

Psidium Guajava Ameliorates Testicular Distortions Triggered by Aluminium Chloride in Adult Male Wistar Rats

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Introduction: Aluminium (Al) is a trivalent cation that does not undergo redox changes. Psidium guajava, the guava tree is widely distributed as a native plant in Latin America and Sub-Saharan Africa, with its leaves being deployed in folk medicine to manage several diseased conditions. The study was conducted to evaluate the effects of an aqueous extract of Psidium guajava on Aluminium (AlCl₃) induced testicular damage.

Materials and Methods: Thirty (30) adult male Wistar rats were divided randomly into six (6) groups of five (5) animals each and used for this study. Group A was orally administered with 0.5 ml of normal saline from the 1st to the 14th day. Group B got 0.5 ml of 100mg/kg body weight of aluminium chloride (AlCl₃) only, from the 1st to 14th day. Group C got 0.5 mls of 250mg/kg body weight of Psidium guajava leaf extract from the 1st to 14th day, Group D got 0.5 mls of 500 mg/kg body weight of Psidium guajava from the 1st to 14th day. Group E got 0.5 mls of 100mg/kg body weight of aluminum chloride from the 1st to 14th day, and 0.5 mls of 250mg/kg body weight of Psidium guajava leaf extract from the 15th to 28th day. Group F got 0.5mls of 100mg/kg body weight of aluminum chloride from the 1st to the 14th day, and 0.5mls of 500 mg/kg body weight of Psidium guajava leaf extract from the 15th to the 28th day. Administration was via the oral gavage.

Results: Oral administration of aluminum chloride adversely compromised the testicular histomorphology of Wistar rats, causing testicular atrophy associated with degeneration of germ cells within the seminiferous tubules, and enlarged lumen with fewer spermatids compared with the non-treated animals.

Conclusion: Guava leaf extract reversed the damage caused by aluminum chloride, restoring the distorted seminiferous tubules and presenting the lumen with densely packed primary spermatocytes.

Keywords: Aluminium chloride, Psidium guajava, testes, histomorphology, oxidative stress

Introduction

Man is exposed to many environmental contaminants at different stages of life and exposure to aluminium (Al) due to industrial and environmental pollution poses severe ecological hazards (1). It has been adjudged the most widely distributed metal in the environment. It is believed to be the most prevalent metal and the third most crucial

abundant element in the earth's crust; only oxygen (49.5%) and silicon (26%) occur more commonly than Al (8%) (2). In biological systems, Al is present only in trace amounts, shown to have no definite biological function and is poorly absorbed but efficiently eliminated when absorption does occur. Al is described mainly in bone, liver, testes, kidney, and brain, while its

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accumulation in tissues and organs results in dysfunction and toxicity (3).

The wide distribution of this element ensures the potential to exert harmful effects on human health upon exposure (4). Al compounds are widely used in medicines as antacids, vaccines, anti-diarrhea, phosphate binders, and allergen injections (5); food additives and toothpaste (6); water purification agents (7). It is still a metal of choice in making various household cookware and storage utensils.

Al contributes to various cognitive impairments in mice, rabbits and rats (8,9). Epidemiological studies have indicated a link between Al in drinking water and Alzheimer's disease, and a variety of human and animal studies have implicated learning and memory deficits post-Al exposure (10,11). $AlCl_3$ can generate reactive oxygen species (ROS) (12). In another instance, Al has been reported to have induced toxicity in the epididymis, vas deferens, seminal vesicle, and ventral prostate of mice. $AlCl_3$ -induced free radicals and inhibited antioxidant enzymes in blood and seminal plasma, liver, testes, kidney, lung, and brain of rabbits (12, 13).

Antioxidants are substances that protect cells against the adverse effects of xenobiotics, carcinogens, drugs, and toxic agents (14). Since spermatogenesis and Leydig steroidogenesis are vulnerable to oxidative stress, the low oxygen tension that characterizes this issue may be an essential component of the mechanisms by which the testis protects itself from free radical-mediated damage (15).

The use of plants as medicines predates written human history. Nigeria has a vast repository of medicinal plants. It has thus paved the way for synchronizing traditional medicine into the healthcare delivery system, and the guava plant is one such example (16). The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing expensive modern pharmaceuticals (17).

Psidium guajava, the common guava tree, is widely distributed as a native plant in Africa and America from Mexico to Brazil. Whose leaves have been used in folk medicine for many years in different parts of the globe in managing various disease conditions and overcome several disorders, such as; rheumatism, diarrhea, diabetes mellitus, cough, and ulcers (18-21). The plant has also been reported to exhibit antimicrobial, analgesic and anti-inflammatory properties (22, 23).

Materials and Methods

Ethical approval for the study was given by the ethical committee of the College of Health Sciences, University of Ilorin, in line with the globally approved guidelines for the use of animals for scientific studies.

Thirty male adult Wistar rats, weighing between 160 to 250g, were obtained from a private animal farm in Ogbomoso, Oyo State, Nigeria, and maintained in the animal house of the College of Health Sciences, University of Ilorin. They were housed in cages with adequate space to encourage free movement under natural and dark cycles (12hr light and 12hr dark) at room temperature. They were bred on conventional commercial standard rat pellets. They were also supplied with water *ad libitum* and allowed to acclimatize to experimental conditions for 14 days before commencement of the experiments. Interval weighing of animals was done throughout. Strict hygiene was maintained by clearing their wastes daily.

Fresh leaves of *P. guajava* were obtained from a private farm at Tanke, a suburb of Ilorin, Kwara State-Nigeria. Aqueous extraction of guava leaf was achieved according to an existing procedure (24). In this procedure, fresh guava leaves were harvested from the botanical garden of the University of Ilorin, washed thoroughly, air-dried and pulverized using an electric blender. Two hundred grams (200g) of the powder obtained was macerated with 600mls of distilled water for 72 hours at

30±4°C. The mixture was sieved and filtered with Whatman No. 1 filter paper and the filtrate was concentrated in an electrically regulated oven at 45°C. Finally, concentrated crude aqueous extract was dried to a solid mass. The testing samples were prepared by dissolving the solid mass with normal saline to obtain a final concentration of 250 mg/kg body weight (bw) and 500 mg/kg body weight (bw) used for the experiment. Laboratory grade AlCl₃ was locally sourced from Dennis and Dennis Laboratory, Taiwo Road, Ilorin-Nigeria. Other reagents & chemicals were of analytical grade.

The thirty (30) adult male Wistar rats were divided randomly into six (6) groups of five (5) animals per group. Group A was orally administered with 0.5 ml of normal saline from the 1st to 14th day and served as the control group; Group B was orally administered with 0.5 ml of 100 mg/kg body weight of AlCl₃ only from the 1st to 14th day. Group C was orally administered with 0.5 mls of 250mg/kg body weight of *Psidium guajava* leaf extract from the 1st to 14th day; Group D was orally administered with 0.5mls of 500 mg/kg body weight of *Psidium guajava* leaf extract from 1st to 14th day, Group E was orally administered with 0.5 mls of 100 mg/kg body weight of AlCl₃ from 1st to 14th day, followed by the oral administration of 0.5 mls of 250 mg/kg body weight of *Psidium guajava* leaf extract from 15th to 28th day, Group F was orally administered with 0.5mls of 100 mg/kg body weight of AlCl₃ from 1st to 14th day, followed by the oral administration of 0.5mls of 500 mg/kg body weight of *Psidium guajava* leaf extract from 15th to 28th day. All administration was achieved via oral gavage.

