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Effect of Crude Methanol Extract of Heliotropium indicum on Certain Biochemical Parameters in Rats with Monosodium Glutamate-Induced Fibroids

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Abstract
Background: Uterine fibroids or uterine leiomyoma are the commonest non-cancerous tumor affecting mostly women of age between 30-45 years. Hormones such as estrogen and progesterone produced by the ovaries cause the regeneration of the uterine lining which may stimulate the growth of fibroids. It has been established that monosodium glutamate (MSG) (a commonly used food seasoning) induces fibroids by increasing the levels of cholesterol, estradiol and total protein. Heliotropium indicum has been indicated in folkloric medicine to be anti-tumoric. The effect of crude Methanol Extract of Heliotropium indicum (MEHI) on some biochemical parameters in MSG-induced fibroid was studied. **Materials and Methods:** Twenty-five female Wistar rats were divided into five study groups; A(Control), B(MEHI – 50mg/kg bdwt), C(MSG – 50mg/kg bdwt), D(MSG - 50mg/kg bdwt + MEHI - 50mg/kg bdwt) and E(MSG - 50mg/kg bdwt + MEHI - 100mg/kg bdwt). The administration was carried out through intraperitoneal injection for 10days. The animals were fasted for 24hours before sacrifice after which the blood was collected into appropriate sample bottles. The levels of progesterone, estradiol, cholesterol, liver enzymes, antioxidant and hematological parameters were estimated using standard procedures. **Results:** The results showed that the MEHI has an ameliorative effect on MSG-induced fibroid as seen in its ability to reduce elevated levels of cholesterol, progesterone and estradiol. Also the extract elevated the levels of liver enzymes and antioxidants. Although there was insignificant decrease in the levels of antioxidants, this might be because the extract competes with the antioxidants for active site. Also, the plant extract improved packed cell volume and thus, elicits its ability as co-treatment for anaemia. Lesions at histopathology of uterus exposed to MSG alone revealed striking lesions ranging from moderate diffuse cellular infiltrates and hyperplastic endometrial glands in uterine mucosa to degenerate and necrotizing endometrial mucosa with atrophy of glands, while the ovaries exhibited lesions ranging from obliteration of ovarian follicles to attenuation of germinal epithelium (diffuse oophoritis). On the other hand the inclusion of MEHI in MSG-fibroid induced rat led to acute endometritis, moderate diffuse endometritis and glandular hyperplasia (uterus), while lesions in the ovaries ranged slightly from follicular atresia, atrophy of the gonads to normal ovaries in a dose dependent manner.**Conclusion:** These findings showed that the plant extract can be used for the treatment of uterine fibroid and its use could lead to improved packed cell volume of the patient, antioxidants activities and regulation of female reproductive hormones. Also, MSG has high fibroid generation tendencies which could lead to destruction of ovaries and uterus when consumed incessantly, while MEHI, showed a great potential in ameliorating the effect of MSG on ovaries and uterus, hence, MEHI is ovariohysteric protective.
Keywords: Uterine Fibroids, MSG, Heliotropiumindicum, PCV, Antioxidant

INTRODUCTION

Childlessness and infertility are health issues that affect couples globally and it has been defined by World Health Organization (WHO) as inability to conceive after two years of exposure to copulation which is expected to lead to pregnancy. WHO noted that women who are unable to conceive are termed as 'infertile' and these set of women live hopelessly with intense stigmatization in Africa [1].

There are quite a number of articles that are dedicated to help couples who have been diagnosed with infertility, as a couple of reasons have been ascribed to infertility, some of which are; blocked fallopian tubes, ovulation disorder, low body weight, uterine fibroids, ectopic pregnancy etc [2].

Uterine leiomyoma also known as uterine fibroid affects 20-50% of reproductive age women and its affiliation to infertility is quite divisive [3].Fibroid is a benign smooth muscle tumor of the uterus lining that is common in about 20 - 40% of women in their reproductive age. The classifications of these leiomyoma however are in line with their location with respect to the layers of the uterus; subserous, intramural, or submucous [4].

Studies have assessed the prevalence of uterine fibroids based on clinical assessment as 33%, ultrasound scan as 50% and histological examination of hysterectomy specimen as 77% [5].

Although the pathogenesis that leads to the formation of fibroid is still largely unknown, there is however risk factors that serves as pointers to the onset of its formation, some of these factors are; early menarche, parity

and pregnancy, caffeine intake, age, familial history, ethnic origin, obesity, diet, lack of exercise etc [6,7].

Large number of women with uterine leiomyoma may not experience any symptom (asymptomatic) and therefore leaves the fibroid medically unattended to through a long period of time given that it is benign, however from reviews on fibroids in recent years, some presentations of uterine fibroids have been highlighted; asymptomatic, abnormal bleeding, pelvic pressure, pelvic mass, pelvic pain, infertility, obstetric complications, malignancy, and benign metastasizing [8-9].

Emerging technologies are used in obtaining the diagnostic views of fibroids; one of such is Magnetic Resonance Imaging (MRI). MRI is able to diagnose uterine fibroid because it basically contains smooth muscle cells and fibrous connective tissue. They therefore form enlarged masses that easily outgrow their blood supply and thereby undergo necrosis creating the signal intensities read by MRI. Other technological methods are: ultrasonography and saline infusion sonohysterography [4, 10-11].

