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Serum Antioxidant, Sex Hormones and Histopathological Examination of the Ovarian Tissues of Diabetic Rats Treated with Polyherbal Therapy

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Authors' contributions

This work was carried out in collaboration between all authors. Author EEE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EHI and PEE managed the analyses of the study. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To access the impact of single and polyherbal therapy of *Vernonia amygdalina, Moringa oleifera, Ocimum gratissimum* and *Gongronema latifolium* on oxidative stress indices and ovary of diabetic rats.
Study Design: An experimental diabetes mellitus model was created in 78 rats.
Place and Duration of Study: Department of Biochemistry, University of Calabar and Department of Chemical Pathology, University of Calabar Teaching Hospital, Calabar, 2013-2014.
Methodology: Thirteen groups of 6 rats each were used. Groups 1 and 2 Normal and Diabetic Control received 0.5 ml Dimethylsulphoxide; 3 and 4 received 5 UI/Kg b.w insulin and 5 mg/Kg b.w glibenclamide; 5, 6, 7 and 8 received 500 mg/Kg b.w of *Vernonia amygdalina*, *Moringa oleifera*, *Gongronema latifolium* and *Ocimum gratissimum* extracts respectively; 9, 10 and 11 received 250

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mg/ Kg b.w of *M. oleifera*/*V. amygdalina*, *M. oleifera*/ *G. latifolium* and *M. oleifera*/*O. gratissimum* respectively; 12 received 166.66 mg/kg b.w of *V. amygdalina*/ *G. latifolium*/ *O. gratissimum* while 13 received 125 mg/kg b.w of all extracts.

Results: After 28 days of treatment, the animals were anaesthetized and sacrificed to obtain blood by cardiac puncture. Serum was collected and assayed for malondialdehyde, glutathione peroxidase and sex hormones while the ovaries were collected for histopathological examination. Malondialdehyde and Gluthathione peroxidase increased and reduced respectively in the diabetic control group and reduced and increased respectively in the treated groups except group 8. Combination of *O. gratissimum* with *M. oleifera* ameliorated the effect observed in group 8. Sex hormone reduced for diabetic control but increased in the treated groups. Group 5 and 12 showed better results than others. Synergy was observed more in group 12 than in others. Histological observation confirmed this.

Conclusion: The findings demonstrated that groups 5, 11 and 12 were more potent at removing oxidative radical produced in diabetes as well as correct the injury to the ovary.

Keywords: Ovary; diabetes; phytomedicine; antioxidants; malondialdehyde; gluthathione peroxidase; female sex hormones; streptozotocin; histology.

1. INTRODUCTION

Diabetes is characterized by hyperglycaemia, which gives raise to oxidative stress and other complications ranging from nephropathy, retinopathy, sexual dysfunction and cardiovascular disorders. Oxidative stress is considered the most important factor in the onset and progress of diabetic complications [1]. It is the imbalance between production and removal of reactive oxygen species (ROS). Increased oxidative stress contributes greatly to the development and progression of tissue and vascular complications of diabetes, either by enhanced ROS production or attenuated ROS scavenging ability. Catalase, Cu/Zn superoxide dismutase (SOD), and glutathione peroxidase (GPx) are biological antioxidant enzymes that participate directly in scavenging free radicals. Thus, preventing the conversion of free radicals to toxic products [2]. According to Stohs and Bagchi [3], the evaluation of lipid peroxidative activities of antioxidant enzymes such as GPx is a useful biomarker for tissue damage.

The antioxidant defence system is a complex network of interactions, synergism and specific tasks for a given antioxidant [4]. Research reports have shown alterations in antioxidant enzymes in diabetes [5,6] which is considered a major cause of diabetes-related complications and onset of other disease conditions like atherosclerosis and coronary heart disease [7].