While all the rats from Groups A to D were sacrificed on the 15th day of the experiment, Groups E and F were sacrificed on the 29th day of the experiment. At the end of the treatment protocols, the animals were euthanized with intramuscular injection of ketamine hydrochloride (20 mg/kg). The testes were identified,

removed, cleared of fat, and weighed using a sensitive analytical weighing balance and were placed in a solution of Bouin's fluid and processed for histological light microscopic examination using routine hematoxylin and eosin, and immunohistological examination using Ki67 marker.

The right testes were placed in 0.25 mM of sucrose solution in the ratio of 1:4. Each of these tissues was separately transferred to a glass homogenizer containing the cold sucrose solution and were homogenized using an electrical homogenizer (Remi 8000 RPM). The unbroken cells and cell debris were removed by centrifuging at 3000 RPM for 15 minutes. The obtained supernatant(s) were used for the enzymatic estimations (antioxidant enzyme levels). Determination of follicle stimulating hormone (FSH), luteinizing hormones (LH), and testosterone were quantitatively measured by adopting an enzyme-linked immunosorbent assay (ELISA) technique using the Accu-Bind Microwell kit. Lake Forest, CA 92630, USA.

Results

The effect of the oral administration of aqueous extract of *P. guajava* leaves on body weight of AlCl₃-induced testicular toxicity is presented in Table 1. The weight of the experimental animals was measured once a week, and the analysis of the data obtained throughout the experiment indicates an increase in body weight in the control group and the *P. guajava* groups (250mg/kg and 500 mg/kg bw). The extract showed no significant changes ($p < 0.05$) in body weight at all doses administered compared to the control group. However, the animals orally administered AlCl₃ for 14 days recorded the lowest body weight, which was reversed when treated with the aqueous extract of guava leaves (250 mg/kg and 500mg/kg bw). However, the reversal effect was dose-dependent.

The effect of the oral administration of aqueous extract of *P. guajava* leaves on testicular weight of $AlCl_3$ -induced testicular toxicity is presented in Table 2. There was an increase in testicular weight in the control and *P. guajava* groups (250 and 500 mg/kg). The extract showed no significant changes ($p < 0.05$) in testicular weight at all doses administered compared to control. However, the animals given oral administration of $AlCl_3$ for 14 days recorded the lowest testicular weight, which was reversed when treated with an aqueous extract of guava leaves (250 and 500 mg/kg); however, the reversal effect was dose-dependent.

Biochemical evaluation

Quantification of reproductive hormones, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) was achieved via competitive enzyme immunoassay technique utilizing a polyclonal anti-LH, anti-FSH, and anti-T. These were done with ELISA at 450nm using the microplate readers, according to manufacturer's specifications.

FSH

Follicle-stimulating hormones act directly on the seminiferous tubules, critical in stimulating both mitotic and meiotic DNA synthesis in

spermatogonia (25). The effect of the oral administration of an aqueous extract of *P. guajava* leaves on FSH of the $AlCl_3$ -induced testicular toxicity is presented in Chart 1. An increment in FSH level was observed in the control group and the *P. guajava* groups (250 mg/kg and 500 mg/kg). The extract showed no significant changes ($p < 0.05$) in FSH at all doses administered compared to control. However, the animals given oral administration of $AlCl_3$ for 14 days recorded the lowest level of follicle-stimulating hormone, which was reversed when treated with an aqueous extract of guava leaves (250 mg/kg and 500 mg/kg). However, reversal effect was dose-dependent.

LH

Luteinizing hormone is a gonadotropic hormone primarily secreted by the anterior pituitary gland. It is a glycoprotein that regulates testosterone production from the Leydig cells' interstitial cells. The effect of the oral administration of an aqueous extract of *P. guajava* leaves on the luteinizing hormone of $AlCl_3$ -induced testicular toxicity is presented in chart 2. There was an increase in the level of luteinizing hormone in the control group and the *P. guajava* groups (250 mg/kg and 500

Table-1. The mean and standard error of body weights' mean (SEM) on the Effect of *P. guajava* leaves extract on aluminum chloride-induced Wistar rats' testicular toxicity

Groups	Group A (g)	Group B (g)	Group C (g)	Group D (g)	Group E (g)	Group F (g)
Initial weight	227.3±2.67	171.3±5.7	205.3±7.86	202.67±13.38	212±8.72	226±14
Final weight	330.67±3.71	267.3±5.46	306±12	303.67±16.33	309.67±9.68	324.6±14.6
Weight differences	103.3±3.71	96±4	100.67±8.19	101±3	96.67±8.19	98.67±2

** N=5; Values are expressed as mean ± S.E.M followed by One Way ANOVA –Turkey's multiple Comparison test. Comparison was between Group A=Control, Group B= $AlCl_3$, Group D=250 mg/kg *P. guajava*, Group C=500 mg/kg *P. guajava*, Group E= $AlCl_3$ +250 mg/kg *P. guajava*, Group F=500 mg/kg + $AlCl_3$ The level of significance value is $P < 0.05$

Table-2. The mean and standard error of mean (SEM) on the Effect of *P. guajava* leaves extract on testicular weight of $AlCl_3$ induced toxicity of Wistar rats

Groups	Group A	Group B	Group C	Group D	Group E	Group F
Testicular weight (g)	1.247±0.07	1.197±0.03	1.200±0.07	1.203±0.06	1.317±0.13	1.340±0.02

** N=5; Values are expressed as mean ± S.E.M followed by One Way ANOVA –Turkey's multiple Comparison test. Comparison was between Group A= Control, Group B= $AlCl_3$, Group D=250 mg/kg *P. guajava*, Group C=500 mg/kg *P. guajava*, Group E= $AlCl_3$ +250 mg/kg *P. guajava*, Group F=500 mg/kg + $AlCl_3$ Level of significance value is $P < 0.05$

mg/kg). The extract showed no changes in luteinizing hormone at all doses administered compared with control. However, the animals given oral administration of $AlCl_3$ for 14 days recorded the lowest luteinizing hormone, which was reversed when treated with an aqueous extract of guava leaves (250 and 500 mg/kg); however, reversal effect was dose-dependent.

TESTOSTERONE

Testosterone (T) is a C_{19} steroid and the most potent naturally secreted androgen. In the male, it is synthesized in the interstitial Leydig cells and regulated by luteinizing hormone. It is responsible for the development of secondary sexual characteristics. The effect of the oral administration of aqueous extract of *P. guajava* leaves on testosterone level of $AlCl_3$ -induced testicular toxicity is presented in chart 3. There was an increase in the level of testosterone in the control group and the *P. guajava* groups (250 and 500 mg/kg). The extract showed no changes in T levels at all doses administered compared to control. However, the animals given oral administration of $AlCl_3$ for 14 days recorded the lowest testosterone, which was reversed when treated with an aqueous extract of guava leaves (250 and 500 mg/kg); however, the reversal effect was dose-dependent.

