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Monosodium glutamate-induced follicular atresia and the ameliorative role of *Allium sativum* in Albino Wistar rats.

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ABSTRACT

Food and environmental toxins have increased female infertility in recent years. Dietary monosodium glutamate is ovotoxic. *Allium sativum* is reputed for its great therapeutic value. Based on this premise, this study attempted to evaluate *Allium sativum*'s potential protective or ameliorative function in the adverse effect of monosodium glutamate-induced ovarian toxicity. Twenty-five female Wistar rats (150-200g) were utilized for this study. The experimental rats following acclimatization were assigned into five groups, A-E (n=5). The control as placebo received 0.03mL saline for 28 days. Monosodium glutamate (4mg/kg) solution was administered to rats in groups B-E for 14 days. Following that, groups C and D received 200 mg/kg and 500 mg/kg of *Allium sativum* ethanolic extract respectively, while group E received 200 mg/kg of Vitamin C for 14 days respectively. Administrations were via the orogastric route. Data were analyzed by One-way ANOVA and mean comparisons done by the Student Neuman-Keul's test. Graph pad Prism version 5.1 was used for analysis, *p*-values ≤ 0.05 were considered statistically significant. The administration of monosodium glutamate in the monosodium glutamate-only group (group B) showed a significant (p=0.05) increase in the number of atretic follicles as compared to *Allium sativum* treated groups and control. Administration of monosodium glutamate resulted in a high level of ovarian histological derangement and loss of glycogen components which was ameliorated by *Allium sativum* extract treatments, however dose-dependent. *Allium sativum* demonstrated dose-dependent ameliorative potentials as evident in its role in remedying the damage caused by monosodium glutamate-induced follicular atresia, ovarian histological derangement, and glycogen degradation.

Keywords: Ovarian toxicity; *Allium sativum*; Histomorphometry; Monosodium glutamate; follicular atresia

INTRODUCTION

Environmental chemicals, industrial pollutants, and food additives have all been linked to female reproductive system damage.[1] The female reproductive system is extremely sensitive to various harmful environmental factors. Increased use of food additives such as monosodium glutamate (MSG), poses a significant risk. Most food additives function as preservatives or flavor enhancers.

Monosodium glutamate (MSG) is a well-known and widely used flavor enhancer all around the world. Ajinomoto is a brand name for monosodium glutamate (MSG), which is the hydrated sodium salt of naturally occurring L-glutamic acid. It is widely used as a common food ingredient, in many countries, to give foods a distinct flavor that attracts consumers. According to reports, the food industry is gradually increasing the use of MSG in the production of food items while ignoring the potential human health risks.[2] Flavored chips and snacks, soups or sauces (canned, packed), prepared meals, frozen foods and meals, fresh sausages, marinated meats, stuffed or seasoned chicken, bottled soy or oriental sauces, manufactured meats, some hams, luncheon chicken and turkey, flavored tuna, vegetarian burgers, and sausages contain this flavor enhancer.[3] Its neurotoxic effect, obesity-inducing, metabolic derangements, and reproductive toxicity have all been documented in the literature.[4] According to Ismail, ovarian pathologies observed after MSG treatment were attributed to oxidative damage.[5] A reasonable explanation for this is the fact that MSG leads to the generation of oxygen-derived free radicals and related reactive oxygen species (ROS). Reactive oxygen species are dangerous substances for biological systems as they react with DNA, proteins, and lipids, leading to cellular damage which has been previously shown by Singh and Ahluwalia.[6] In the study of Farombi et al., MSG-induced oxidative damage in rats elevated serum levels of serum alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transferase. Treatments with vitamin C, E, and quercetin counteracted MSG-induced oxidative damage which points to the anti-oxidative potentials of these dietary antioxidants.[7]

The ovaries are the primary female reproductive organs responsible for the production of oocytes and hormones that control various reproductive and sexual functions in females.[8] Ovarian toxicity is due to exposure to ovotoxins. Prolonged exposure of the ovary to ovotoxins has been known to cause follicular degeneration which leads to partial or total interruption of reproductive function.[2] This degeneration is due to a gradual depletion of the oocyte reserve following apoptotic cell death and ovarian atrophy with the disappearance of resting primordial follicles.[9] Therefore, ovarian toxicity increases the chances of infertility in women. The level of impact on reproduction caused by exposure to these toxins is dependent on the stage of the ovarian cycle.[10] Oxidative stress is implicated among the causes of follicular damage in the ovary. Chemotherapeutic agents like cyclophosphamide and doxorubicin, metals such as lead acetate, and food additives like MSG among others have been reported to induce various degrees of ovarian damage.[11] Therefore, MSG is a predisposing factor in the pathogenesis of anovulatory infertility.[12]