Some potential biomarkers of uterine leiomyomas has been documented, some of which are; Prolactin, Total Protein, Soluble Serum HLA-G, VEGF (Vascular Endothelial Growth Factor), Ghrelin and Obestatin, LHDA (Lactate Dehydrogenase A), DAPK (Hypermethylated Death-Associated Protein Kinase), Cancer Antigen-125, HGF (Hematopoietic Growth Factors), HE4 (Human Epididymis Protein 4) and Gonadal Hormones and Growth Factors [12].The most common however of the biomarkers is estrogen, which isinvolved in the mediation of receptors, transcription factors, kinase proteins, growth factors, and numerous autocrine and paracrine factors [13].

Research has suggested existing relationship between diet and the growth of uterine fibroids. More so, over a decade ago, a study by Chiaffarino and colleagues published in ***Obstetrics & Gynecology*** reported that uterine fibroids were associated with the consumption of ham and beef. The study further indicated that a high intake of green vegetables has a protective effect against fibroids. Although various risk factors have been associated with leiomyoma development, nutritional aspect in recent times has formed important aspect in the study of the development of fibroid [14].

Nutrition has been linked to fertility; it determines the fertility status of humans [15]. With respect to nutrition, a common food additive known globally to effectively add suiting taste to food is Monosodium Glutamate (MSG). MSG is sodium salt of glutamic acid and being a flavor enhancer it is able to increase the flavorsome of food. However, this taste-bud friendly substance has pose health threat to consumers which in this study will burrow down to fibroids [16, 17]. Although it is categorized as 'generally recognised as safe' (GRAS), scientific evidence suggests that the taste and palatability are facilitated through explicit glutamate receptors located both in the stomach and the taste buds and these plays physiologic actions advantageous to gut function by stimulating the gastric vagus nerve [18-20]. However there are reports indicating toxicity of MSG on various organs of experimental animals such as; the liver, brain, thymus, and kidneys [21-24] and some of such other toxic effect has been established in increasing cholesterol levels leading to endocrinological disorder and cardiovascular diseases [15, 25], decreased levels of major anti-oxidant enzymes and increased lipid peroxidation in the kidneys of chronic MSG-exposed rats [26-27], sleep disordered breathing (SDB) was also associated with the consumption of MSG in the Jiangsu Nutrition (JIN) Cohort Study amount Chinese adults in normal weight and also, MSG was associated with snoring in normal weight [28-29].

Further reports established that hyperglycemia and taste repugnance are some of the adverse effect of abuse of the use of MSG, so also, MSG administration has been shown to induce hyperphagia; a condition associated with abnormally great desire for food and excessive eating, this also explains reason for recorded obesity with respect to high intake of MSG [16, 30-31].

Heliotrope (*Heliotropiumindicum*) is a common orchard plant that has been studied to have about 250 species andwidely distributed in temperate zones of all continents [32]. There are numerous therapeutic values associated with this plant; analgesic, antipyretic; anti-asthmatic effects, extracted juice is also used as eye drop for conjunctivitis [33-37]. Other folkloric use of this plant includes treatment of fever using its decoction [38], insect bites, stings, diarrhoea, skin rashes, menstrual disorder and urticarial [39]. Prominent among the folkloric use of the plant is the wound healing ability of the plant and this has been scientifically confirmed by various reports [40-41],also the gastroprotective effects of the aqueous extracts of the dried leaves of *H. indicum*on indomethacin- induced gastric ulcerated mucosa has been reported [42, 43].

Various extracts of *H. indicum*have been studied for their biological activities in different animal models and reports have it that the plant extract has significant anti-inflammatory, diuretic, abortifacient, wound healing and antitumor activities [41, 44].The aim of this study however is to assess the effect of crude Methanol Extract of *Heliotropium indicum* (MEHI) on some biochemical parameters in MSG-induced fibroid in Wistar rats.

MATERIAL AND METHODS

Collection of Fresh *Heliotropium indicum*

Freshly harvested heliotrope whole plant was obtained from a farm located in Ibadan, Oyo State, Nigeria. The plant was authenticated at the Herbarium, Department of Botany, University of Ibadan as *Heliotropium indicum*

and was allotted a Voucher No: UIH-22509 on deposition of a specimen of the plant.

Preparation of Crude extract of *Heliotropium indicum*

Eight (8) kilogram of the freshly obtained plant was washed and air-dried after which it waspulverized. The powdery form obtained after pulverization was weighed and recorded to be 508grams. The pulverised plant was soaked with ample methanol (purchased from Sigma Aldrich Chemical Co. St Louis USA)in all glass jars for 72hrs (seventy-two hours) at room temperature.It was filtered and the filtrate obtained was concentrated under reduced pressure using rotary evaporator (Stuart-make). The crude extract obtained after concentration was placed in water bath (at 37^{o}C) to obtain a solvent-free crude extract.

Monosodium Glutamate

The MSG used for this study was obtained from Sigma Aldrich Chemical Co. St Louis USA. A stock solution was prepared by weighing 0.5g of MSG into 5mls of distilled water. This stock solution was based on the weight of the experimental animal.Group C was administered 50mg/kg of MSG only while Groups D and E were co-administered 50mg/kg MSG with 50mg/kg and 100mg/kg of the crude extract respectively.

Experimental animals

Twenty-Five (25) female albino rats (Wistar strain) weighing between 100-120g each were obtained from the Pre-Clinical Animal House, Faculty of Basic Medical Science, University of Ibadan, Ibadan, Nigeria.