SOD

Superoxide Dismutase (SOD) presents the first line of defense against superoxide. It dismutates (O_2^-) anion to form O_2 and H_2O_2 . SOD protects spermatozoa against spontaneous O_2 toxicity and Lipid peroxidation (26). Several reports suggest that $AlCl_3$ may inhibit the activity of SOD and CAT (27,28). The effect of the oral administration of an aqueous extract of *P. guajava* leaves on SOD activity of $AlCl_3$ -induced testicular toxicity is presented in chart 4. There was an increase in the level of SOD in the control group and the *P. guajava* groups (250 mg/kg and 500 mg/kg). The extract showed no changes in SOD at all doses administered when compared with control. However, the animals

given oral administration of $AlCl_3$ for 14 days recorded the lowest SOD, which was reversed when treated with an aqueous extract of guava leaves (250 mg/kg and 500 mg/kg); however, the reversal effect was again, dose-dependent.

MDA

Malondialdehyde (MDA) is a well-known lipid peroxidation indicator that increases in the testis and epididymis after Al exposure (29,30). Increased lipid peroxidation might be caused by interference with mineral metabolism distribution. The capacity of Al to displace other biological cations such as calcium, iron, zinc, copper, and magnesium from their binding sites is a potential target for the adverse effects of Al (31). The effect of the oral administration of aqueous extract of *P. guajava* leaves on MDA activity of $AlCl_3$ -induced testicular toxicity is presented in chart 5. There was a decrease in the level of MDA in the control group and the *P. guajava* groups (250 mg/kg and 500 mg/kg). The extract showed no significant changes ($p < 0.05$) in MDA level at all doses administered compared to control. However, the animals given oral administration of $AlCl_3$ for 14 days recorded the highest level of MDA activity, which was reversed when treated with an aqueous extract of guava leaves (250 mg/kg and 500 mg/kg). However, the reversal effect was equally dose-dependent.

Discussion

Body and testicular weight observations

During close observation, the group of rats given 0.5ml of normal saline, 250mg/kg, and 500mg/kg B.W of *Psidium guajava* leaves extract orally for 14 days appeared normal and active all through the experiment, while the group treated with aluminum chloride only for 14 days were generally weak for about 5 to 10 minutes before picking up again, this contributed to the reduction of feed intake and in essence decrease in weight gain compared to the control, 250mg/kg and 500mg/kg BW of *Psidium guajava*. However, *Psidium guajava*-

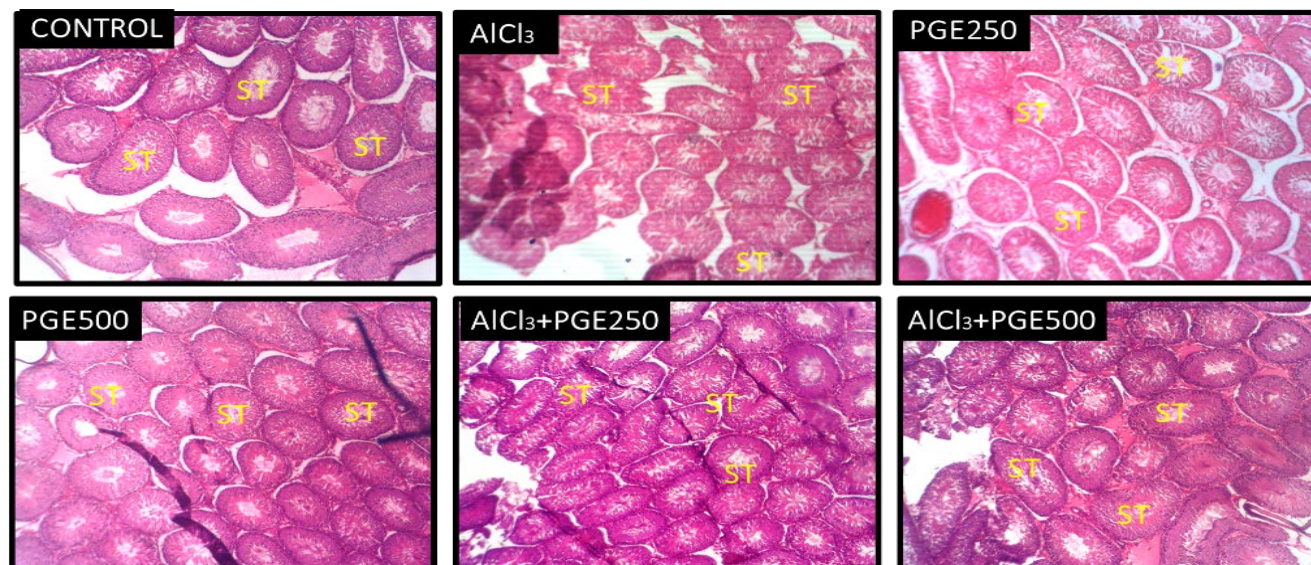


Figure-1. Representative photomicrograph of the testes of rats revealing a panoramic view of multiple seminiferous tubules (ST). control=normal saline, AlCl_3 =100mg/kg body weight of AlCl_3 , PGE250=250mg/kg bodyweight of *Psidium guajava* extract and PGE500=500mg/kg bodyweight of *Psidium guajava* extract. Control, PGE250, and PGE500 present with normal seminiferous tubules with typical interstitial space. The seminiferous tubules of the AlCl_3 -treated animals are laconically packed with atypical interstitial space and irregular condensation of the lumen. AlCl_3 +PGE250 and AlCl_3 +PGE500 presented with packed seminiferous tubules with typical morphological architecture similar to what is obtainable in the control group: Hematoxylin and Eosin stain x40 magnification.

