Glucosidase Inhibition*

α-glucosidase is one of the enzymes found on the brush border membranes of the intestinal mucosa and participates in carbohydrate digestion by hydrolyzing glucose residues from oligosaccharides (Voet and Voet, 2011). Thus, the modulation of the activity of this enzyme represents on key strategy in the control of blood glucose levels in the management of

diabetes mellitus (Qaisar *et al.,* 2014). All four hydrolysates demonstrated a concentration-dependent inhibition of α-glucosidase in hydrolyzing p-nitrophenyl glucopyranoside to p-nitrophenol, with tryptic hydrolysates displaying the highest activity of 80.62% at a maximum concentration of 1.00mg/ml which was higher than 54.54% obtained by Arise *et al.,* (2019) for tryptic hydrolysates of *Luffa cylindrica* seed protein hydrolysates. This suggests that *M. oleifera* seed protein hydrolysates encode bioactive peptides which could work synergistically to cause effective inhibition of α-glucosidase in vitro. In addition, as with the inhibition of hemoglobin glycosylation, the presence of certain residues in specific positions in peptides appear to play vital roles in α-glucosidase inhibition. Ibrahim *et al*.,(2018) reported that peptides containing proline, basic or hydroxy aminocyl residues are strong inhibitors of α-glucosidase. It is known that trypsin, being a residue-specific endopeptidase, cleaves peptide chains at C-terminal basic aminoacyl residues, chymotrypsin hydrolyzes proteins at residues having aromatic side chains while papain and pepsin non-selectively cleave at hydrophobic residues(Voet and Voet, 2011); and *M. oleifera* seed proteins are rich in positively charged amino acids and hydrophobic amino acids(Okereke and Akaninwor, 2013). These could, in part explain the ability of these peptides to inhibit α-glucosidase. In addition, tryptic and chymotrypsin hydrolysates had lower IC_{50} values, when compared to peptic and papain hydrolysates, exhibiting better inhibitory activities.

c) Kinetic Analysis of α-glucosidase Inhibition

Kinetic parameters obtained from the double-reciprocal plots in Figures 5-8 were summarized in Table 1. The Michaelis constant, k_m, of α-glucosidase for p-nitrophenyl glucopyranoside in the absence of inhibitor was determined to be 0.297mg/ml p-NPG in this study. This is slightly higher than 0.211mg/ml (0.7mM) p-NPG obtained by Awosika and Aluko (2019) and lower than 6.31mg/ml reported by Arise et al., (2019). $V_{max,}$ in the absence of inhibitory hydrolysates was 270.27mM/mg/ml. The Lineweaver Burk plots indicate that hydrolysates derived from peptic digestion displayed a mixed type of inhibition at 0.5mg/ml and an uncompetitive type of inhibition at 1.0mglml. This partly compares to the uncompetitive mode of inhibition obtained by Arise *et al.,* (2019) for *Luffa cylindrica* seed protein hydrolysates. Papain hydrolysates on the other hand, showed mixed mode of inhibition at all concentrations. This means that the peptides in the hydrolysate preparations tend to bind and inhibit the α-glucosidase in both its free form and p-NPG bound forms, creating dead-end complexes. Chymotrypsin and tryptic hydrolysates on the other hand exhibited a competitive type of inhibition, which was in contrast to an uncompetitive mode of inhibition for the 1kD fraction of Chymotrypsin hydrolysates derived from yellow field pea proteins as reported by Awosika and Aluko (2019). This could be because the hydrolysates used in this study were unfractionated, thus containing peptides of different lengths and molecular sizes. In addition, the presence of proline, basic and bulky aminoacyl residues in the peptide chains could confer on them, the ability to lock into the enzyme active site (Yu *et al.,* 2011), thereby preventing substrate binding.

Maximal rate of reaction, Vmax, as well as catalytic efficiency, CE, of the enzymatic reaction were reduced by the four hydrolysates, which is usually seen with the different modes of inhibition. The enzyme-inhibitor dissociation constant, $k_{i,}$ of 0.193mg/ml, 0.203mg/ml and 0.305mg/ml determined for chymotryptic, and peptic and tryptic hydrolysates respectively, and was lower than 10.51mg/ml and 49.83mg/ml obtained for tryptic and peptic hydrolysates Arise *et al.,* (2019) for *Luffa cylindrica* seed protein hydrolysates. This indicates that hydrolysates derived from chymotrypsin and tryptic digestion showed higher binding affinity for α-glucosidase when compared to papain hydrolysates.