The rats were kept in ventilated cages with 12 hours light/dark cycling and were acclimatized for two weeks (14 days) in the Animal House of Biochemistry Department, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria. The animals were given water and chow*ad libitum*. Good hygiene was maintained by constant cleaning and removal of feces and spilled materials from cages daily and was kept under standard conditions of temperature and humidity.Stipulated rules guiding animal studies as stated by the Ethical Committee of University of Ibadan were followed. These rules are similar to international guidelines on animal handling.

Experimental Animal Groups and Treatments

The experimental animals (25) were divided into five (5) study groups: A(Control), B(MEHI - 50mg/kg bdwt), C(MSG – 50mg/kg bdwt), D(MSG - 50mg/kg bdwt + MEHI - 50mg/kg bdwt) and E(MSG - 50mg/kg bdwt + MEHI - 100mg/kg bdwt). The administration was carried out through intraperitoneal injection for 10days as a single dose daily.

Sample Collection

The animals were fasted for 24 hours after the last day of administration, and then sacrificed thereafter. The blood sample was collected into appropriate sample bottles through ocular puncture by a professional. The levels of progesterone, estradiol, cholesterol, liver enzymes, antioxidant and haematological parameters were estimated using standard procedures.

Sample preparation:

The animals were euthanized by inhalation of overdose of chloroform after 24hrs of termination of the experiment. Blood was collected by ocular puncture into EDTA sterilized sample bottles. Plasma was prepared by centrifugation (3000 x g, 20 min) and used for the analysis of total estradiol (estrogen), progesterone and total cholesterol. Blood was also collected into plain bottle for serology

Haematological analysis:

The packed cell volume (PCV) and haemoglobin (Hb) were determined using the micro haematocrit method and cyanmethehemoglobin method respectively as described by Mitruka and Rawnsley (1997). Erythrocyte count (RBC) and Leukocyte count (WBC) were determined using the improved neubauershaemocytometer after the appropriate dilution (Schalmet al., 1975).

ANTI-OXIDANT ASSAY

Assay of catalase (CAT):

Catalase activity was assayed by the method of Sinha (1972). The enzyme extract (0.5ml) was added to the reaction mixture containing 1ml of 0.01ml phosphate buffer (PH 7.0), 0.5ml of 0.2M H_2O, 0.4ml H_2O and incubated for different time period. The reaction was terminated by the addition of 2ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium di-chromate with glacial Acetic Acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagents. All the tubes were heated for 10mins and the absorbance was read at 610nm.

Assay of Superoxide Dismutase (SOD):

The assay of superoxide dismutase was done according to the method of Das (2000). In this method, 1.4ml aliquots of the reaction mixture (comprising 1.11ml of 50Mm phosphate buffer of pH 7.4, 0.075ml of 20Mm L-Methionine,0.04ml 0f 1%{v/v} Triton X-100, 0.075ml of 10Mm Hydroxylamine hydrochloride and 0.1ml of 50Mm EDTA) was added to 100ul of the sample extract and incubated at 30^{o}c for 5mins. 80ul of 50uM riboflavin was added and the tubes were exposed for 10mins to 200 W-Philips fluorescent lamps. After the exposure time, 1ml of Greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphonic acid)

was added and the absorbance of the colour formed was measured at 543nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrile formation under assay conditions.

Assay of Glutathione S- Transferase (GST)

Glutathione transferase activity using 2,4-dichloronitrobenzene as substrates was assayed spectrophotometrically essentially as described by Habiget al, (1974). The reaction mixture (3 mL) contained 1.7 mL of 100 mM phosphate buffer (pH 6.5) and 0.1 mL of 30 mM CDNB. After preincubating the reaction mixture at 37°C for 5 min, the reaction was started by the addition of diluted cytosol (0.1 mL) and the absorbance was followed for 5 min at 340 nm. Reaction mixture without the enzyme was used as blank. The specific activity of GST is expressed as nmoles of GSH-CDNB conjugate formed/min per milligram protein using an extinction coefficient of 9.6 mM/cm.

Serum Enzyme Assay

Biochemical parameters were assayed according to standard methods. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were estimated using commercial kits purchased from Randox Laboratories Ltd, West Virginia, USA.

Statistical analysis:

Data collected were expressed as means ±Standard Deviation (SD) and One way ANOVA was used for analysis. Values of $p<0.05$ were regarded as significant.

HISTOLOGY

Histological evaluation of the colon and liver of control and treated rats were carried out with the light microscope. Colon and liver biopsies were fixed in 4% formalin, dehydrated in serial alcohol, cleared in chloroform and embedded in paraffin. Sections of 4–5 µm were cut by a microtome and stained with hematoxylin and eosin. The slides were subsequently observed under a light microscope and photographed using a digital camera. All slides were coded before examination by investigators who were blinded to control and treatment groups.

Results and Discussion

Results

Table 1 - Effect of MSG and Methanol Extract of Heliotropium indicum on haematological parameters in rats.