Allium Sativum, also known as garlic is reputed for its therapeutic functions. It possesses antioxidant, antimicrobial, antiprotozoal, antimutagenic, antiplatelet, and antihyperlipidemic among other properties. Thus, it is a promising remedy for the amelioration of ovarian toxicity as it contains anti-oxidant compounds, notably, allicin.[13] To date, there is no available literature that has established its role in MSG-induced ovotoxicity, especially on follicular integrity. This study, therefore, employed histological and histochemical approaches in filling this knowledge gap.

METHODS

Chemicals and Reagents Utilized: MSG salt was obtained from Sigma Aldrich USA, Ascorbic acid (Emzor brand) was obtained from AGRAM pharmaceuticals, Sagamu, Ogun State Nigeria. All other chemicals used were of analytical grade.

***Allium sativum* (AS) Extract Preparation:** Bulbs of AS were procured from Ilishan Main Market Nigeria (6.8940° N, 3.7187° E, rain forest zone). A sample was taken to a taxonomist at the Department of Basic Sciences, Babcock University Ilisan-Remo for authentication. The bulbs were washed in distilled water and allowed to drain for an hour at room temperature (27°C) before peeling with a clean knife, air-dried, and thereafter pulverized in a blender (PHILIPS, Model HR-1724, Brazil) to obtain a smooth powder. A known weight (250 g) of the powder was extracted in 1000 mL of ethanol for 72 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper (Maidstone, UK), and the resulting filtrate was concentrated in a Rotary Evaporator. The mixture was further transferred into a steam bath where it was evaporated to give the required residue. The residue was stored in a cool environment until used.

***Allium sativum* Extract Stock preparation**: 10g of the ethanolic AS extract was weighed and dissolved in 250 mLs of distilled water. It was administered at a dose of 200 mg/kg and 500 mg/kg to groups C and D respectively. Oral LD_{50} of *Allium sativum* has been reported to be 30 mL/kg[14] and this informed the choice of the doses used in this study.

Monosodium Glutamate stock preparation: 3g of MSG was dissolved in 100 mL of distilled water and administered at a dose of 4 mg/kg body weight orally. The LD_{50} of MSG: 15.000– 18.000 mg/kg body weight had been earlier reported.[15]

Vitamin C stock preparation: 500 grams of Vitamin C was dissolved in 50 mLs of distilled water.

Animal Care and grouping: Twenty-five (25) female adult Wistar rats (*Rattus norvegicus*) weighing between 150-200 grams were procured from the animal housing facility, Babcock University, Ilishan-Remo, Ogun State, and used for this experiment. The rats were allowed to acclimatize for 7 days before the commencement of the experiment. They were kept under natural light and at room temperature. Distilled water and pelletized standard rat chow were given to the rats, daily *ad libitum*. The body weights of the rats were taken using a Camry Electronic Kitchen Scale every two days. Research approval was obtained from the Babcock University Health Research Ethical Committee and the clearance number (BUHREC: 036/19) was given. Rats were

randomly assigned into five groups (A-E) n = 5. Rats in group A served as the control and were administered 0.03 mL of normal saline for 28 days. Rats in groups B, C, D, and E were administered MSG at a dose of 4 mg/kg body weight for 14 days. After ovarian toxicity induction for 14 days, groups C and D received 200 mg/kg and 500 mg/kg of *Allium sativum* ethanolic extract respectively while group E received 200 mg/kg of Vitamin C for 14 days while group B was untreated. All administrations were via oral route using an oral cannula mounted on a 2 mL syringe.