Diabetes has also been associated with alteration in the sexual activity in men and women such as reduced libido and fertility [1], inadequate lubrication and pain on sexual intercourse [8,9]. Diabetes when uncontrolled causes ovarian dysfunction, changes in estrous behaviour, follicular growth, oocyte maturation and decline in ovulation. There is also alteration in the timing of the estrous cycle which is involved with modifications in ovary function, thus, inducing a decrease or even absence of ovulated oocytes and oocyte maturation in female rats [10,11,12]. Women with diabetes have higher prevalence of menstrual irregularities than nondiabetic women of similar age [13]. The irregularities observed in the menstrual cycle in women may be attributed to the modification of the hypothalamus-pituitary signal pathway which leads ultimately to the synthesis of female sex hormones [14].

The control of blood glucose level in diabetics leads to the delayed onset and control of diabetic related complications. Antidiabetic plants are being used to correct hyperglycaemia and may decrease the side effect of diabetes mellitus on ovary. Several plants have been reported to possess fertility regulating properties and a few have been tested for such effect [15]. These medicinal plants are rich sources of antioxidants which are capable of scavenging free radicals and ameliorating the damage of diabetes on tissues. However, polyherbal therapy [16] has proven more beneficial in the management of various diseases [17] because of the synergistic effect of the different plant principles. Examples of plants used as antidiabetic treatment are *Vernonia amygdalina, Moringa oleifera, Ocimum gratissimum* and *Gongronema latifolium* [18,19].

V. amygdalina is also known as African bitter leaf. It is used traditionally to manage diabetes in the African sub–Region and Asia. It belongs to

the Compositae family Asteraceace and grows extensively in a range of ecological zones in tropical Africa [20]. *G. latifolium* (Asclepiadaceae) is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine [18]. The leaves have protective role against diabetes, hypertension, stomach upsets, pains, and typhoid fever [21]. *O. gratissimum* is also called Africa basil/sweet basil. It is a plant belonging to Lamiceae family known in Nigeria as nton, efinrin, Nehonwu, and ai daya ta guda by the Efiks, Yoruba, Igbo and Hausa, respectively. The Ocimum species are widely found in tropical and subtropical regions and commonly used as food spice and traditional herb [22]. *M. oleifera* is present worldwide but was originally native to the sub-Himalayan tracts of India, Bangladesh, Afghanistan and Pakistan where it is used in folk medicine [23]. It is referred to as a "miracle tree" or a "wonder tree" of significant socio economic importance because of its several nutritional, pharmacological and industrial applications [24].

These plants are used traditionally in the management of diabetes and their hypoglycaemic activity has been reported by Ugochukwu et al. [18], Mohammed et al. [25], Jaiswal et al. [26] and Ebong et al. [27] for G. latifolium, O. gratissimum, M. oleifera and V. amygdalina respectively. These plants produces a variety of flavonoids and bitter sesquiterpene lactones which contribute to their bioactivities [28].

It is therefore intended in this study to evaluate the antioxidant effect of single and different combined extracts from *V. amygdalina, M. oleifera*, *O. gratissimum* and *G. latifolium* leaves on oxidative stress indices by measuring serum malondialdehyde (MDA) and Gluthatione peroxide (GPX) levels. The impact of the different extracts on the ovarian histology was also investigated.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The fresh leaves (500 g) of *V. amygdalina, M. oleifera*, *O. gratissimum* and *G. latifolium* were collected from the Endocrine Research farm, University of Calabar, Calabar.

2.2 Chemicals Used

All chemicals and drugs used were obtained commercially and of analytical grade.

2.3 Preparation of Extract

Fresh leaves of *V. amygdalina, M. oleifera*, *O. gratissimum* and *G. latifolium* were collected, macerated and allowed to stand in 80% alcohol at room temperature for 48 hours. The filtrate was evaporated in a rotary evaporator and allowed to concentrate in a water bath at 36°C. A greenish paste was obtained. The whole leaves extraction of *V. amygdalina, M. oleifera*, *O. gratissimum* and *G. latifolium* leaves was done in the Department of Biochemistry, University of Calabar. The obtained whole leaf extracts were stored at 4°C.