GROUP	PCV (%)	Hb (g/dl)	RBC ($x10^6$/µL)	WBC ($x10^3$/µL)	PLAT ($x10^5$/µL)	LYM (%)	HETER (%)	MON (%)	EOS (%)
Control	43.33±1.53^{b}	13.97±0.15^{c}	7.17±0.22^{c}	6.33±0.98^{a}	1.58±0.14^{c}	69.33±2.52^{b}	25.33±1.53^{a}	2.33±0.58^{ab}	2.67±0.58^{a}
Extract	52.33±2.52^{a}	16.20±0.40^{b}	10.55±0.65^{a}	5.97±0.67^{b}	1.96±0.29^{ab}	69.33±3.79^{b}	26.33±2.08^{a}	2.67±0.48^{a}	2.33±0.58^{a}
MSG	35.31±1.53^{c}	10.33±0.50^{d}	5.48±0.49^{d}	3.71±0.57^{b}	1.67±0.17^{bc}	74.23±2.08^{a}	20.67±1.56^{b}	1.33±0.48^{b}	1.33±1.16^{a}
MSG + Extract (50mg/kg)	42.33±1.43^{b}	16.73±1.06^{b}	8.59±1.08^{b}	4.46±0.15^{a}	2.51±0.14^{ab}	69.67±1.53^{b}	26.00±1.00^{a}	2.33±0.58^{ab}	1.67±0.58^{a}
MSG + Extract (100mg/kg)	45.67±1.53^{b}	19.50±0.82^{a}	9.94±0.76^{a}	5.16±0.33^{a}	2.84±0.25^{a}	69.51±2.08^{b}	26.33±1.53^{a}	2.67±0.48^{a}	2.33±0.58^{a}

N/B- Values with different superscripts were statistically significant across the groups

Table 2 - Effect of MSG and Heliotropium indicum on the activities of CAT, SOD, GST and GSH

GROUP	Catalase activity (Units/mg protein)	SOD activity (Units/mg protein)	GST activity (Units/mg protein)	GSH (µg/mg protein)
Control	5.87±0.51^{c}	12.63±0.46^{c}	35.45±1.08^{d}	47.83±7.94^{a}
Extract	4.09±1.00^{d}	12.51±0.27^{c}	36.41±1.13^{d}	46.11±6.40^{a}
MSG	10.25±0.83^{a}	18.62±0.40^{a}	107.11±1.88^{a}	26.71±1.19^{c}
MSG + Extract (50mg/kg b.dt)	8.20±0.96^{b}	16.57±0.48^{b}	72.54±2.25^{b}	36.18±3.70^{b}
MSG + Extract (100mg/kg b.w)	5.92±0.41^{c}	16.26±0.04^{b}	57.27±1.32^{c}	42.01±2.06^{a}

N/B- Values with different superscripts were statistically significant across the groups

Table3 – Effect of MSG and Heliotropium indicum on liver function indices

GROUP	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Control	5.87±0.51^{c}	12.63±0.46^{c}	35.45±1.08^{d}
Extract	4.09±1.00^{d}	12.51±0.27^{c}	36.41±1.13^{d}
MSG	10.25±0.83^{a}	18.62±0.40^{a}	107.11±1.88^{a}
MSG + Extract (50mg/kg b.dt)	8.20±0.96^{b}	16.57±0.48^{b}	72.54±2.25^{b}
MSG + Extract (100mg/kg b.dt)	5.92±0.41^{c}	16.26±0.04^{b}	57.27±1.32^{c}

N/B- Values with different superscripts were statistically significant across the groups

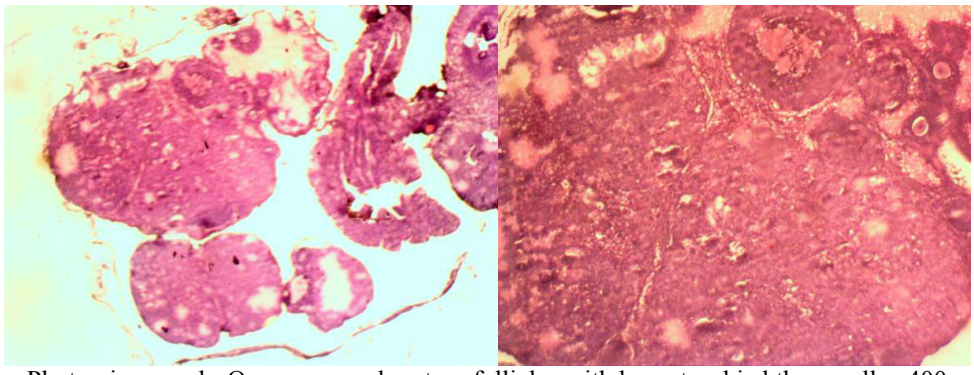
Table 4: Effect of MSG and Heliotropium indicum on levels of estradiol, progesterone and cholesterol

GROUP	Estradiol (mg/dL)	Progesterone (mg/dL)	Cholesterol (mg/dL)
Control	1.76±0.10^{d}	0.78±0.04^{d}	53.70±1.30^{d}
Extract	1.64±0.06^{d}	0.71±0.03^{d}	50.01±0.55^{d}
MSG	3.41±0.12^{a}	2.51±0.19^{a}	131.29±1.67^{a}
MSG + Extract (50mg/kg b.dt)	2.52±0.05^{b}	2.04±0.07^{b}	82.82±1.64^{b}
MSG + Extract (100mg/kg b.dt)	2.11±0.07^{c}	1.30±0.06^{c}	63.75±5.85^{c}