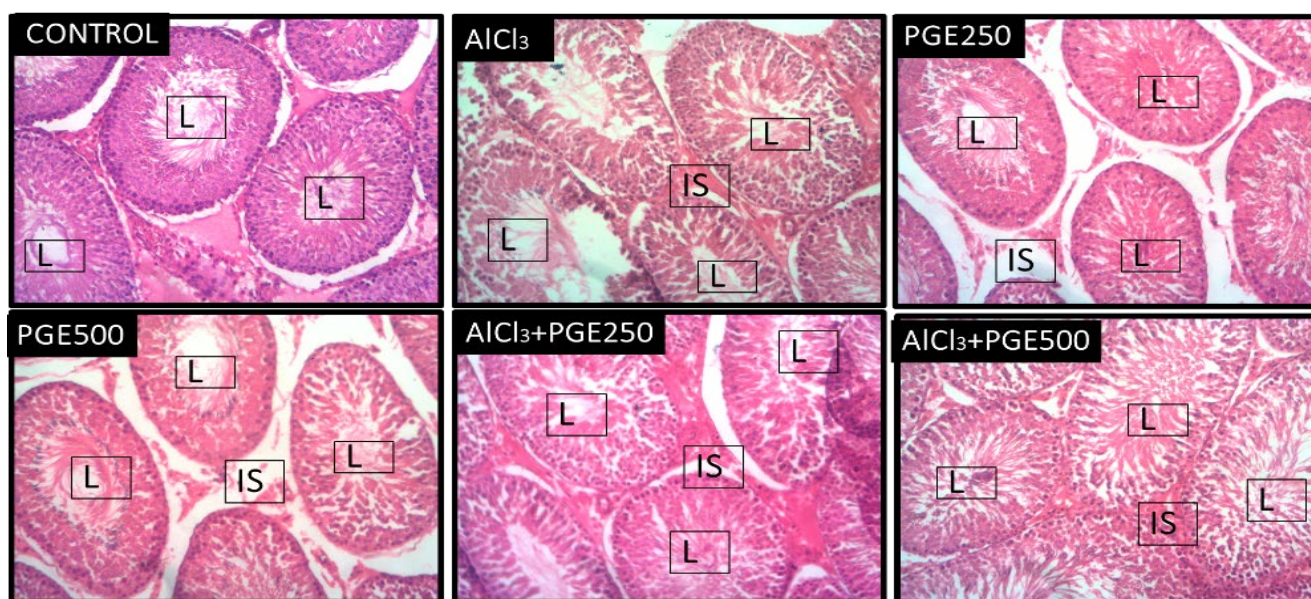


Figure-2. Representative photomicrograph of the testes of rats revealing a detailed view of seminiferous tubules (ST), Lumen of seminiferous tubule (L), as well as the interstitial spaces (IS). control=normal saline, AlCl_3 =100mg/kg body weight of AlCl_3 , PGE250=250mg/kg bodyweight of *Psidium guajava* extract and PGE500=500mg/kg bodyweight of *Psidium guajava* extract. Control, PGE250, and PGE500 present with isoconcentrated lumen, intact basal membrane, and typical interstitial space with no signs of testicular degeneration. The seminiferous tubules of the AlCl_3 treated animals are hypercondensed with poorly outlined basal membrane, enlarged lumen and distorted interstitial architectural presentation. AlCl_3 +PGE250 and AlCl_3 +PGE500 were present with trimly sorted seminiferous tubules with morphological architecture and smoothly outlined interstitial space similar to what is obtainable in the control group: Heamatoxylin and Eosin stain x100 magnification.

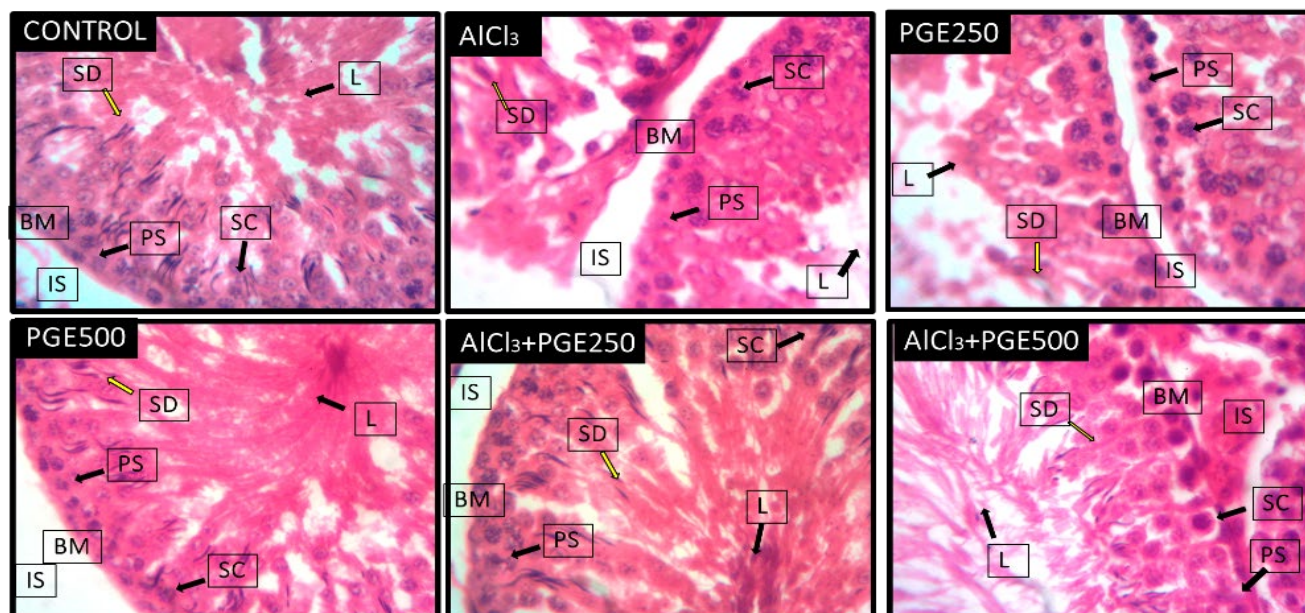


Figure-3. Representative photomicrograph of Wistar testes showing a high-power magnification of the seminiferous tubules (ST), Lumen of seminiferous tubule (L), spermatids (SD), primary spermatocytes (PS), supporting cells, basement membrane (BM) and the interstitial spaces (IS) depicting the centripetal spermatogenic process. control=normal saline, AlCl_3 =100mg/kg body weight of AlCl_3 , PGE250=250mg/kg bodyweight of *Psidium guajava* extract and PGE500=500 mg/kg bodyweight of *Psidium guajava* extract. Control, PGE250, and PGE500 present densely populated primary spermatocytes and supporting cells in the basement membrane. Lumen is densely packed with matured spermatocytes. The basement membrane is well delineated with adjoining interstitial comprising intact interstitial cells. The seminiferous tubules of the AlCl_3 -treated animals at this high-power magnification present tubular degeneration with laconically expressed spermatogonial cells and very few supporting cells in the degenerated seminiferous tubule. The spermatids near lumen are very few relative to what is obtainable in other photomicrographs. AlCl_3 +PGE250 and AlCl_3 +PGE500 present normal seminiferous tubules with characteristic morphological Architecture, densely packed cells, and smoothly outlined interstitial space similar to what is accessible in the control: Heamatoxylin and Eosin stain x400 magnification.