Table 1: **Grouping and experimental design**

Groups	**Agent administered**	**Dosing**	**Duration**	**Administration Route**
Control (A)	Normal saline as placebo	0.03 mL	28 days	Orally
B (Negative control)	Monosodium glutamate (MSG) only	4 mg/kg	14 days	Orally
C	MSG and *Allium sativum* (AS)	4 mg/kg and 200 mg/kg respectively	14 days respectively; drug first, treatment after	Orally
D	MSG and *Allium sativum*	4 mg/kg and 500 mg/kg respectively	14 days respectively; drug first, treatment after	Orally
E (standard drug)	MSG and Vitamin C	4 mg/kg and 200 mg/kg	14 days respectively each; drug first, treatment after	Orally

Animal Sacrifice and Organ Harvest: Following the 28 days experimental period, the rats were euthanized and sacrificed and the inferior abdominal cavity was then opened and the ovaries were identified, excised, and weighed.

Histological Analysis: The excised ovaries were fixed in 10% formol-saline solution and processed using routine histological tissue analysis procedures. To demonstrate general histoarchitecture and histomorphometry of the ovary, Hematoxylin, and eosin stains were employed, while the Periodic Acid Schiff (PAS) was used to demonstrate the glycogen components of the tissue.

Photomicrography: The hematoxylin and eosin and the periodic acid Schiff stained Slides were viewed using LEICA DM 750 microscope connected to a digital camera (LEICA ICC50) and a desktop computer. The images taken were archived.

Histomorphometry Analysis: Image J software (National Institutes of Health (NIH), U.S.A) was used for histomorphometry studies. The follicular

count was carried out by importing the archived photomicrographs into the software, 5 photomicrographs for each group, and different stages of the follicular development were noted and recorded in Excel. We used photomicrographs taken with x40 objective lens for the primordial follicular count while x10 was used for the other types of follicles.

Statistical Analysis: Statistical analysis was carried out using GraphPad Prism 5.0. Data were represented as mean ± SEM (standard error of the mean) and analyzed using descriptive and inferential statistics. One-way ANOVA was used to analyze data, followed by Student Newman-Keul (SNK) test for multiple comparisons. P-values <0.05 were considered statistically significant.

RESULTS

Mean Body Weight and Relative Ovarian Weights: Ovarian toxicity was accompanied by weight gain following the administration of monosodium glutamate within the first two weeks in the experimental animals. The result shows weight gain across all the groups. A slight decrease in weight difference was observed, though not significant in the MSG + 200 mg/kg *Allium sativum* group (19.35±2.082) when compared to the control (23.70±6.936), MSG (21.88±5.450), and treatment groups D and E, (23.98±9.507 and 23.83±8.755 respectively). Weight gain continued across all groups even with the administration of *Allium sativum* ethanolic extract and Vitamin C (Figure 1).

The mean relative ovarian weight of monosodium glutamate + 200 mg/kg *Allium sativum* (0.068 ± 0.011%) was significantly ($p<0.05$) higher when compared to that of the control group (0.545 ± 0.102%). However, the mean relative ovarian weight of the monosodium glutamate + 500 mg/kg *Allium sativum* group (0.041 ± 0.003%) was significantly lower when compared to the control (0.545 ± 0.102%), MSG (0.633 ± 0.006%) and monosodium glutamate + 200 mg/kg *Allium sativum* (0.068 ± 0.011%) groups (Figure 2).