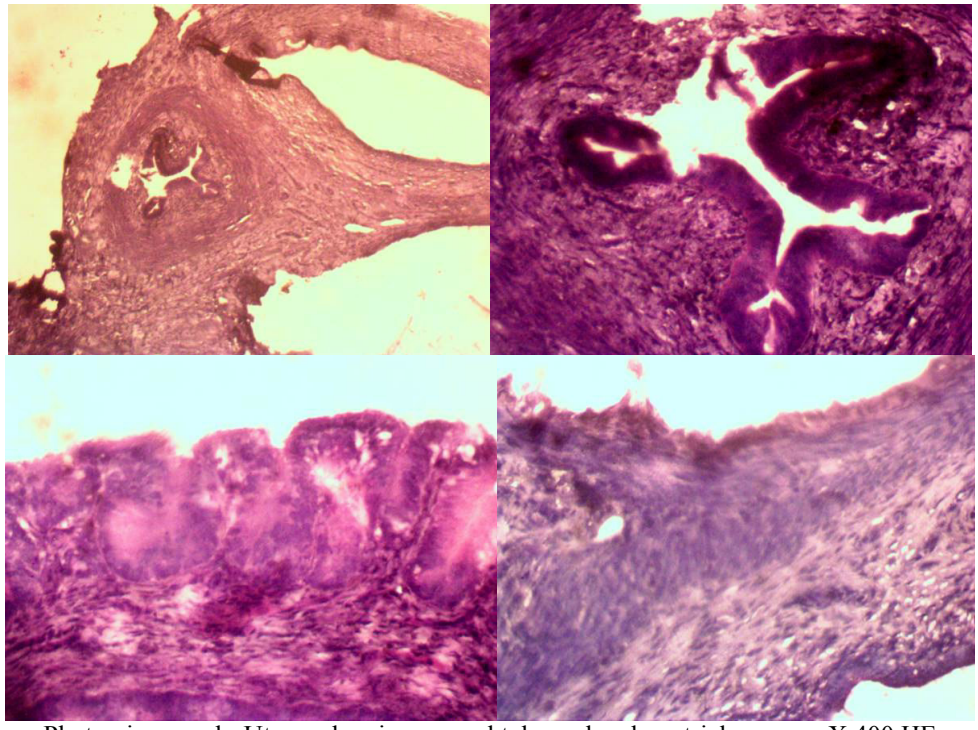
N/B- Values with different superscripts were statistically significant across the groups

HISTOPATHOLOGICAL LESIONS OF OVARIES AND UTERUS OF WISTAR RATS SUBJECTED TO DIFFERENT TREATMENTS OF THE EXTRACTS AND MONOSODIUM GLUTAMATE

CONTROL GROUP



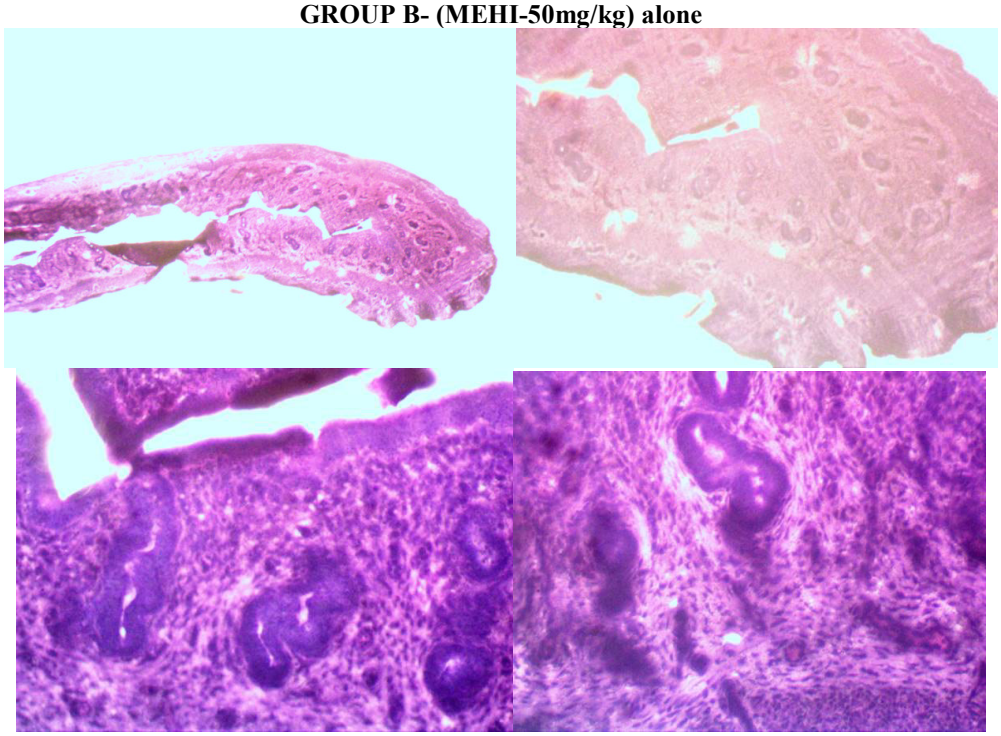
Photomicrograph- Ovary- normal mature follicles with hypertrophied theca cells x400



Photomicrograph- Uterus showing normal tube and endometrial mucosa. X 400 HE

In the control group where neither MSG nor MEHI was administered, the ovaries and the uterus photomicrographs appeared apparently normal.