2.4 Experimental Animals

Seventy eight (78) female rats weighing between 120- 180 g, were obtained from the Department of Biochemistry animal house, University of Calabar. These were divided into 13 groups of 6 rats each. Before the experiment, the rats were allowed to acclimatize to the animal house for 7 days. Standard environmental conditions such as temperature (26 ± 2°C), relative humidity (45-55%) and 12 hours dark/light cycle were maintained. All the animals were fed with standard rat chow and water was allowed *ad-libitum* under strict hygienic conditions.

2.5 Acute Toxicity Test (LD_{50})

The oral acute toxicity of the ethanol extract (EE) was determined in mice as described by Lorke [29].

2.6 Induction of Diabetes

STZ was prepared in citrate buffer (0.1 M, pH 4.5). STZ solution was injected intraperitoneally at a concentration of 40 mg/kg of body weight in a volume of 0.5 ml/rat. Diabetic condition (type I) was confirmed in fasting rats from blood glucose level more than 150 mg/100 ml determined 72 hours after day of injection.

2.7 Experimental Design

The 78 adult female wistar albino rats weighing 120- 180 g were grouped into thirteen (13) as shown in Table 1.

2.8 Histopathological Examination of the Ovary Tissues Using Haematoxylin and Eosin Staining Technique

2.8.1 Procedure

Tissue blocks were sectioned at 5 micron with a microtome. Sections were stained with

Table 1. Animal grouping

S/No	No of animals	Treatment
1	6	Normal control
2	6	Placebo (Diabetic control)
3	6	Insulin (5 IU/kg b.w s.c.)
4	6	Glibenclamide (0.5 ml)
5	6	*V. amygdalina* (500 mg/kg b.w)
6	6	*M. oleifera* (500 mg/kg b.w)
7	6	*G. latifolium* (500 mg/kg b.w)
8	6	*O. gratissimum* (500 mg/kg b.w)
9	6	*M. oleifera/ V. amygdalina* (250 mg/kg b.w each)
10	6	*M. oleifera/ G. latifolium* (250 mg/kg b.w each)
11	6	*M. oleifera/ O. gratissimum* (250 mg/kg b.w each)
12	6	*V. amygdalina/ G. latifolium/ O. gratissimum* (166.66 mg/kg b.w)
13	6	*V. amygdalina/ G. latifolium/ O. gratissimum/ M. oleifera* (125 mg/kg b.w)

Treatment was administered twice daily (12 hourly) for 28 days

haematoxylin and eosin (H and E) technique. Sections were brought to xylene for two minutes per two changes. The xylene was cleared in 95% alcohol for 1 minute per two changes and then in 70% alcohol for another minute. This was then hydrated in running tap water for 15 minutes, stained with haematoxylin for 15 minutes, differentiated in 1% alcohol (3 dips) and blue in running tap water for 10 minutes. The slides were then counterstained with 1% alcohol eosin for 1 minute followed by rapid dehydration in ascending grade alcohol, clearing in xylene and mounting with DPX mountant. The properly stained slides were then mounted on a light microscope and photomicrographed at X 100 magnificaion.

2.9 Statistical Analysis

The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett's method. The values of $p<0.05$ were taken as significant.

3. RESULTS

3.1 Effect of Treatment on Glutathione Peroxidase

From Table 2, there was a reduction in glutathione peroxidase in the diabetic untreated group. The reverse was the case for the treated diabetic groups. VA, MO and GL recorded high levels, while OG level was not significantly (*P*> .05) different from DC. Combination of OG with MO reversed the result of OG. However, there was no synergistic effect in the double extract groups (MV, MG and M/O). Result for the combination of three extracts of VA, GL and OG and four extracts MO, VA, GL and OG gave a significant (*P*> .05) synergistic effect. The 3xt group showed a higher synergy than 4xt.