V. Conclusion

To summarize, the hydrolysates derived from enzymatic digestion of *M. oleifera* seed proteins demonstrated potential anti-diabetic activities in-vitro by inhibiting both the formation of glycosylated haemoglobin and α-glucosidase activity. Peptic and chymotrypsin hydrolysates displayed better inhibitory effects against non-enzymatic glycosylation of haemoglobin, while chymotrypsin and tryptic hydrolysates demonstrated higher α-glucosidase inhibitory properties. This not only justifies the use of *M. oleifera* seeds for alernative therapeutic purposes, but may also indicate that these proteins could be potential sources of biologically active peptides which could be optimized to formulate new and potent anti-diabetic agents. Further studies such as fractionation of these hydrolysates and characterization of resulting peptides responsible for the observed biofunctional properties are suggested, and are currently underway.

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Histological Effects of Aqueous Stem Bark Extract of Cadaba Farinosa on Gastrointestinal Tract of Wistar Rats

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Abstract- *Background:* Cadaba farinosa fork is widely used traditionally as mucosae plant medicine for treatments of diarrheal, dysentery, intestinal parasites, and ulcerative peptic diseases that are hard to cure even with conventional medicines.

Aim and Objective: The main aim of the study is to evaluate the possible histological effects of aqueous stem bark extract of Cadaba farinosa on the gastrointestinal tract of Wistar rats.

Materials and Methods: The lethal dose (LD50) of aqueous stem bark extract of Cadaba farinosa was determined using the Lorke's method. Thirty (30) male and female Wistar rats were selected and randomized into five groups of six rats per group. Group 1 served as the control group, and no extract was administered to the experimental animals while the rats in groups 2, 3, 4, and 5 were administered by gavage dose levels 100, 200, 300, and 400 mg/kg extract for twenty-eight days.

Keywords: *cadaba farinosa, stem bark, wistar rats, intestinal goblet cells, mucin, and cyclooxygenase.*

GJMR-B Classification: *NLMC Code: QV 4*

Strictly as per the compliance and regulations of:

Histological Effects of Aqueous Stem Bark Extract of *Cadaba Farinosa* on Gastrointestinal Tract of Wistar Rats

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Result: The lethal dose (LD_{50}) of aqueous stem bark extract of *Cadaba farinosa* is above 5000mg/kg. Sub-chronic oral administration of aqueous stem bark extract of *Cadaba farinosa forsk* at the tested doses showed numerous intestinal goblet cells that secrete mucin and cyclooxygenase.

Conclusion: Acute oral administration of aqueous stem bark extract of *Cadaba farinosa forsk* is safe up to 5000mg/kg body weight/day. Sub-chronic oral administration of aqueous stem bark extract of *Cadaba farinosa forsk* at the tested doses showed numerous intestinal goblet cells that secrete mucin and cyclooxygenase (COX) responsible for the synthesis of prostaglandin. Hence, *Cadaba farinosa forsk* is a possible source of anti-peptic ulcer drug since prostaglandins deficiency plays a critical role in the background of gastrointestinal lesions.

Keywords: cadaba farinosa, stem bark, wistar rats, intestinal goblet cells, mucin, and cyclooxygenase.

I. Introduction

A peptic ulcer disease is major diseases of the gastrointestinal tract seen throughout the world[1]. The formation of peptic ulcer diseases depends on the presence of acid and peptic activity in gastric juice with a breakdown in mucosal defenses of the gastrointestinal tract[2]. The prevalence of Helicobacter pylori infection and widespread use of acetylsalicylic acid and other nonsteroidal anti- inflammatory drugs (NSAIDs) are known etiologic agents disrupting mucosal resistance to injury[3,4]. Several other pathogenic elements postulated for gastrointestinal lesions, include prostaglandins deficiency, bile acids, bacterial flora, and nitric oxide[5,6], yet the precise mechanisms remain unknown.

Currently, the prevention and cure of peptic ulcer diseases are among global health challenges confronting medicine[2]. In our review, most reported studies give the general idea of peptic ulcer and its management using synthetic drugs demonstrated intermittent relapses and adverse drug interactions[7,8,9].