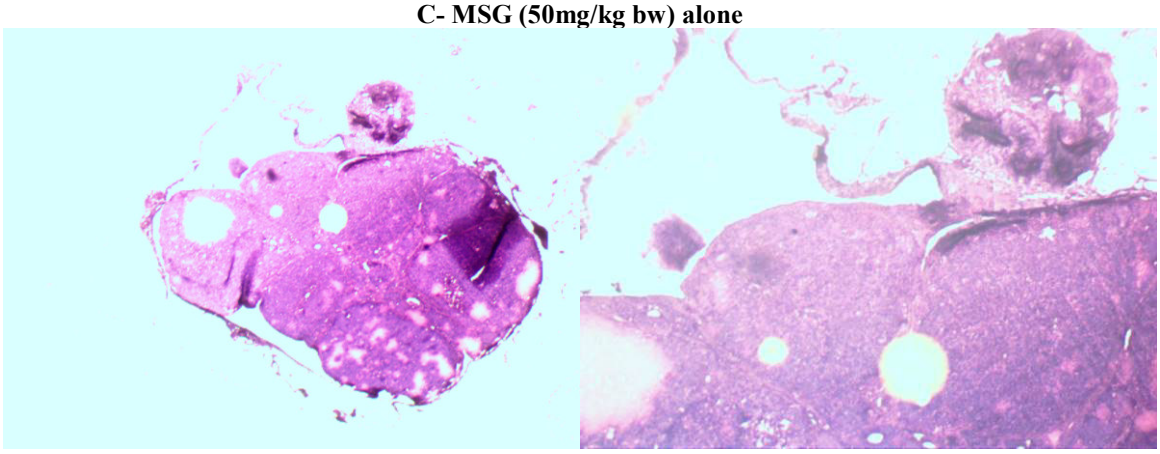
GROUP B- (MEHI-50mg/kg) alone



Uterus- Moderate diffuse cellular infiltrates and hyperplastic endometrial glands in uterine mucosa x400 HE

The inclusion of 50mg/kg of MEHI led moderate physiological challenge of the endometrial gland, causing infiltration and hyperplastic proliferation of the cells.

C- MSG (50mg/kg bw) alone

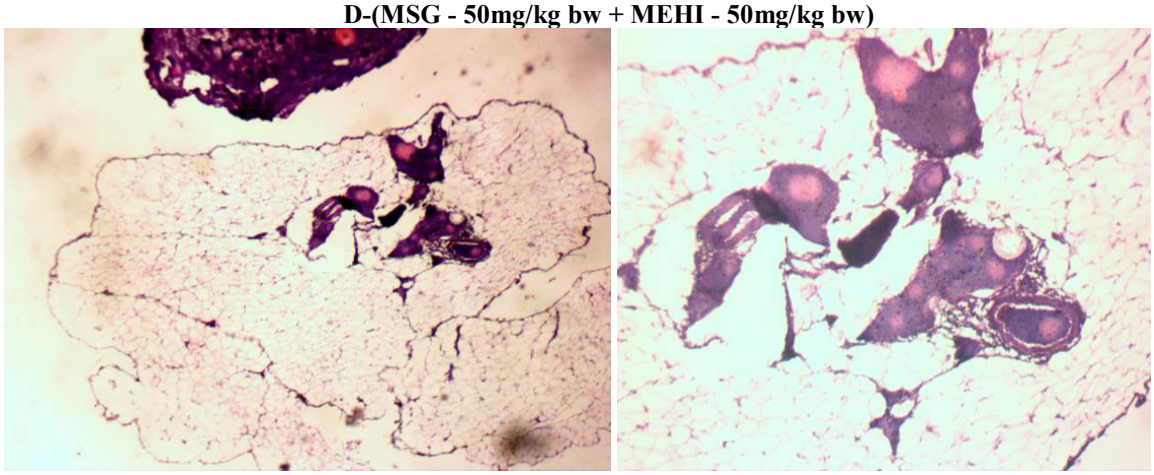


Ovary- there is obliteration of ovarian follicles, attenuation of germinal epithelium (diffuse oophoritis)

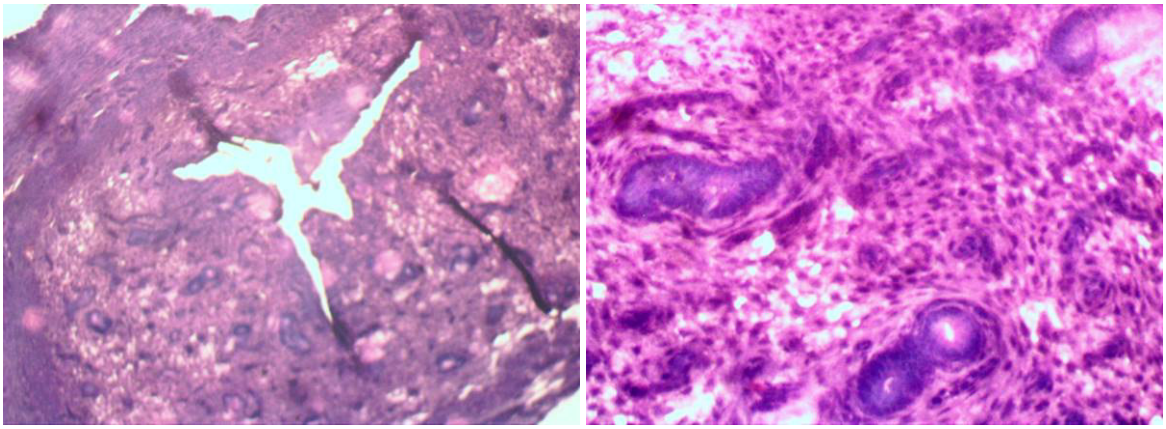
Uterus- degenerate and necrotizing endometrial mucosa with atrophy of glands x400

The inclusion of MSG markedly caused development of pathologies in the ovarian cells and the uterus, which were observed as obliteration of ovarian follicles, attenuation of germinal epithelium (diffuse oophoritis) and necrotic degeneration of endometrial mucosa with atrophy of glands respectively.