3.2 Effect of Treatment on Malondialdehyde

From Table 2, there was an increase in malondialdehyde levels in the diabetic untreated group. The reverse was the case for the treated diabetic groups. The reduction in MDA was not significant (*P*< .05) for OG when compared with the diabetic control group. Combining OG with MO reversed the result of OG. Nevertheless, the double extract groups (MV, MG and M/O) did not show a synergistic effect. Synergism was observed for the three and four extract groups with a better result noticed for 3xt group.

Table 2. Serum MDA and GPX concentrations

	GPX (nmol/min/ml)	MDA (nmol/ml)
NC	10.20 ± 0.8	1.50 ± 0.11
DC	4.56 ± 0.6*	1.60 ± 0.16
INS	7.84 ± 0.96 *,a	1.20 ± 0.57*,a
GB	5.12 ± 0.4 *	1.59 ± 0.19 *,a,b
VA	12.96 ± 0.9 *,a	0.95 ± 0.35 *,a,b
MO	19.7 ± 0.5 *,a,b	0.13 ± 0.12 *,a, b
GL	14.6 ± 0.7 *,a, b	0.43 ± 0.01 *,a,b
OG	4.58 ± 0.9 *,b	1.59 ± 0.00 *,a,b
MV	5.01 ± 0.2 *,b	1.34 ± 0.00 a
MG	5.21 ± 0.6 *,b	1.40 ± 0.18*,b
M/O	7.76 ± 0.17 *,a	1.25 ± 0.18 *,a
3xt	22.75 ± 0.91 *,a, b	0.38 ± 0.06 *,a,b
4xt	20.12 ± 0.78 *,a,b	0.45 ± 0.02 *,a,b

Values are expressed as mean ± SEM.
**significantly different from NC at P< .05*
a = significantly different from DC at P< .05
b = significantly different from INS at P< .05

3.3 Effects of Treatment on Female Sex Hormones

From Table 3, testosterone level reduced in the diabetic control group when compared with the normal control group. Among the single extract groups, testosterone level increased in VA, MO and OG groups but reduced for GL when compared with DC. However, when each extracts of VA, GL and OG were combined with MO, no synergy was observed except for M/O group. Similar result in M/O group was observed for 4xt group. There was significant (*P*> .05) synergy observed for the 3xt group. Testosterone was highest in VA group followed by 3xt group.

Estradiol reduced in DC but increased in the treated groups. Among the single extract groups, its level was highest for VA group. Synergism was recorded for the polyherbal groups except 4xt group. There was significant synergy in 3xt group.

LH reduced in DC but increased in the treated groups except 4xt group. There was no significant (*P*> .05) synergy in the double extract groups. 3xt recorded significant (*P*< .05) synergy.

FSH levels reduced in DC and increased in the treated groups. Among the single extracts, the highest level of FSH was observed for VA, while among the double extract groups only M/O showed synergy.

3.4 Effects of Treatment on Ovary

Plates 1 to 13 are photomicrographs of the experimental rat ovaries showing different treatments. Haematoxylin and Eosin (mag. X 100).

Plate 1: Photomicrograph of normal rat ovary receiving placebo showing developing follicles (PRF, PF) at the cortex and secondary follicles (MGF) with viable oocytes, stroma is cellular and contain blood vessels (BV). Haematoxylin and Eosin (mag. X 100).

Plate 2: Photomicrograph of diabetic rat ovary receiving placebo showing few primary follicles (PF). Haematoxylin and Eosin (mag. X 100). BV = Blood Vessel, FT = Fallopian Tube, S = Stroma

Plate 3: Photomicrograph of diabetic rat ovary receiving insulin showing a vascularized cellular stroma with numerous corpus albican, primordial follicle (PRF), primary follicles (PF), secondary follicle without oocytes. Haematoxylin and Eosin (mag. X 100). BV = Blood Vessel

Plate 4: Photomicrograph of diabetic rat ovary receiving glibenclamide showing primordial (PRF), primary follicles (PF), matured graffian follicle (MGF) and corpus albican (CA). Haematoxylin and Eosin (mag. X 100).