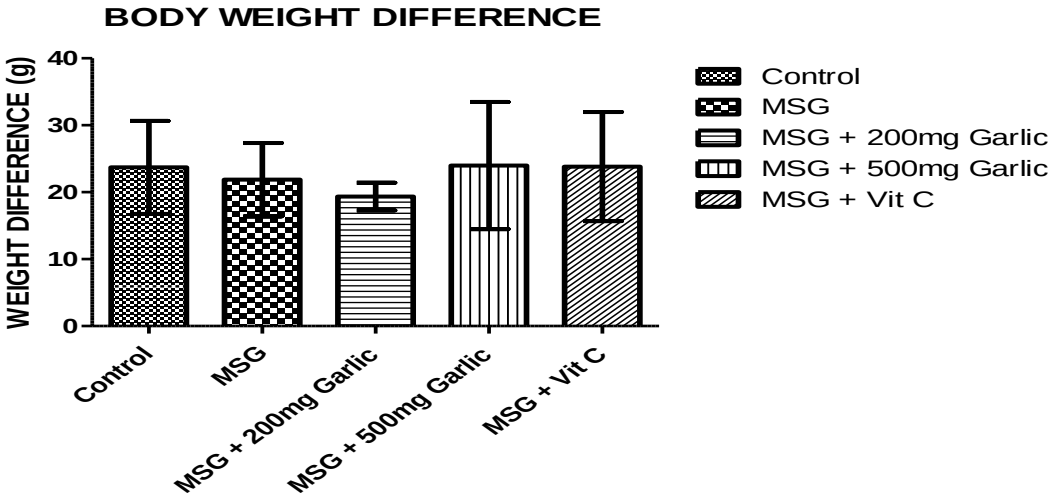

Figure 1: Effect of *Allium sativum* on the body weight change in MSG-induced ovarian toxicity in rats. Data are expressed as mean ± SEM (n=5) and analyzed by One-way ANOVA followed by the Student Neuman-Keuls test for each parameter separately.*$p<0.05$ as compared to the control group.

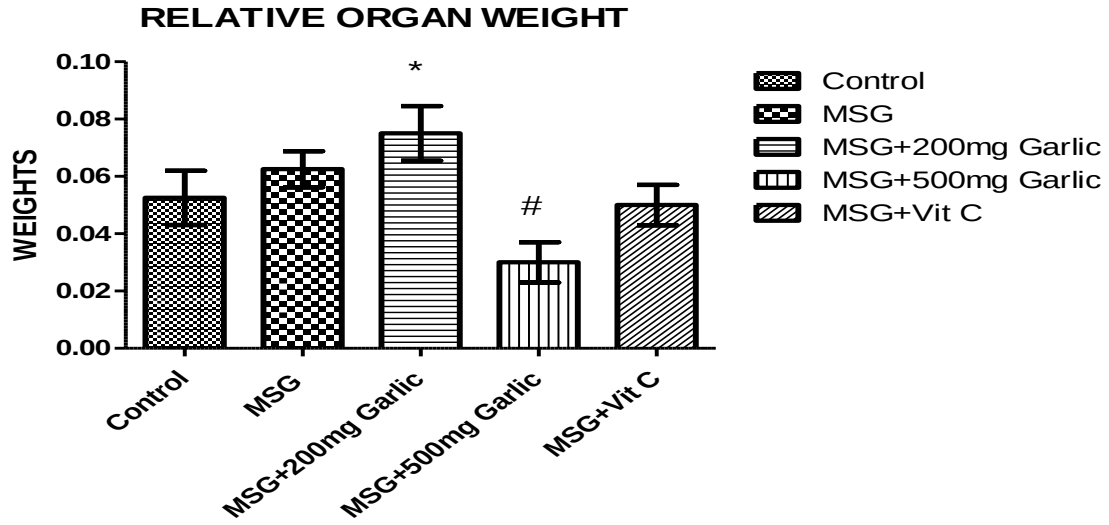

Figure 2: Effect of *Allium sativum* on the relative organ weight in MSG-induced ovarian toxicity in rats. Data are expressed as mean ± SEM (n=5) and analyzed by One-way ANOVA followed by the Student Neuman-Keuls test for each parameter separately. *p<0.05 as compared to the control group. # p<0.05 compared to the Control.

Effect of MSG and *Allium sativum* on Follicular count: The data obtained from the comparison between the numbers of the follicles of the different stages of follicular development showed that there was a significant decrease in the numbers of primordial and primary follicular count in the MSG-only treated group when compared to the control. The number of atretic follicles was significantly (p<0.05) higher in the MSG-only group when compared to the control with a reversal of this trend in the *Allium sativum* and Vit. C treated groups. The reduction in the number of atretic follicles was more pronounced (p<0.05) in the group treated 200 mg/kg *Allium sativum* when compared to the higher dose (Figure 3).