D-(MSG - 50mg/kg bw + MEHI - 50mg/kg bw)



Follicular atresia and atrophy of ovaries

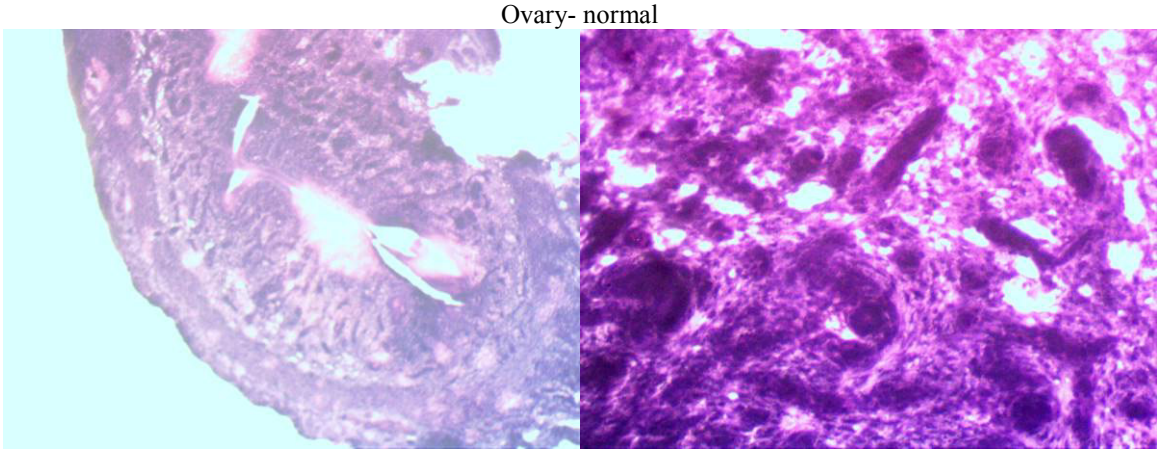


Moderate diffuse endometritis and glandular hyperplasia

Inclusion of 50mg/kg MEHI, had slight effect on the pathologies observed in the ovaries and uterus, which was not significant.

E (MSG - 50mg/kg bdwt + MEHI - 100mg/kg bw)

Ovary- normal



Uterus- endometrial gland hyperplasia

With the inclusion of 100mg/kg of MEHI intraperitoneally to the rats exposed to MSG, there was a marked protective and regenerative effect of the gonads seen as normal histology of the gonads with endometrial hyperplasia seen in the uterus.

Discussion

Treatment of experimental animals with crude methanol extract of *Heliotropium indicum* as shown in Table 1 indicated increase in the values of PCV, Hb, RBC with marked increase in Platelets ($p<0.05$) to the tune of 24.1% when compared to the control group. The extract also increased the values of HETER, and MON although insignificantly. The administration of MSG however decreased the values of PCV, Hb, RBC, WBC and EOS, but increased PLAT by 12.0% when compared to the control group. Also the result as displayed in Table 1 shows a reversed effect of MSG on some parameters that were measured..

Table 2 presents the results of the effect of MSG and *Heliotropiumindicum*on the activities of CAT, SOD,

GST and levels of GSH. MSG increased the activities of CAT, SOD and GST by 74.6%, 63.3% and 202.1% respectively. However, MSG decreased the level of GSH by 50.5% as compared to the control group, while there was no significant ($p<0.05$) increase in theactivities of SOD and GST in the groups administered the extract. The results further showed that the co-administration the extract alongside MSG reversed the increased activities recorded for CAT, SOD and GST, while there was increase in the level of GSH when compared to the group given MSG only in the increasing graded doses.

The results of the effect of MSG and *Heliotropium indicum* on liver enzymes are presented in Table 3. The results show that MSG significantly ($p<0.05$) increased the serum levels of the alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymes assayed while the graded doses of *Heliotropium indicum* reversed this effect on co-administration with MSG.

Table 4 gives a summary of the effect of the MSG and the graded doses of *Heliotropium indicum* on the levels of estradiol, progesterone and cholesterol. MSG significantly ($p<0.05$) increased the levels of estradiol, progesterone and cholesterol by 93.8%, 221.8% and 144.5% respectively. There was however, mitigation of this effect with co-administration of the extract.

THE HISTOPATHOLOGICAL LESIONS OVARIES AND UTERI IN THE VARIOUS GROUPS

In the control group where neither MSG nor MEHI was administered, the ovaries and the uterus photomicrographs appeared apparently normal.

Inclusion of 50mg/kg of MEHI led moderate physiological challenge of the endometrial gland, causing infiltration and hyperplastic proliferation of the cells. It was observed that inclusion of MSG markedly caused development of pathologies in the ovarian cells and the uterus, which were seen as obliteration of ovarian follicles, attenuation of germinal epithelium (diffuse oophoritis) and necrotic degeneration of endometrial mucosa with atrophy of glands respectively.