However, many medicinal plants have been reported to possess beneficial effects in gastrointestinal disorders, especially ulcerative peptic diseases with a high level of safety compared to most synthetic drugs[10]. In developing countries, most people still rely on medicinal plants to meet their health needs, especially in cases where synthetic medicines could not provide relief from hard-to- cure illnesses[11,12]. *Cadaba farinosa* belongs to the capparidaceae (capparaceae) family[13]. The plant is enriched with abundant phytochemicals, including flavonoids and alkaloids are widely used in traditional medicine as antibacterial, antiprotozoal and anthelminthic agents to treats diarrheal, dysentery, and gastrointestinal parasites[13,14]. The stem bark of *Cadaba farinosa* served as aperients, purgative, and stomachic stimulants. In that desert of India and Pakistan, its extract is externally applied to fresh wounds to prevent sepsis, thereby assisting in healing[15,16]. In Nigeria, the analgesic and anti-inflammatory properties of *Cadaba farinosa* was reported among the people of Maiduguri, Jimeta, and Nguru[17].The plant was also used in the management of gastric and duodenal ulcers by inhibition of carbonic anhydrase[18].Hence, these findings prompted us to study the possible histological effects of the aqueous stem bark extract on the gastrointestinal tract.

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II. Materials and Method

a) Experimental Animals

Wistar rats were procured from the Animal House, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University Sokoto, and maintained with free access to standard animal pellets and water. The permission and approval for animal studies with Reg. NO: PTAC/Cf/OT/004-18 was obtained from the Faculty of Pharmaceutical Sciences, Animal Ethics Committee, Usmanu Danfodiyo University Sokoto.

b) Plant Collection

The fresh stem bark of *Cadaba farinosa* was harvested from its natural habitat at the Faculty of Pharmaceutical Science, Usmanu Danfodiyo University Sokoto. The plant was authenticated and deposited at the Department of Pharmacognosy and Ethno medicine, Usmanu Danfodiyo University Sokoto, Nigeria.

c) Plant Extraction

The fresh inner stems bark was shadow dried and pounded into small pieces using pestle and mortar. About 210g powdered plant material was soaked and extracted in 600mL of water at room temperature for 24 hours[19]. The liquid filtrates were concentrated and evaporated to dryness at 45°C in a water bath. The aqueous extract was stored at -4°C until used.

d) Experimental Design

The acute toxicity and LD_{50} determination were carried out using Lorke's method[20]. According to guideline 423 of the Organization for Economic Cooperation and Development (OECD), the first phase consists of nine Wistar rats that were separated into three groups of three rats each and the aqueous extract was administered by gavage at dose levels 10, 100, and 1000 mg/kg/day. A cage side observation was done to detect any behavioral signs of toxicity salivation, erection of the hair, diarrhoea or mortality.

Following the absence of toxicity sign, the second phase according to Lorke's consisted of three rats that were administered with dose levels 1600, 2900, and 5000mg/kg/24 hours. The animals were observed for signs of toxicity.

In the sub-acute study, thirty (30) male and female Wistar rats were selected and randomized into five groups of six rats per group. Group 1 served as the control while the rats in groups 2, 3, and 4 were administered with plant extract by gavage at dose levels 100, 200, 300, and 400mg/kg for twenty-eight days.

Table 1: Acute toxicity study and LD_{50} determination of aqueous stem bark extract of *Cadaba farinosa* on Wistar rats (n=12)

Dose	Mortality	
mg/kg body weight	Phase I	Phase II
10		-
100		-
1000		-
1600	-	
2900	-	
5000	-	

e) Tissue Histology

The intestines of Wistar rats were excised by abdominal incision and the tissues were fixed in 10% formal saline for 24 hours before being processed and embedded in paraffin wax. The tissues were sectioned with a rotary microtome at 5μm, and the cut sections were stained with Haematoxylin and Eosin (H&E) stain[21]. The stained slides were carefully examined under a light microscope at high power magnification, and photomicrographs were taken[22].

III. Results

a) Acute toxicity and LD_{50} determination

The toxicity study and LD_{50} determination result (Table 2) showed that oral administration of the aqueous stem bark extract of *Cadaba farinosa* at dose levels 10, 100, 1000mg/kg/24hours produced no behavioral sign of toxicity or mortality.