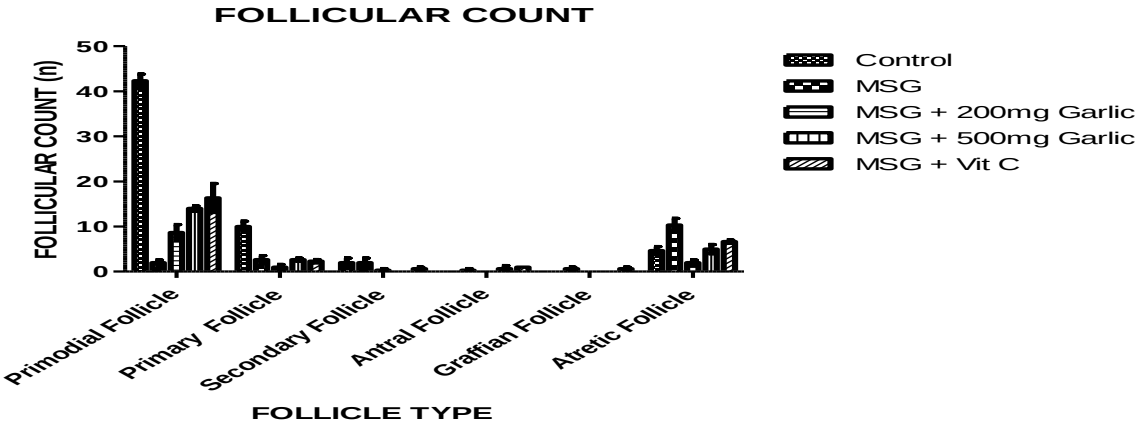

Figure 3: Effect of AS on the follicular numbers at the different follicular stages in MSG-induced ovarian toxicity in rats. Data are expressed as mean ± SEM (n=5) and analyzed by One-way ANOVA followed by the Student Neuman-Keuls test for each parameter separately.

Histological and Histochemical Assessments: To assess ovarian histoarchitecture or dynamics following monosodium glutamate-induced ovarian toxicity and treatments with *Allium Sativum*, we employed the hematoxylin and eosin staining procedure for general histoarchitectural layout and the periodic acid Schiff (PAS) reaction for glycogen demonstration in the ovarian tissues. With the hematoxylin and eosin stains, it was observed that the photomicrographs of the control group revealed normal undistorted histoarchitecture of the ovary, illustrating a well-defined zona granulosa surrounding the oocyte and compact theca folliculi, the presence of a large number of primordial follicles and clusters of unilaminar primary follicles. In the monosodium glutamate-only group (Group B), there was a multi-layered primary follicle with deranged granulosa cells in addition to the mild hemorrhagic areas observed. Cellular hypertrophies of the theca folliculi, and complete distortion/destruction of the basement membrane separating the theca folliculi from the zona granulosa were also observed in this group. The hemorrhagic feature in group D (MSG + 500mg garlic) is highly noticeable while mild hemorrhage can also be seen in group E (MSG + Vit C). However, in group C administered MSG and treated with 200mg/kg of *Allium sativum* there was a near to normal histology of the ovary indicating better protection of the follicles at this dose of AS (**Plate 1**). With the periodic acid Schiff (PAS) reaction, there is a strong reactivity for PAS within the follicles and the around the zona pellucida in the control, AS-high dose, and Vit. C-treated groups while MSG-only and the AS-low dose groups showed negative PAS reactivity (**Plate 2**).