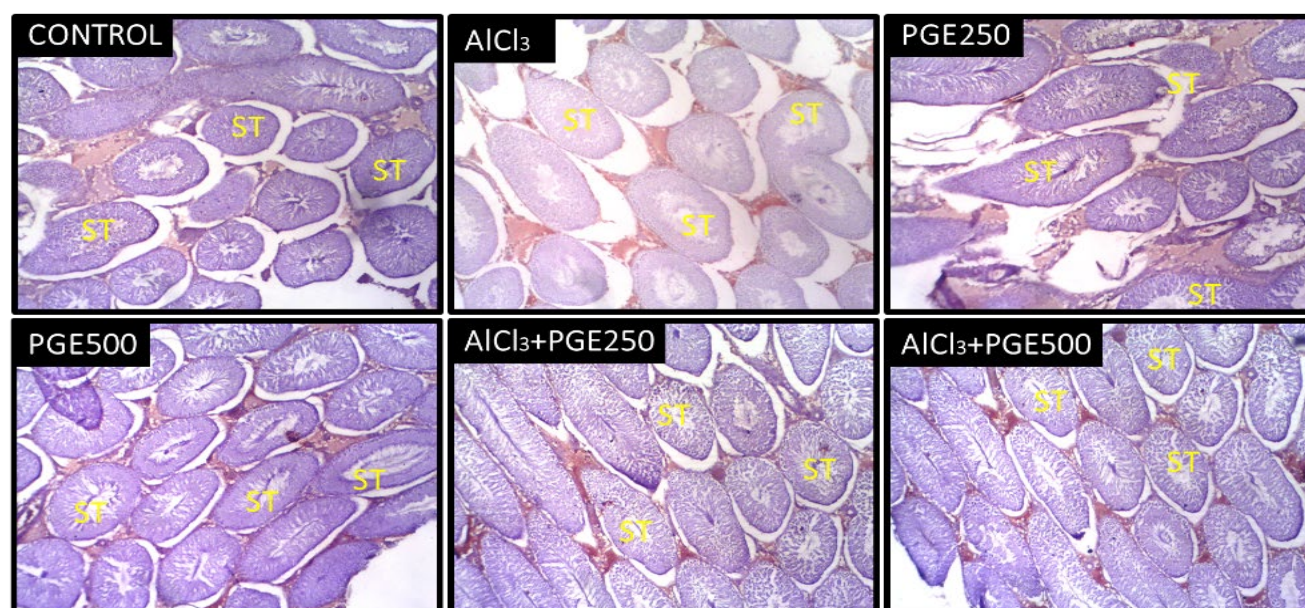


Figure-4. Representative photomicrograph of the testes of rats revealing a panoramic view of multiple seminiferous tubules (ST). Control=normal saline, AlCl_3 =100mg/kg body weight of AlCl_3 , PGE250=250mg/kg body weight of *Psidium guajava* extract, and PGE500=500mg/kg body weight of *Psidium guajava* extract. AlCl_3 -treated rats presented with poor staining intensity relative to other experimental groups showing expression of Ki67. Immunohistological staining for paraffin-embedded tissues at x40 magnification.

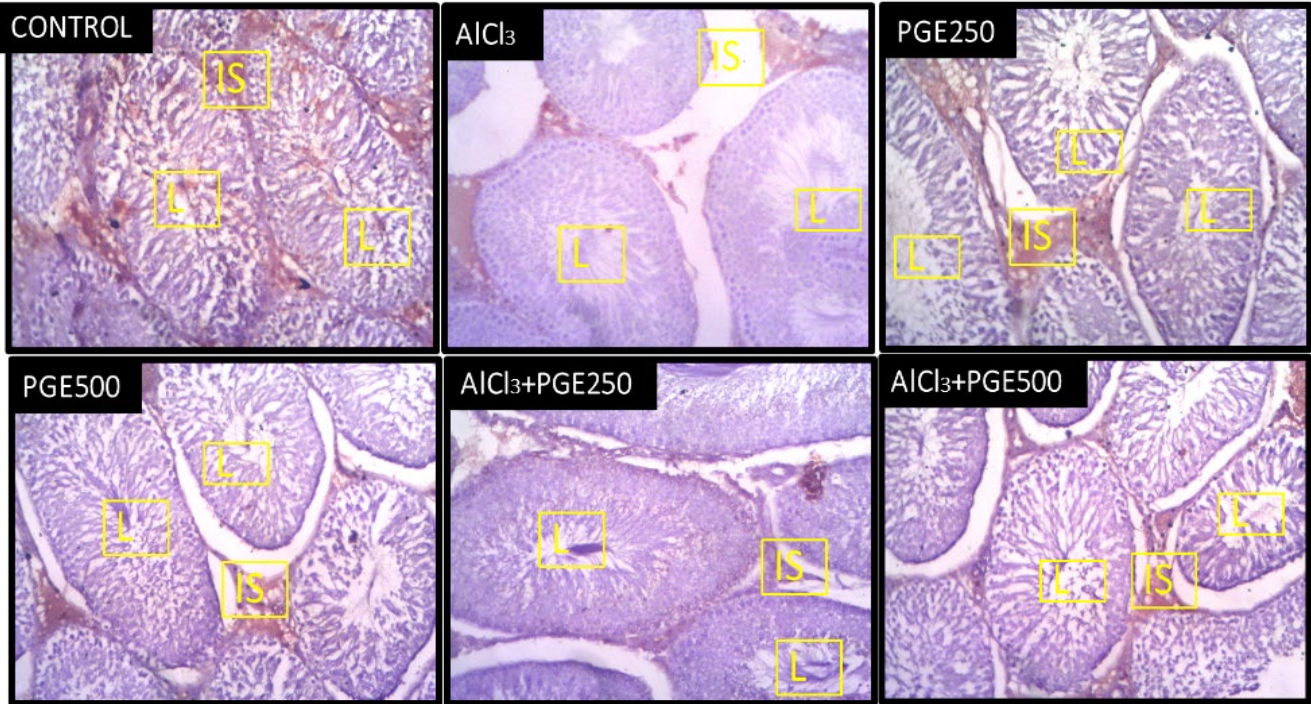


Figure-5. Representative photomicrograph of the testes of rats revealing a panoramic view of multiple seminiferous tubules (ST). Control=normal saline, AlCl₃=100 mg/kg body weight of AlCl₃, PGE250=250 mg/kg body weight of *Psidium guajava* extract, and PGE500=500 mg/kg body weight of *Psidium guajava* extract. AlCl₃-treated rats presented with poor staining intensity relative to other experimental groups showing expression of Ki67. Immunohistological staining for paraffin-embedded tissues at x100 magnification.

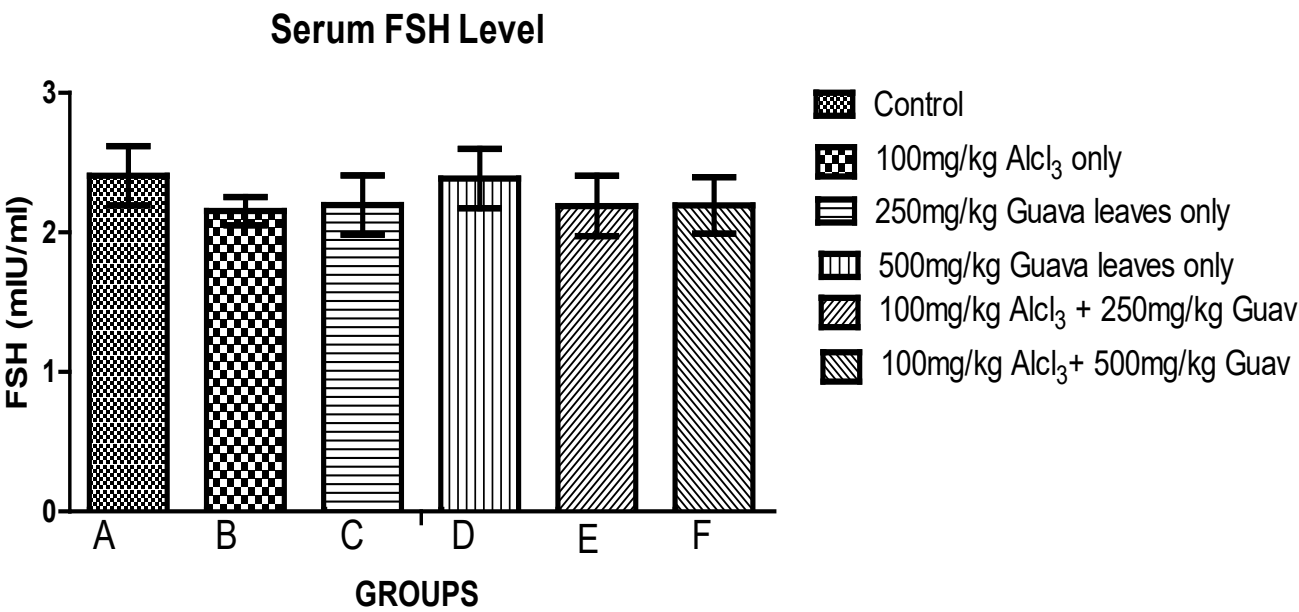


Chart-1. Bar Chart showing the level of FSH in the serum of Wistar rats. All values were represented as the mean and standard error of the mean (SEM), followed by One Way ANOVA –Turkey's multiple Comparison test. A=Control, B=100 mg/kg AlCl₃ only, C=250 mg/kg guava leaves only, D=500 mg/kg guava leaves only, E=100 mg/kg g AlCl₃+250 mg/kg guava leaves, F=100 mg/kg AlCl₃ + 500mg/kg guava leaves.