In Phase II, oral administration of the extract at dose levels 1600, 2900, and 5000mg/kg/day, indicated neither behavioral change nor death. The animals were as active as control. Therefore, the median lethal dose (LD_{50}) of aqueous stem bark extract of *Cadaba farinosa* is above 5000mg/kg.

Table 2: Acute toxicity study following oral administration of aqueous stem bark extract of *Cadaba farinosa* on adult Wistar rats (n=12)

Dose	Mortality	
mg/kg body weight	Phase I	Phase II
10	0/3	-
100	0/3	-
1000	0/3	-
1600	-	0/1
2900	-	0/1
5000	-	0/1

b) Tissue effects of plant extract

Our histological finding showed normal intestinal goblet cells lined by epithelial cells and intact submucosa and smooth muscle layers (Plate 1).

Extract administration of 100mg/kg/28days showed considerably increased intestinal goblet cells lined by epithelial cells and well-preserved submucosa and smooth muscle layers (Plate 2).

Extract administration of 200,300 and 400mg/kg/28 days showed numerous intestinal goblet cells, and well-preserved submucosa and smooth muscle layers (Plate 3, 4, and 5).

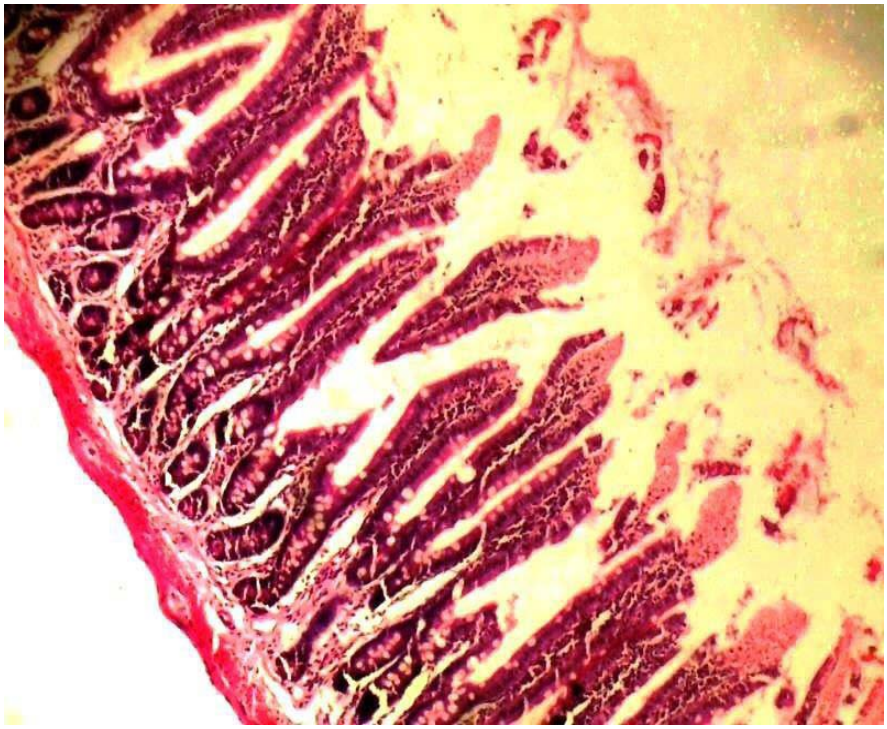

Plate 1: Normal control showed a mucosa layer with few intestinal goblet cells lined by epithelial cells and intact submucosa and smooth muscle layers. (H&E. X100).