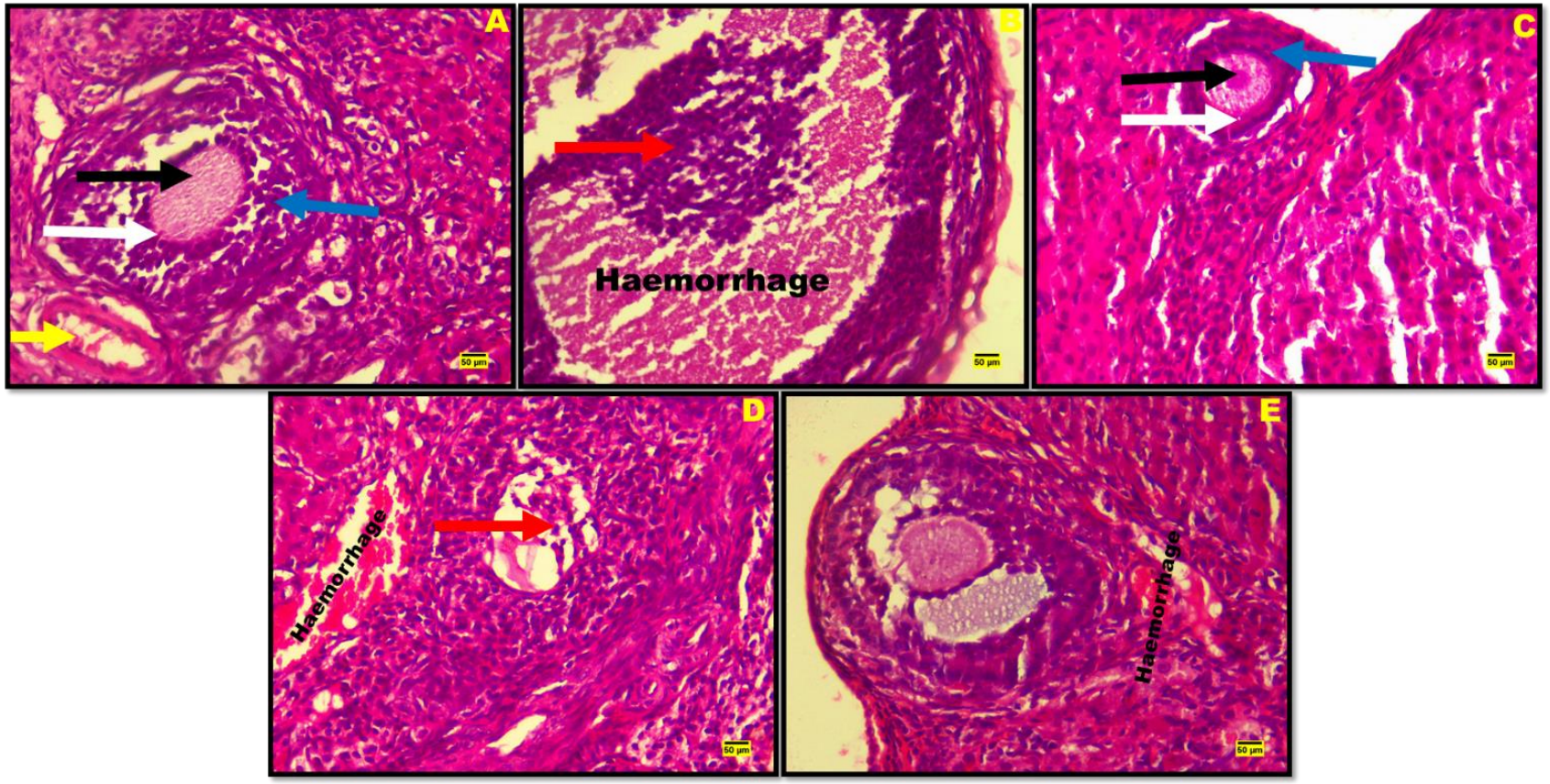

Plate 1: Representative photomicrographs of the sections of the ovary (H and E x400). Observe in the control section [A], the normal histoarchitecture of the ovary, delineated by the oocyte (black arrow) within the primary follicle surrounded by granulosa cells (blue arrow). Also present is a glycoprotein coat, the zona pellucida (white arrow) between the granulosa cells and oocyte and blood vessel (yellow arrow). The MSG-only group [B] is outlined by deviation from the cytoarchitectural framework of the ovary. Observed here are an atretic follicle (red arrow) and severe haemorrhagic areas. Evident in 200mg/kg AS treated group [C] is a near to-normal ovarian architecture. A primordial follicle is seen here with an oocyte (black arrow), intact zona pellucida (white arrow), and follicular cells (blue arrow). Groups [D] and [E] show areas of vascular haemorrhage, atretic follicles (red arrow), and mild haemorrhagic areas respectively. Scale Bars- 50µm