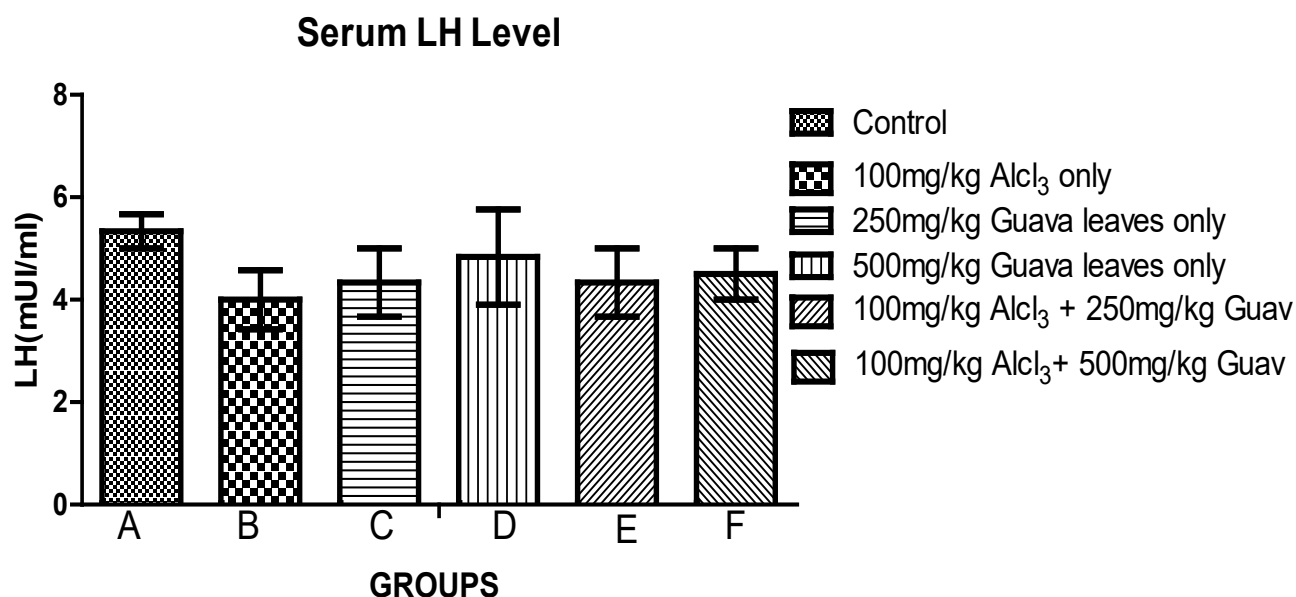


Chart-2. Bar Chart showing the level of luteinizing hormone in the serum of Wistar rats. All values were represented as the mean and standard error of the mean (SEM), followed by One Way ANOVA –Turkey's multiple Comparison test. A=Control, B=100 mg/kg AlCl₃ only, C=250 mg/kg guava leaves only, D=500 mg/kg guava leaves only, E=100 mg/kg g AlCl₃+250 mg/kg guava leaves, F=100 mg/kg AlCl₃ + 500mg/kg guava leaves.

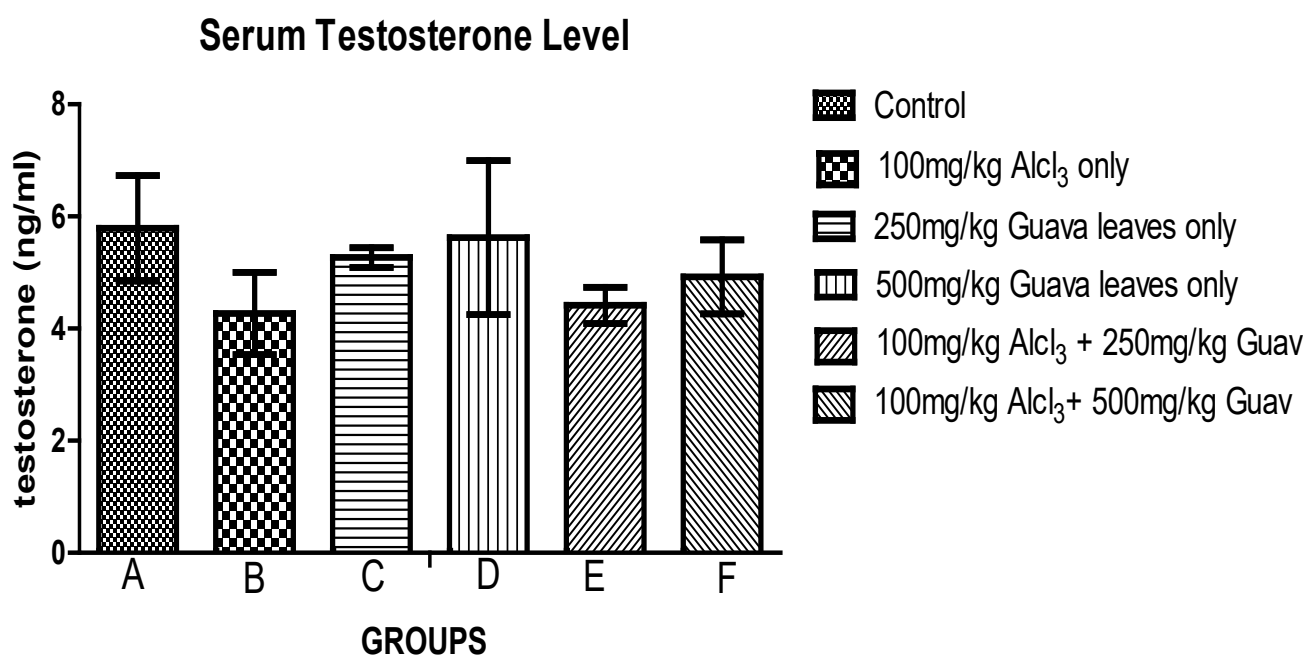


Chart-3. Bar Chart showing the level of luteinizing hormone in the serum of Wistar rats. All values were represented as the mean and standard error of the mean (SEM), followed by One Way ANOVA –Turkey's multiple Comparison test. A=Control, B=100 mg/kg AlCl₃ only, C=250 mg/kg guava leaves only, D=500 mg/kg guava leaves only, E=100 mg/kg g AlCl₃+250 mg/kg guava leaves, F=100 mg/kg AlCl₃ + 500 mg/kg guava leaves.