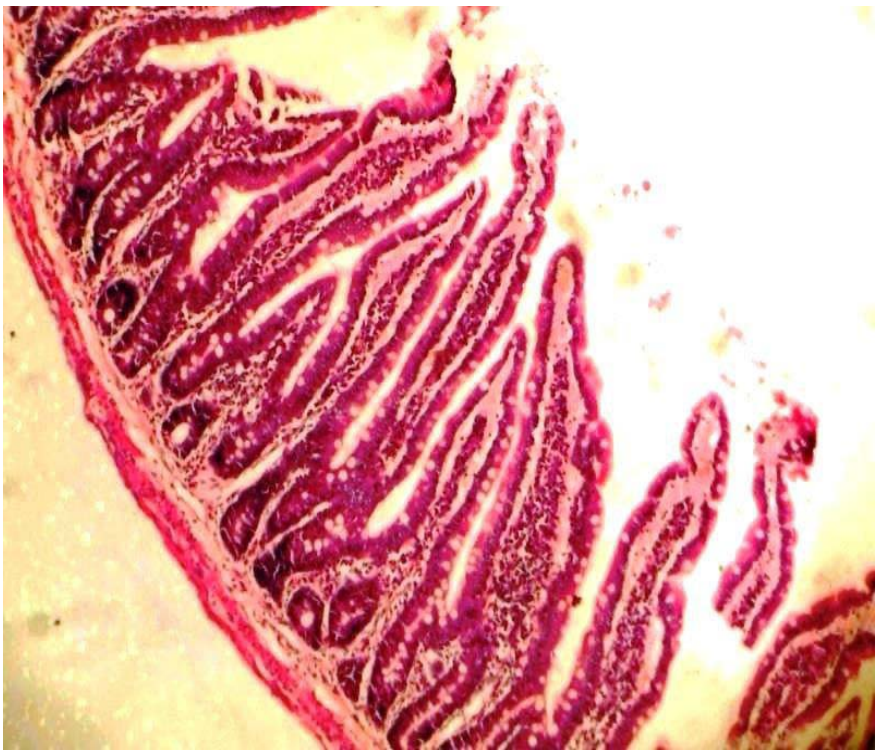

Plate 2: Administration of 100mg/kg/28days showed a mucosa layer with considerably increased intestinal goblet cells lined by epithelial cell and well- preserved submucosa and smooth muscle layers. (H&E. X100).

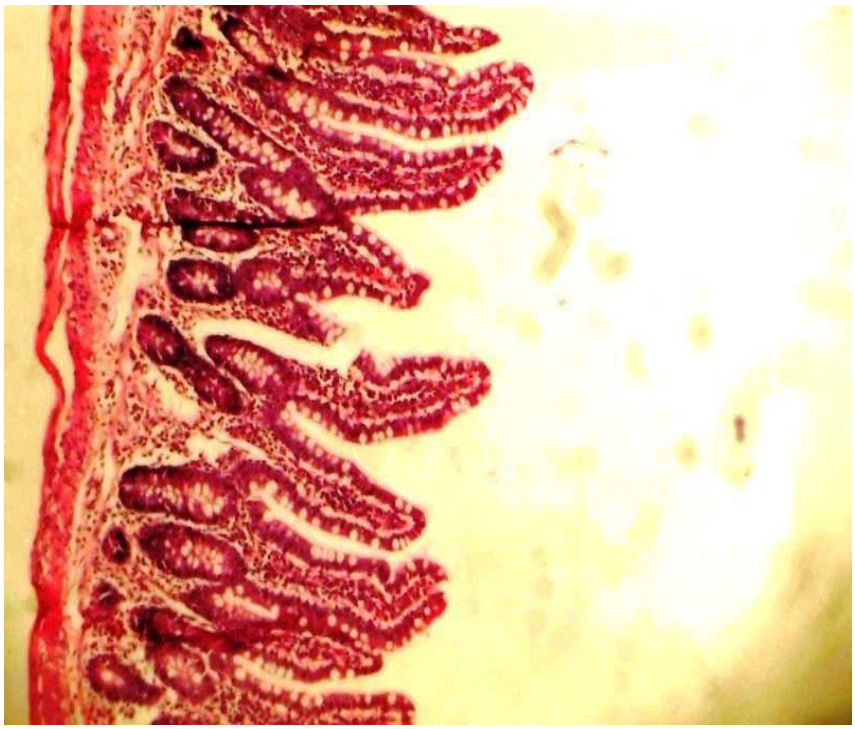

Plate 3: Administration of 200mg/kg/28days showed a mucosa layer with numerous intestinal goblet cells lined by epithelial cells and well-preserved submucosa and smooth muscle layers. (H&E. X100).