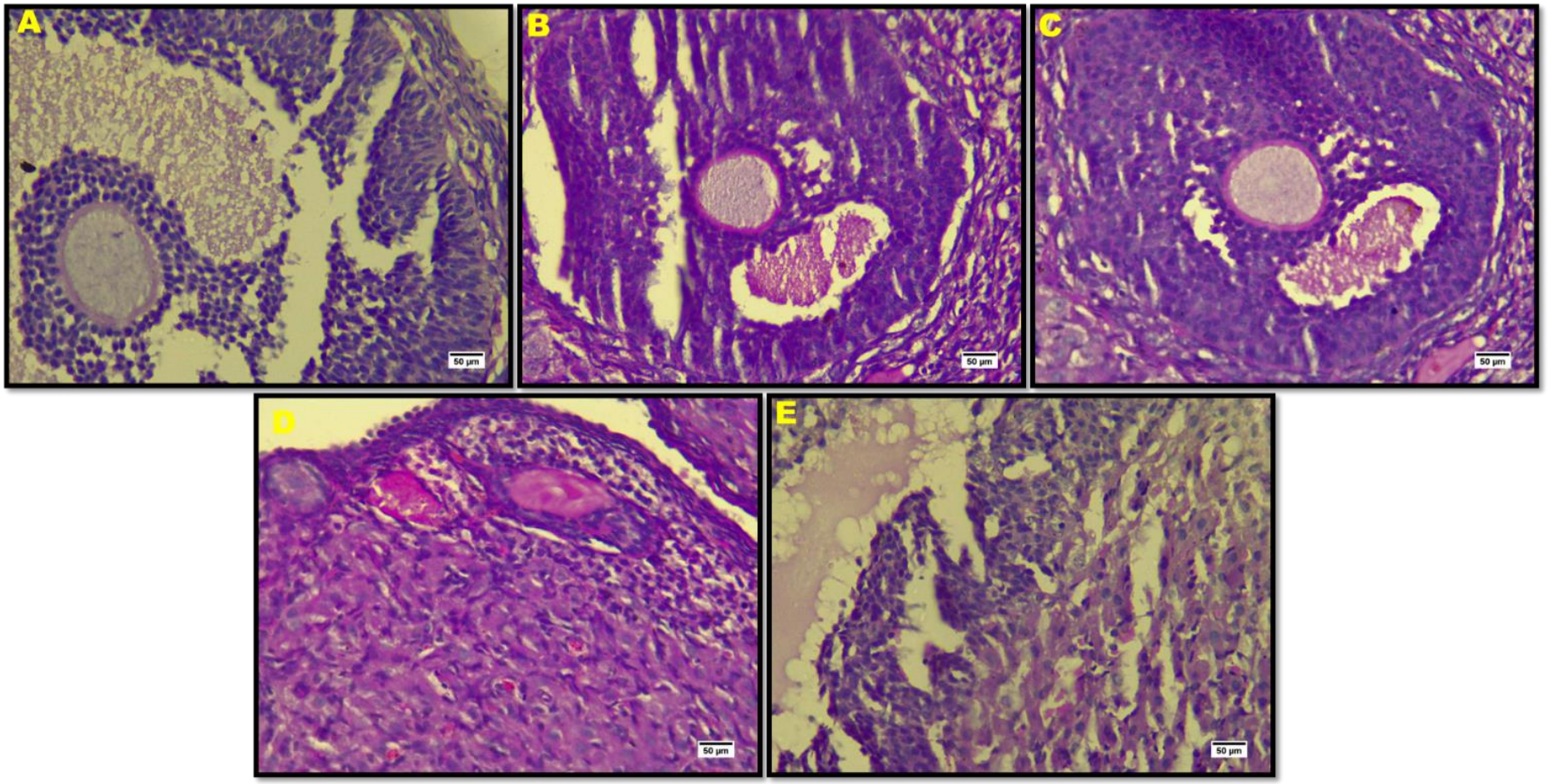

Plate 2: Representative photomicrographs of the sections of the ovary (Period Acid Schiff (PAS- x400) demonstrating glycogen contents in the basement membrane of the ovarian follicles. Observed in the control [A], MSG+500mg/kg AS treated [D], and MSG+Vit.C treated [E] groups is normal glycogen content deposition around the basement membrane, while the MSG-only group and MSG+ 200mg/kg AS treated group [C] shows negative PAS expression. Scale Bar-50µm

DISCUSSION

In this study, the induction of ovarian toxicity with MSG resulted in a slight decrease in the body weight of the MSG-only group and the low-dose *Allium sativum*-treated group. The initial reduction in body weight change of the rats contradicts previous reports that MSG intake causes weight gain due to its mechanism of leptin-resistance which inhibits the satiety center in the hypothalamus thereby encouraging excess food intake leading to obesity.[16,17] It is plausible that the weight loss that was observed in this study could be linked to allicin, a major constituent of AS which has been reported to possess anti-obesity properties; which is in tandem with the previous report of Shang et al. (2019).[18] Our result also presents a significant increase in the relative ovarian weight in MSG-only and AS (200mg/kg) treated groups which is in agreement with the report of Ahmed et al. (2014).[19] MSG caused an increase in relative ovarian weights in rats but contradicts another report that ovarian weight in MSG-induced ovarian toxicity was significantly reduced (Abd and Osman 2011).[20] The increase in ovarian weight in our present study may be attributed to the induction of oxidative stress and subsequent inflammatory response which could lead to edema which could accompany MSG intake. Interestingly, the increase in ovarian weight was restored to normal by AS (500mg/kg), confirming its antioxidant and anti-inflammatory activities.