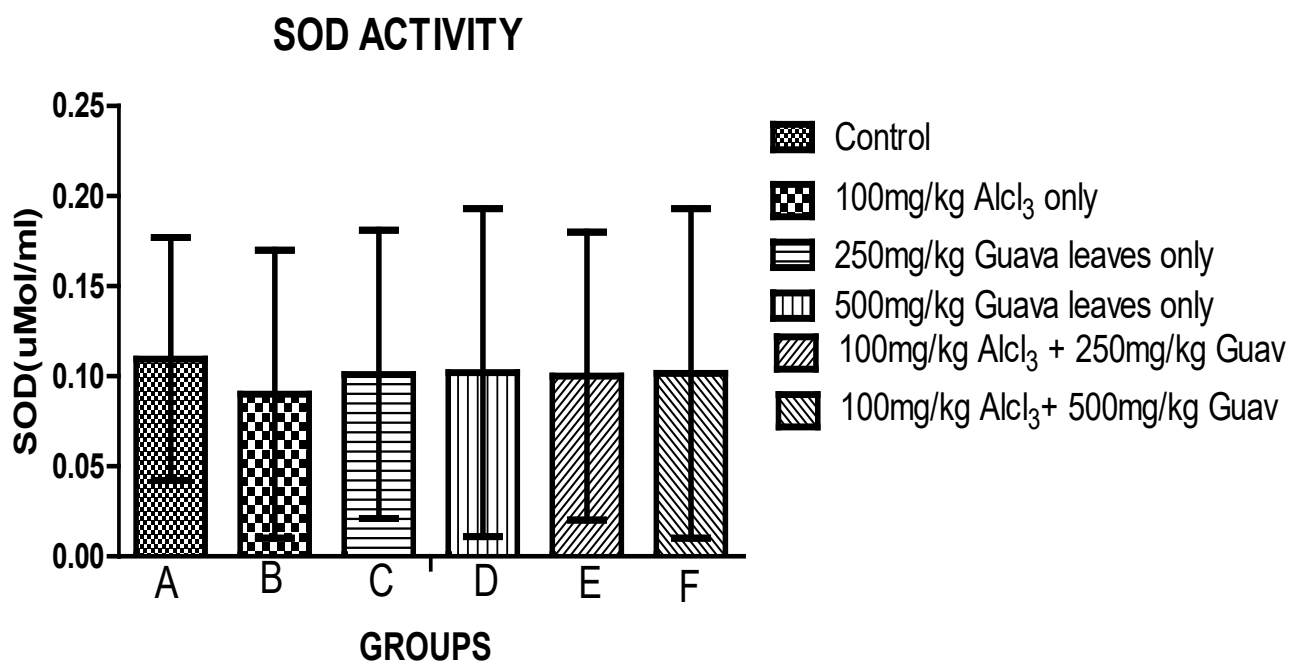


Chart-4. Bar Chart showing Superoxide Dismutase Activity (SOD) in the testicular homogenate of Wistar rats. All values were represented as the mean and standard error of the mean (SEM), followed by One Way ANOVA –Turkey's multiple Comparison test. A=Control, B=100 mg/kg AlCl₃ only, C=250 mg/kg guava leaves only, D=500 mg/kg guava leaves only, E=100 mg/kg g AlCl₃+250 mg/kg guava leaves, F=100mg/kg AlCl₃ + 500 mg/kg guava leaves.

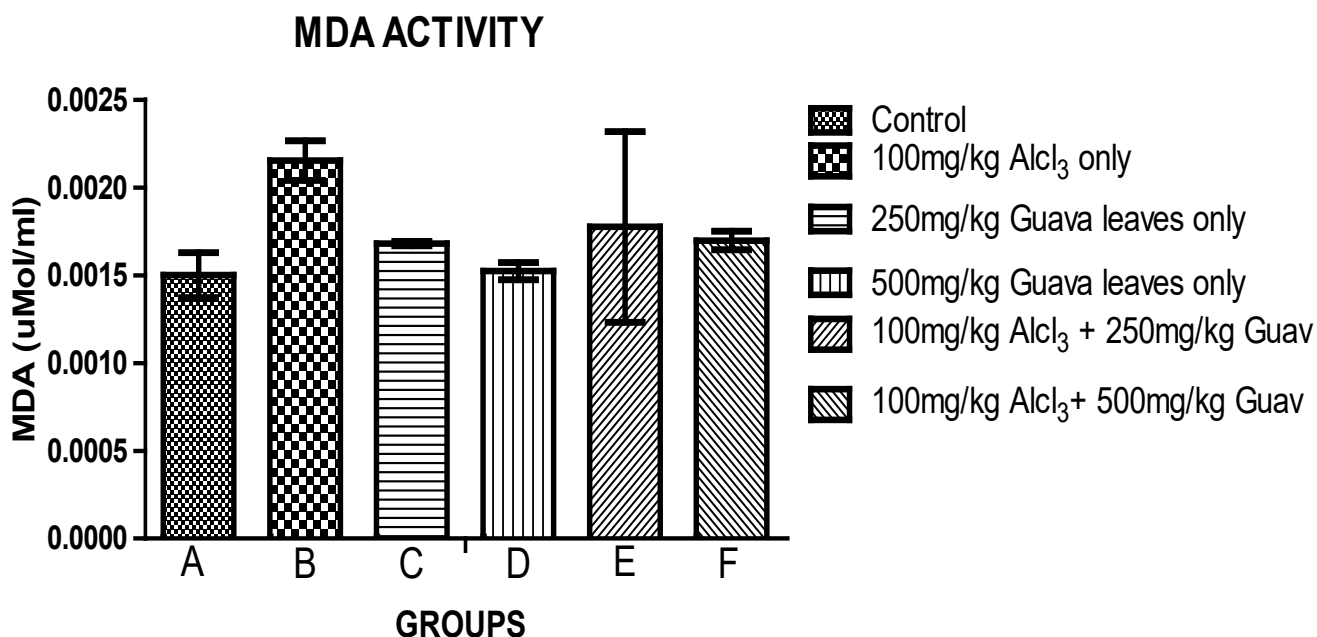


Chart-5. Bar Chart showing Malondialdehyde Activity (MDA) in the testicular homogenate of Wistar rats. All values were represented as the mean and standard error of the mean (SEM), followed by One Way ANOVA–Turkey's multiple Comparison test. A=Control, B=100 mg/kg AlCl₃ only, C=250 mg/kg guava leaves only, D=500 mg/kg guava leaves only, E=100 mg/kg g AlCl₃+250 mg/kg guava leaves, F=100 mg/kg AlCl₃ + 500 mg/kg guava leaves.

treated rats were normal all through the experiment, depicting that aqueous extract of guava leaves ameliorated the inactiveness caused by aluminum chloride. The inactiveness observed in the aluminum-treated rats might be due to the induction of oxidative stress. Oxidative stress is recognized as the major mode of action of aluminum exposure as this has been observed in vitro in several types of cells and also in vivo in soft tissues such as the liver, kidney, brain, lung, and testes of experimental animals (7). Guava leaves, with its antioxidant properties, were observed to have ameliorated the effect induced by $AlCl_3$, as all the groups that took 250 mg/kg and 500 mg/kg bw aqueous extract of the leaves appeared active and healthy. Also, the ultimate effect of testicular damage on body weight indicates its toxicity to health (32). However, there have been conflicting reports of correlations between testicular damage and weight changes in the body. In this current study, the weight of the experimental animals was measured once a week. Analysis of the data obtained from animal body weights monitored throughout the experiment indicates that animals in all the experimental groups gained weight. The group that gained weight most was the control group, which had a weight gain of 103.33 ± 3.71 . An increase was observed in the group given 250 mg/kg and 500 mg/kg BW of *Psidium guajava* leaves extract only, which had a rise of 100.67 ± 8.19 and 101 ± 3 , respectively. A similar increase was observed in the treated groups given 250 mg/kg and 500 mg/kg BW of *Psidium guajava* leaves extract after a 100 mg/kg dosage of $AlCl_3$ for 14 days. The last increase in weight was that of the group given 100 mg/kg of $AlCl_3$ only for 14 days. However, the animals given oral administration of $AlCl_3$ only for 14 days recorded the lowest body weight gain; this result is in tandem with previous findings that reported that during three months of observation of rats receiving $AlCl_3$, a decrease in water and food intake and transient diarrhea occurred, which