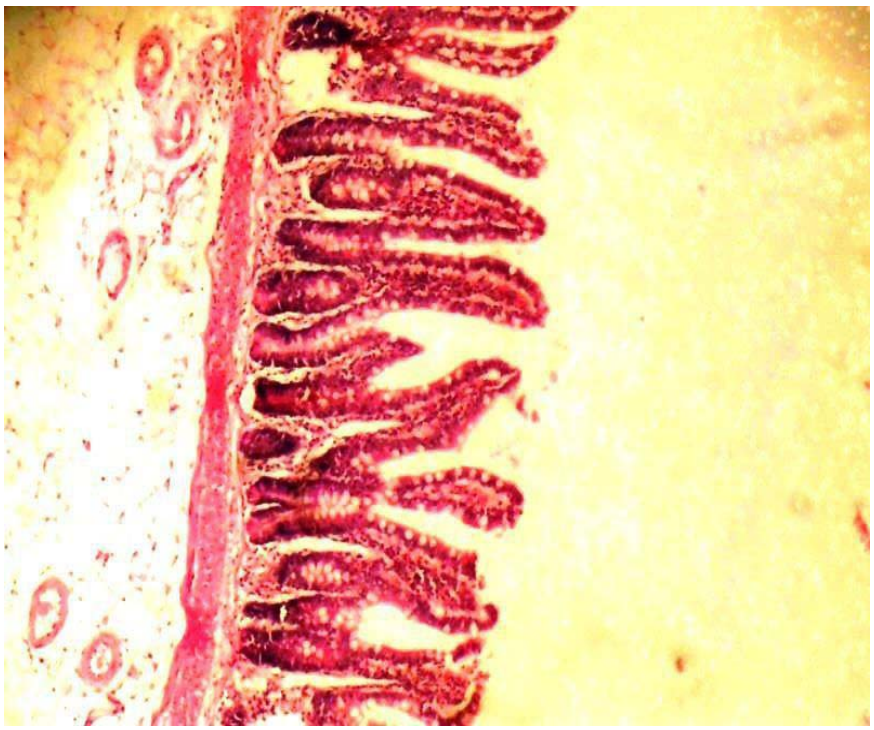

Plate 4: Administration of 300mg/kg/28days showed a mucosa layer with numerous intestinal goblet cells lined by epithelial cells, and well-preserved submucosa and smooth muscle layers. (H&E. X100).

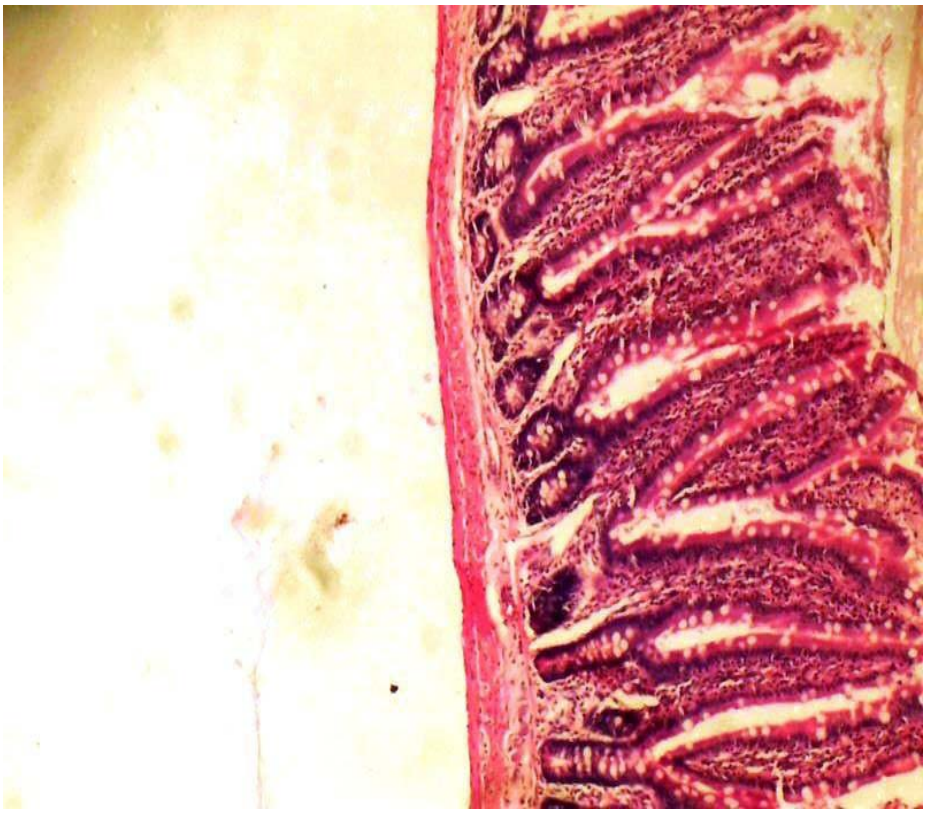

Plate 5: Administration of 400mg/kg/28days showed a mucosa layer with numerous intestinal goblet cells lined by epithelial cells, and well-preserved submucosa and smooth muscle layers H&E. X100).