Plate 5: Photomicrograph of diabetic rat ovary receiving *V. amygdalina* showing multiple graffian follicles (GF) at various levels of maturation- primary follicles (PF) and mature grafian follicles (MGF). The follicles are located within the cortex and consists of mainly granulose cells. Follicular antrum, primary oocytes and the theca layers are prominent. The medulla is cellular and highly vascularized. FT = Fallopian Tube, GF = Graffian Follicle, PRF = Primordial Follicle, S = Stroma.

Table 3. Effect of treatment on female sex hormones

	TEST (ng/ml)	E2 (pg/ml)	LH (MIU/ml)	FSH (MIU/ml)
NC	0.68±0.00	39.00±6.9	1.50±0.10	4.60±0.00
DC	0.20±0.00*	8.50±0.32*	0.10±0.00	0.10±0.00*
INS	0.31±0.08*	19.00±1.41*, a	0.15±0.04	6.05±0.04
GB	0.20±0.20*	56.00±0.00*, a,b	0.65±0.04*	0.09±0.01*, b
VA	0.50±0.00a,b	57.50±0.95*, a,b	0.15±0.03*	27.50±0.32*, a,b
MO	0.25±0.05*	10.00±4.00*, b	1.00±0.00	5.50±0.50 a
GL	0.15±0.04*	14.50±0.35*, b	0.90±0.07	5.50±0.35a
OG	0.23±0.04*	23.00±4.00*, a	1.00±0.00	0.60±0.08*, b
MV	0.19±0.00*	56.00±25.40*, a,b	0.95±0.03	3.90±1.85a,b
MG	0.19±0.00*	56.00±25.40*, a,b	0.75±0.03*	3.90±1.85a,b
M/O	0.24±0.04*	26.75±15.38*, a,b	0.15±0.04*	5.75±0.18a
3XT	0.41±0.15*, a	111.25±7.95*, a,b	1.20±0.14 a,b	5.80±0.14a
4XT	0.25±0.04*	9.00±0.71*,b	0.10±0.00*	5.05 ±0.74a

*Values are expressed as mean ± SEM; *significantly different from NC at P< .05; a = significantly different from DC at P< .05; b = significantly different from INS at P< .05*

Plate 6: Photomicrograph of diabetic rat ovary receiving *M. oleifera* showing developing ovarian follicles within the cortex- mature grafian follicle (MGF) with a prominent follicular antrum, with a primary oocytes and theca cell layers, numerous primary follicles (PF). The medulla is cellular and highly vascularized. CA = Corpus Albican.

Plate 7: Photomicrograph of diabetic rat ovary receiving *G. latifolium* showing multiple ovarian follicles (GF and PF) at various levels of maturation. Some of the follicles are atretic and degenerated. Corpus albican are seen. The stroma (S) appears hyalinized. Haematoxylin and Eosin (mag. X 100). BV = Blood Vessel.

Plate 8: Photomicrograph of diabetic rat ovary receiving *O. gratissimum* showing developing ovarian follicles (PF, GF, MGF) located at the cortex at various stages of development. Haematoxylin and Eosin (mag. X 100). FT = Fallopian Tube.

Plate 9: Photomicrograph of diabetic rat ovary receiving *M. oleifera* and *V. amygdalina* showing mainly primary follicles (PF) without oocytes. The medulla is highly vascularized and cellular. Corpus albican seen. Haematoxylin and Eosin (mag. X 100). BV = Blood Vessel, FT = Fallopian Tube, S = Stroma.

Plate 10: Photomicrograph of diabetic rat ovary receiving *M. oleifera* and *G. latifolium* showing developing follicles consisting of primary, secondary and mature grafian follicles (GF) within which are viable oocytes, the medulla is cellular and rich in blood vessels (BV). No pathology seen. Haematoxylin and Eosin (mag. X 100). S = Stroma.