Our result on follicular count showed a significant increase in the number of atretic follicles in MSG-only when compared with the control, AS, and vitamin C-treated groups (Figure 3). This result agrees with the previous report that a 14-day MSG administration led to an increased number of atretic follicles with no corpora luteum.[19] The atretic incidence is greatly reduced in AS-treated groups pointing to the probable antioxidant capacity of the extract which helped to preserve the follicles in those groups. We also noticed a decrease in the number of primordial follicles and the primary follicles in MSG-only group as compared to the control and AS-treated groups. The disappearance of resting primordial follicles observed in this study is in agreement with the work done by Chapman (1982)[9] which reported that ovarian toxicity is characterized by follicular degeneration due to a gradual depletion of the oocyte reserve following apoptotic cell death and ovarian atrophy with the disappearance of resting primordial follicles. The histological assessment showed that MSG-induced ovarian toxicity is associated with histopathological changes such as follicular degeneration (atresia), increased vacuolation, and inflammation which is characterized by interstitial edema, vascular hemorrhage, and interstitial stroma cell hypertrophy (Plate 1). The medulla appeared degenerated, and have multiple vacuoles with congested blood vessels some of which were full of hyaline material. These pathological derangements were very prominent in the MSG-only and MSG + 500 mg/kg AS groups when compared with the control and other treated groups. The degeneration of ovarian follicles and their oocytes detected in this study might be due to oxidative stress caused by MSG. This agreed with the report of (Ismail, 2012)[5], which attributed ovarian pathologies after MSG treatment to oxidative damage. Worthy of note however is the normal

appearance of the histoarchitecture of the AS (200mg/kg) and vit. C treated groups.

PAS-stained ovarian sections of rats in the control group showed a high level of PAS-positive reaction within the oocytes indicating high amounts of glycogen in the basement membrane of the ovary. However, ovarian sections of rats treated with MSG only and the group that received a low dose of AS showed negative PAS reactivity within some oocytes with complete absence in zona pellucida (Plate 2). This result indicates a reduction or even depletion of glycogen within the oocytes and their surrounding zona pellucida. Our finding is in concord with that of El-sherbiny[21], which demonstrated marked depletion in carbohydrate content in the cortex and medulla of kidneys of MSG-treated rats. Disturbances in carbohydrate metabolism could be suggested to be achieved by modifying the activities of the enzymes of the glycolytic pathways that led to metabolic degradation and inhibition of carbohydrate synthesis in the ovarian follicles, as previously reported.[22] Of novel observation in this present study is the action of AS extract in preserving the glycogen contents in the high-dose group when compared with the MSG-only and low-dose groups. This suggests that our extract possesses the ability to normalize the glycolytic pathway that was disrupted by MSG intake.

CONCLUSIONS

By putting together the findings from our study, *Allium sativum* extract possessed dose-dependent ameliorative properties evidenced by its role in remedying the damage caused by monosodium glutamate-induced follicular atresia, histological derangement, and glycogen degradation.

LIST OF ABBREVIATIONS

- Allium sativum- AS
- Monosodium glutamate- MSG
- Periodic acid Schiff- PAS
- Reactive oxygen species- ROS

DECLARATIONS

Ethics approval and consent to participate: All experimental procedures were Research Approval was gotten from the Babcock University Health Research Ethical Committee (BUHREC: 036/19)

Consent for publication: Not applicable

Availability of Data and Material: Not applicable

Competing Interests: We declare no conflict of interest

Funding: Not applicable

Authors' contributions: Taiye S. Adelodun-conceptualized and designed the study, **Anointing E. Esim** was involved in data acquisition, **Sunday Y. Olatunji**- data analysis, **Kehinde O. Adeniji** was involved in manuscript preparation, **John A. Olanrewaju**- data analysis, **Ayodeji Z. Abijo** was involved in writing and editing the manuscript and **Oluseyi S. Fabiyi** was involved in histological slides preparation.

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