Also, inclusion of 50mg/kg MEHI, had slight effect on the pathologies observed in the ovaries and uterus, which was not significant, while the inclusion of 100mg/kg of MEHI intraperitoneally to the rats exposed to MSG, there was a marked protective and regenerative effect of the gonads seen as normal histology of the gonads with endometrial hyperplasia seen in the uterus.

Discussion

It is important to assess the hematological effect of a plant extract as it gives first information about the presence of dangerous foreign substance that can cause potential health hazards to humans [45],it is also imperative to establish safety in the use of most folklorically relevant plant decoction, and a part of the safety measure is observing the effect of the use of such extract on hematological indices, as an extract that will boost hematological indices is desirable especially in complications involving anemic conditions. However, MSG has been noted for its characteristic adverse effect on some hematological indices [46, 47], and the tendencies of triggering fibroid growth in the uterus with associated lesions in the ovaries.

In this present study *Heliotropium indicum* was able to boost PCV to 53.33±2.52 at the 50mg/kg b.wt dose and also significantly ($p<0.05$) increased the platelet level of the blood by 20.2%, while the group administered MSG only increased the platelets level by 8.4%. Also a significant ($p<0.05$) increase was observed for the co-administration of MSG and the extract was suggestive of a synergistic effect on the platelet levels and this actually supports the folkloric use of *Heliotropium indicum* i.e. wound healing property as an increase in platelet culminates to better ability to clot blood. The group administered MSG only also recorded significant ($p<0.05$) increase in platelet level and this was coherent to the study reported by……..where it was recommended that MSG be used as a short-term medication for patients undergoing chemotherapy. Likewise, there were ameliorative effects of MEHI on the lesions caused by MSG in the ovaries and uteri of the Wistar rats in a dose-dependent manner.

Antioxidants have been reported to play a substantial role in the defense against oxidative stress as a balance between free radicals and antioxidant is important for favorable physiological function (Lobo *et al,* 2010).There is however a defense system comprising of enzymes such as Catalase - CAT, Superoxide dismutase-SOD, Glutathione S-transferase – GST) and decreased the activity of Glutathione– GSH and the therapeutic desirability of a plant extract is dependent on its ability to induce the activity of this defense mechanism. In this present study antioxidants such as catalase, superoxide dismutase, glutathione and glutathione S-transferase were considered as literature as established them as important self-defense system (Celik et al, 2009; Uzun et al, 2010; Demir et al, 2011). These defense systems are important because they practically scavenge free radicals (I.A. Adedara and E.O. Farombi). The result in this present study shows that MSG significantly ($p<0.05$) increased the levels/activity of CAT, SOD and GST) but on the other hand decreased the activity of GSH and these findings are in accordance to with Farombi and Onyema, 2006. This study revealed that GSH level decreased in the group treated with MSG only and this may be ascribed to increased levels of free radicals as Onyema et al, 2006 reported that MSG is capable of inducing lipid peroxidation. GSH, however, is effective in attacking electrophilliccentres by reacting with superoxides, singlet oxygen or hydroxyl.5,6

The increase in the activities of CAT and SOD may be due to their recognition of MSG as an oxidant that can cause stress to the liver and therefore required increased activity. Moreso, there was already reduced activity of GSH a compensation was indeed needed to reduce supposed oxidative stress, therefore increase in the activity of CAT, SOD and GST as defense systemsmay have been necessary as a palliative for the decrease in GSH as these are left to defend the system against stress, this present study agreed with Farombi and Onyema 2006; Onyeama*et al*., 2006 and Waiz et al, 2014. However, the graded doses of *Heliotropiumindicum* co-administered with MSG were able to overturn the undesirable effect of MSG on the activities of these enzymes by being hepatoprotective against MSG.

The activities of ALT, AST and ALP from our results increased in the group given MSG only; these enzymes are biomarkers of hepatocellular damage. The elevated values of these enzymes may indicate injury of the liver and it may additionally indicate injury of the kidney and heart (42). The graded doses of *Heliotropiumindicum*however abolished these effects by decreasing the levels of these enzymes in the serum when compared to the group given MSG only.

Fibroids have been studied to be hormones-dependent; they thrive on estrogen especially at the peak during ovulation and just before theinitiation of menstrual period and increases in pregnancy when Gonatrophins-ReleasingHormones (GnRH) is at its peak (Benagiano*et al*., 1992; Cohen *et al*., 1994; Obochi et al, 2009). This present study indicates that MSG increased the levels of progesterone but significantly ($p<0.05$) for cholesterol and estradiol. An increase in the levels of cholesterol and estrogen leads to the induction of fibroids (Adamson, 1992; Newton *et al*., 1994; Obochi et al, 2009). MSG could cause the elevation of cholesterol levels with its ability to activate 3-hydroxyl-3-methoxylglutamyl-COA reductase, HMGR, an enzyme that catalyzes the irreversible step in cholesterol synthesis (i.e., HMG-COA is converted to mevalonate). The enzyme which is active in the dephosphorylated state which then enables it to increase the synthesis of cholesterol (Bernard *et al*. 2002). The effects of MSG on estradiol levels could be ascribed to its ability to activate aromatase, which is an enzyme that catalyze the transformationof testosterone to $-estradiol and aromatization of ring A of $-estradiol, resulting in increased estradiol synthesis. Estradiol and its receptor stimulates fibroid and are able to incorporate progesterone by inducing the expression of its receptor (Marsh and Bulun, 2006; Olowofolahan et al, 2017).

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