Plate 11: Photomicrograph of diabetic rat ovary receiving *M. oleifera* and *O. gratissimum*. The cortex and medulla is prominent. The cortex consists of developing follicles at various stages of maturation (PRF, PF and GF). Mature graafian follicles has a distinct cell layer and prominent antrum. The medulla is highly cellular and vascularized. No pathology seen. Haematoxylin and Eosin (mag. X 100). FT = Fallopian Tube, S = Stroma.

Plate 12: Photomicrograph of diabetic rat ovary receiving *V. amygdalina, G. latifolium* and *O. gratissimum* showing ruptured corpus luteum (CL) consisting of polygonal shaped luteal cells, primordial follicles (PRF) lined by cuboidal to columnar epithelium and corpus albican (CA) are seen. Haematoxylin and Eosin (mag. X 100) PF = Primary Follicle.

Plate 13: Photomicrograph of diabetic rat ovary receiving *M. oleifera, V. amygdalina, G. latifolium* and *O. gratissimum* showing distinct cortex and medulla. In the cortex are mature graafian follicles with distinct theca zone, follicular cells and oocytes; a secondary follicle is also seen. The stroma is compact and highly cellular. No pathology; mature graafian follicles are prominent. Haematoxylin and Eosin (mag. X 100). CL = Corpus Luteum, GF = Graffian Follicle, PF = Primary Follicle, PRF = Primordial Follicle.

4. DISCUSSION

This study clearly demonstrated the importance of validating the ethnomedicinal uses of combined ethanolic leaves extracts of *M.oleifera, V. amygdalina, G. latifolium* and *O. gratissimum* as a basis for the continual use and integration into the modern medical system. A medicinal plant is one in which one or more of its organ contain phytochemicals which are precursors for the synthesis of drugs [30].