led to lowering of final body mass of animals compared to controls (33). The low body weight was reversed when treated with an aqueous extract of guava leaves (250 mg/kg and 500 mg/kg bw); however, the reversal effect was dose-dependent, and the weight gained showed no changes. This could be attributed to *Psidium guajava* leaf extract exerting its antioxidant effect; hence, it lessened the effect of $AlCl_3$ on weight. This result corroborates the findings of another study, which also reported an increase in the weight of rats when administered 250 mg/kg, 500 and 750 mg/kg, aqueous extract of guava leaves for 14 days (34). Oral administration of $AlCl_3$ significantly decreased testicular weight with 100 mg/kg bw. At 14 days compared to the control, the groups were administered 250 and 500 mg/kg bw. Aqueous extract of guava leaves. These findings aligned with another study where a decrease in absolute and relative testes and seminal vesicle weights after $AlCl_3$ ingestion was reported (35). The decrease in the reproductive organs' weights could be due to the decrease in testosterone that may be attributed to oxidative damage induced in the rat testes. The decrease in testicular weight was noted to have been reversed when treated with an aqueous extract of guava leaves (250 and 500 mg/kg); however, reversal effect was dose-dependent. These findings are in tandem with another independent study where oral administration of 250 mg/kg and 500 mg/kg of aqueous extract of guava leaves increased body weight (36).

Hormonal and biochemical observations

The complex functioning of reproductive hormones is essential and closely linked with the temperance of spermatogenesis and sperm development. Co-secreted by gonadotrophin cells of the anterior pituitary, LH and FSH trigger T release by the Leydig cells of the testes in the male reproductive system, which affords a handy pointer to hormonal events in scenarios of significant distortions of sperm quality.

The results of the present study indicate that the treated rats with AlCl_3 in a dose of 100 mg/kg daily for 14-day periods have shown a significant decrease in the serum FSH, LH, and testosterone, which are represented in bar charts 1-3, compared with the control group and the groups orally administered with 250 mg/kg and 500 mg/kg of guava leaves only. It also showed a significant decrease compared to the group treated with varying doses of aqueous guava leaves previously administered AlCl_3 orally. AlCl_3 can significantly adversely affect reproductive performance in the animal model (38). This effect can be exerted centrally, peripherally, or both. Aluminium is a voltage-sensitive calcium channel blocker and can affect neurotransmitter release (39). The results aligned with the earlier report that AlCl_3 could generate reactive oxygen species in rabbit testes (12). Another finding equally showed that; exposure to aluminum lowered plasma and testicular testosterone in mice (9). A decrease in the LH release will lead to a reduction in the serum concentration of testosterone, as it is shown in this study. A decrease in testosterone level will eventually be reflected in peripheral sex organs functions, including epididymis and testis (40). The decrease in LH, FSH, and testosterone levels was reversed when treated with an aqueous extract of guava leaves (250 and 500 mg/kg bw). However, the reversal effect was dose-dependent. This finding is in line with a previous investigation, which showed that guava leaf extract improves semen quality in Adriamycin-induced testicular toxicity (41).

Malondialdehyde (MDA) is a well-known lipid peroxidation indicator that increased in testis and epididymis after Al exposure (29,30). Increased lipid peroxidation might be caused by the interference with mineral metabolism distribution. The capacity of Al to displace other biological cations such as calcium, iron, zinc, copper and magnesium from their binding sites is a potential target for the adverse effects of Al (31). Initial findings on intraperitoneal injection

of Al chloride in male mice for two weeks significantly increased the testicular Al burden, broke the balance of essential minerals Zn, Cu and Fe which lead to increased lipid peroxidation, as well as decreased testis ACE (testis-specific angiotensin-converting enzyme) activity. Al ions might gradually displace Zn from induced MT or MT-like proteins and release Zn capable of synthesizing more thionein proteins with further binding of Al and other metals, including copper. Reports point to aluminum's interference with iron homeostasis and severely impeding cellular metabolism. Aluminum also favors the generation of an oxidative environment because it creates a labile iron pool and interacts with membrane lipids (9). In this study, it was observed that testicular SOD activity of orally administered 100 mg/kg bw of AlCl_3 was lower. At the same time, the MDA level was higher compared to the control, 250 mg/kg bw and 500 mg/kg bw of aqueous extract of guava leaves only. The group treated with 250 mg/kg and 500 mg/kg bw after oral administration of AlCl_3 also showed an increased level of SOD and a decreased level of MDA compared to the AlCl_3 -treated group; however, the increase was dose-dependent. These findings aligned with those of a previous study where Al was shown to reduce antioxidants and increase lipid peroxidation (42). Another previous study had also reported Al-induced oxidative damage and the ability of Al to cross the blood-testis barrier after inducing oxidative stress and lipid peroxidation that damaged the biological membrane of the testes (43).

Histoarchitectural observations

Testicular toxicity of AlCl_3 was further confirmed by immunohistological observation in testicular tissues. These immunohistological observations revealed induced testicular atrophy associated with degeneration of germ cells within the seminiferous tubules, enlarged lumen and distortions in normal spermatogenic maturation when compared to the standard testicular

architecture with a conspicuous orderly arrangement of germinal cells and Sertoli cells as observed in the control group and the groups administered 250 mg/kg and 500 mg/kg of aqueous extract of guava leaves.

Testes treated with 100 mg/kg bw present intense necrosis, spermatogenesis disrupts, spermatozoa were absent in the lumen, and the seminiferous tubules' germinal epithelium was degraded. These findings agree with the data obtained from a previous study where in $AlCl_3$ -treated groups, histopathological examinations revealed apparent alterations in the testes, which induced marked lesions in seminiferous tubules (30). In addition, other documented findings reported that administering sodium fluoride and $AlCl_3$ for 30d caused disorganized epithelium cell debris in the lumen and reduced sperm density (44). The immune-histological staining for Ki67 also revealed that the $AlCl_3$ treated group was poorly stained, thereby revealing lesser cell proliferation than the control and treated groups.

Treatment with aqueous extract of *Psidium guajava* (250 and 500 mg/kg bw) showed both the drastic reduction in the histopathological features (testicular atrophy and vascular intimal erosion) and also expressed a large population of immunopositive cells as was obtained in the control groups.

Conclusion

Oral administration of $AlCl_3$ distorted the testicular histomorphology of Wistar rats, causing testicular atrophy associated with degeneration of germ cells within seminiferous tubules, enlarged lumen with fewer spermatids compared with the non-treated animals. Aqueous extract of guava leaves was able to reverse the damage caused by $AlCl_3$, reversing the distorted seminiferous tubules and presenting lumen with densely packed primary spermatocytes. A reversal in weight reduction caused by $AlCl_3$ was also established.

Conflicts of interest

There are no conflicts of interest.

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