IV. Discussion

Gastrointestinal lesions (ulcerative peptic diseases) is associated with several pathogenic elements, including prostaglandins deficiency, bile acids, bacterial flora, and nitric oxide,[5,6] yet the precise mechanisms remain unknown[23]. However, the overwhelming proportions of chemical agents (84.45%) used in most pharmaceutical industries for the production of conventional drugs used for the management of gastrointestinal lesions are gotten from plants[24]. In the present study, the acute toxicity study revealed that oral administration of *Cadaba farinosa* extract up to 5000mg/kg produced no immediate signs of toxicity or mortality indicating that the LD_{50} was above 5000 mg/kg, therefore, this explains that aqueous extract of *Cadaba farinosa* could be administered to animals with some degree of safety, through oral route where absorption might not be complete due to inherent factors limiting gastrointestinal tract absorption[25].

Our histological finding, showed a mucosa layer with few intestinal goblet cells lined by epithelial cells with intact submucosa and smooth muscle layers (Plate 1), while extract dose level 100mg/kg/28days showed considerably increased intestinal goblet cells and well-preserved submucosa and smooth muscle layers (Plate 2). A mucosa layer with numerous intestinal goblet cells that was lined by epithelial cells and well-preserved submucosa, and smooth muscle layers were seen in animals at dose levels 200, 300, and 400mg/kg compared to the normal control with few intestinal goblet cells.

Intestinal goblet cells are unicellular glands[26]. These cells synthesized and secretes mucin and cyclooxygenase (COX) responsible for the synthesis of prostaglandin that is expressed in cyclooxygenase pathway 1 (COX-1) and the inducible cyclooxygenase pathway 2 (COX- 2) isoforms[27]. The intestinal goblet cells following oral administration of aqueous stem bark of *Cadaba farinosa* is indicative of high secretion of mucin COX-2 that would preserve vulnerable cellular compartment of the gastrointestinal tract. The luminal prostaglandin modulates acid concentration by inhibiting acid secretion, alter blood flow, and stimulate mucus and bicarbonate secretion leading to dramatic protection against mucosal damage. Our result is similar to the role of endogenous prostaglandins in gastric secretion and mucosa defence[28], the anti-inflammatory effects of prostaglandins in ameliorating mucosal damage[26,27], and stimulation of duodenal bicarbonate and mucus secretion mediated intestinal mucosal protection[29,30,31]. These physiological functions of mucin COX-2 could modulate major etiologic factors implicated in ulcerative peptic diseases, including lesions caused by NSAIDs were effectively prevented by supplementation of exogenous prostaglandin-endoperoxide synthase (PGE2)[31,32,33].

Therefore, stem bark extract of *Cadaba farinosa* is a possible source of a drug, which modulates secretions of mucus, acid, and bicarbonates preceding dramatic protection against mucosal damage risk factors of ulcerative peptic diseases and gastrointestinal hemorrhage.

V. Conclusion

This study showed that acute oral administration of aqueous stem bark extract of *Cadaba farinosa forsk* is safe up to 5000mg/kg body weight/day. Sub-chronic oral administration of aqueous stem bark extract of *Cadaba farinosa forsk* at the tested doses showed numerous intestinal goblet cells that secretes mucin and cyclooxygenase (COX) responsible for the synthesis of prostaglandin. Hence, *Cadaba farinosa forsk* is a possible source of anti-peptic ulcer drug since prostaglandins plays a critical role in the background of gastrointestinal lesions.

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Conflict of interest
None declared

Ethical approval
No: PTAC/Cf/OT/004-18 was obtained from the Faculty of Pharmaceutical Sciences, Animal Ethics Committee, Usmanu Danfodiyo University Sokoto.

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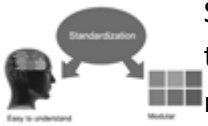

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Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. 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 Medical 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 medical research 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.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
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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