Plates 1 to 13. Effect of treatment on the histology of ovary

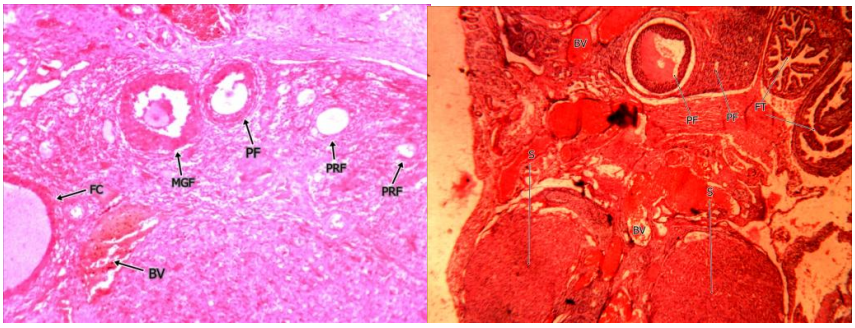

Plate 1. Normal control **Plate 2. Diabetic control**

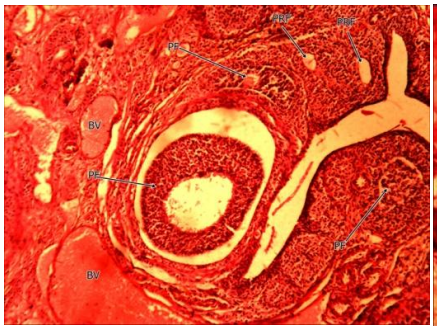

Plate 3. Insulin

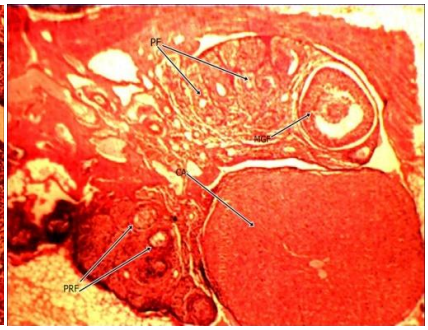

Plate 4. Glibenclamide

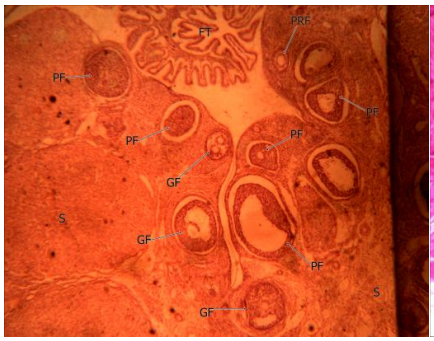

Plate 5. *Vernonia amygdalina*

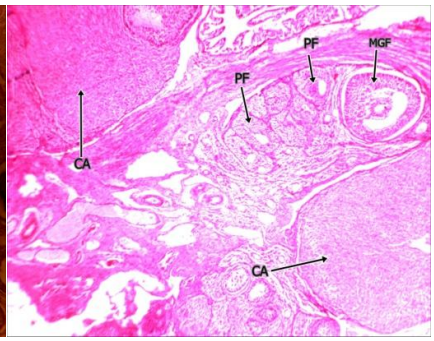

Plate 6. *Moringa oleifera*

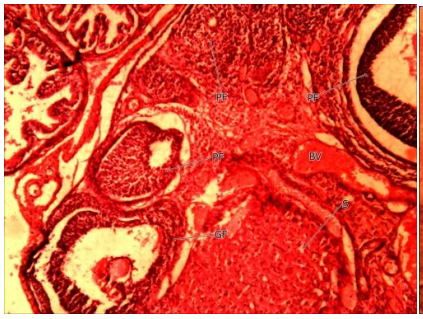

Plate 7. *Gongronema latifolium*

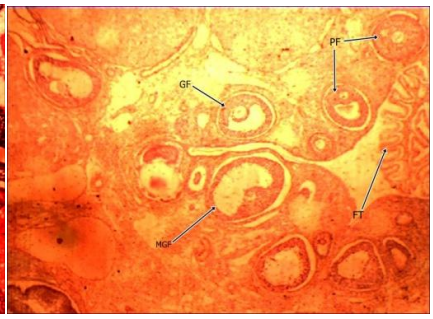

Plate 8. *Ocimum gratissimum*

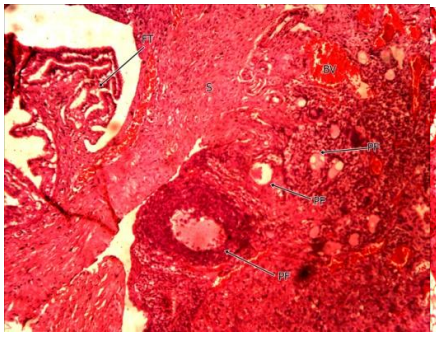

Plate 9. *Moringa oleifera* and *Vernonia amygdalina*

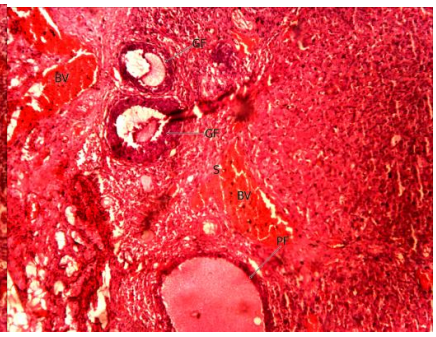

Plate 10. *Moringa oleifera* and *Gongronema latifolium*

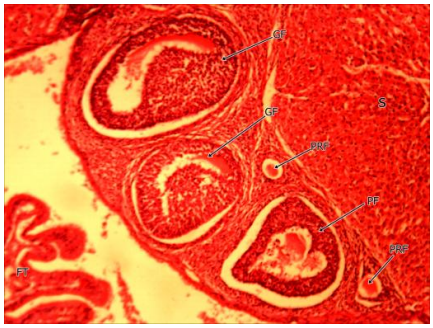

Plate 11. *Moringa oleifera* and *Ocimum gratissimum*

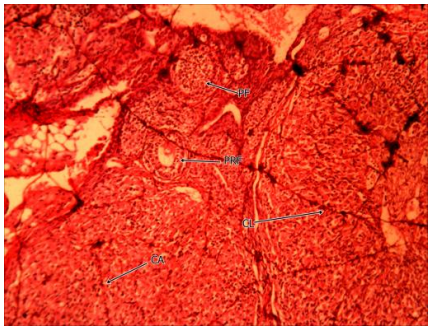

Plate 12. *Vernonia amygdalina,Gongronema latifolium* and *Ocimum gratissimum*

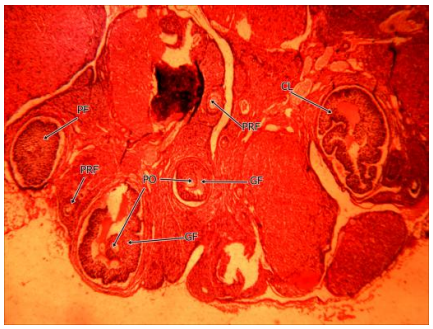

Plate 13. *Moringa oleifera, Vernonia amygdalina, Gongronema latifolium* and *Ocimum gratissimum*

From the results of this study, the reduction in MDA and increase in GPX values for all treated groups shows a remediating effect of the treatments. This is in tandem with another work by Akinola et al. [31]. An exception to this result was for OG treated group where MDA and GPX results were not significantly different from that of DC. However, when OG was combined with MO, there was a reversal of the result which compared well with the INS treated group. A similar observation was made by Ebong et al. [32] where combination of OG with MO ameliorated OG induced damage on the testes of diabetic rats. This finding further agrees with a similar work on OG by Obianime et al. [33]. The histological examination of the ovaries for each group confirms the biochemical results. Diabetic rats treated with a combination therapy in another work showed the maximum restoration in SOD, catalase, and GPx activities [2,27,34] which corroborated with findings from my work. Other reports have also shown that flavonoids, tannins and saponins may play some roles in antioxidative effect [19,35]. Antioxidant are significantly altered in untreated diabetic condition but could be ameliorated after treatment with medicinal plants with hypoglycaemic activities.

Disorders of female sex hormone include infertility, subfertility, hirsutism with the commonest pathological cause being polycystic ovarian syndrome [36]; virilism in which testosterone concentration is markedly increased [37].

LH and FSH acts cooperatively on the ovaries to stimulate sex hormone secretion and reproduction processes. FSH regulates the growth of ovarian follicles and stimulates testicular spermatogenesis [38,39] while LH triggers ovulation. Estradiol is responsible for female secondary characteristics, thus, stimulate follicular growth and development of the endometrium. The ruptured follicle differentiates into the corpus luteum which secretes progesterone and estradiol. The ruptured corpus luteum in the 3xt group accounts for the high levels of LH and E2.

There was a reduction in the female sex hormones in the diabetic untreated group.

According to Bukonla et al. [40], deficiency of testosterone increases the probability of infertility and could result in hypogonadism. There was a reversal of the sex hormones in the treated groups which in most cases compared well and even better than insulin and glibenclamide treated groups. The single as well as the combined extract groups showed protective effects on the ovarian structure of the treated rats and agrees with histological observation and with the underlying knowledge about the roles of the different sex hormones. The improvement in the biochemical result was more prominent for the 3xt and VA groups. The combination of each extracts of VA, GL with MO did not result in remarkable synergistic effect, while combination of OG and MO showed improvement when compared with results for OG group. The extracts have proven their abilities at reversing diabetes induced damage on the ovary of female diabetic treated rats. The histological results confirms the biochemical findings.

5. CONCLUSION

Treatment of diabetes induced ovarian dysfunction was more effective with the combination of *V. amygdalina, G. latifolium* and *O. gratissimum* followed by single treatment with *V. amygdalina*. The damage to the ovary by *O. gratissimum* was reversed when it was combined with *M. oleifera*. The improvement in the oxidative indices could be a possible mechanism for the reversal of diabetes induced injury to ovarian tissues. Thus, confirming the effectiveness of ethnobotanicals for the treatment of diabetes and associated female reproductive organ alterations.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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