ORIGINAL ARTICLE

Effects of Ethanol Extract of *Secamone afzelii* (Schult) K. Schum (Asclepiadceae) Leaves on Aluminum Chloride-induced Reproductive Toxicity in Male Wistar Rats

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Abstract:

Background: The local use of medicinal plants in the management of reproductive abnormalities is a common practice in Africa. One prominent plant used in Nigeria for such purpose is *Secamone afzelii*. *Aim and Objectives:* To investigate the effects of Ethanol Extract of *Secamone afzelii* (EESA) on reproductive dysfunction induced by Aluminum Chloride ($AlCl_3$) in male rats. *Material and Methods:* Thirty matured male Wistar rats equally divided into six groups were used for this study. Group I served as control, group II received $AlCl_3$ (35 mg/kg bw), groups III and IV received 35 mg/kg bw and 100 and 200 mg/kg EESA respectively. Groups V and VI received 100 and 200 mg/kg EEESA respectively. Doses were given once daily via oral route for 14 consecutive days. *Results:* The results revealed that, $AlCl_3$ caused significant decrease in final body weight, sex organs relative weight, sperm concentration, motility and viability, serum follicle stimulating hormone, luteinizing hormone, testosterone concentration and Glutathione (GSH) activity, accompanied with significant increase in sperm abnormalities. $AlCl_3$ also induced apparent alteration in the histoarchitecture of the testis and epididymis. Treatment with EESA modulated the harmful effects of $AlCl_3$. This was proved by improvement in the gross anatomical, hormonal and antioxidant activity of treated rats. There was a noticeable improvement in the histopathology of testicular and epididymal tissues. EESA significantly increased serum testosterone in a dose dependent manner. *Conclusion:* EESA is a possible ameliorative agent in $AlCl_3$-induced reproductive toxicity and oxidative stress in male rat.

Keywords: *Secamone afzelii*, Sperm, Testosterone, Testes, Epididymis

Introduction:

Aluminum (Al) is the most prevalent metal and the third most important abundant element in earth's crust, only oxygen (49.5%) and silicon (26%) occur more commonly than Al (8%). In biosystems, Al is present only in trace amounts. Al is extensively used in daily life and that provides easy exposure to human beings. Food sources of Al are corn, yellow cheese, salt, herbs, spices, tea. Other sources are cosmetics, Aluminum ware and containers [1].

Al absorption and accumulation in humans can occur via the diet, drinking water, ingestion with fruit juices or citric acid and these can cause a marked increase in both gastrointestinal absorption and urinary excretion of Al in healthy subjects [2]. Different forms of Al are environmental xenobiotic

that induce free radical-mediated cytotoxicity and reproductive toxicity. Al ingestion in excessive amount leads to accumulation in target organs and has been associated with damage of testicular tissues of both humans and animals [3]. Testicular Al accumulation has been reported to result in necrosis of spermatocytes/spermatids and significant decrease in fertility in male mice [4].

Secamone afzelii is a creeping woody climber with innately compound leaves, it is known locally in Nigerian languages as Awogba-arun (Yoruba), Utunta (Igbo) and Ewuonkwonegie (Bini) [5]. Traditionally, it is used to treat stomach upset, diabetes, colic, dysentery and kidney problems, cough and catarrh, hence, the name "Awogba-arun" (cure for multiple ailments).

The antioxidant activity of methanolic extract of *Secamone afzelii* stems was tested and reported by Mensah *et al*. The total extract was reported to have effective free radical scavenging activity [6].

Material and Methods:

Animals:

A total of 30 sexually matured male Wistar rats of age range 8-10 weeks (130 ± 10 g) were obtained from the Animal Holding Unit of College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were housed in well ventilated plastic rat cages, maintained on a natural light-dark cycle at room temperature. The rats were fed standard rat chow and given access to water *ad libitum*. The rats were allowed to acclimatize for 2 weeks before the start of the experiment. The international and national guidelines for the care and use of laboratory animals were followed.

Preparation of Extract:

The whole plant of *Secamone afzelii* was obtained at Aba Iya Gani area of Ile-Ife, Osun State, Nigeria and was identified by a taxonomist in the Department of Botany Obafemi Awolowo University, Ile-Ife where a voucher number was obtained (IFE-17721), and a specimen was deposited in the herbarium. The leaves were separated from the plant, air dried, ground to powder form using Warring blender and weighed. One kilogram of the powder was macerated in 5 liters of ethanol for 72 hours with regular shaking with an electric shaker at room temperature. The resulting mixture was filtered with Whatmann No. 1 filter paper (0.2 mm). The filtrate was evaporated under reduced pressure using a Rotary evaporator, weighed and freeze-dried in a lyophilizer (Ilshin Lab. Co. Ltd, Seoul, Republic of Korea) [7]. The extract obtained was kept at 4^0C in a refrigerator. The extract weight was 50 g which is equivalent to a yield of 15%.

Preparation of Stock Solution of Aluminum Chloride:

Aluminum Chloride ($AlCl_3$)was obtained from BDH Chemicals Ltd. Poole England. Seven hundred (700 mg) of $AlCl_3$ was dispensed into a sterile universal bottle, 20 ml of distilled water was added resulting in a solution containing 35 mg/ml. The solution was thoroughly shaken. Fresh solution was prepared at three days interval. It was administered through intraperitoneal injection using insulin syringe to induce reproductive toxicity at 35 mg/kg bw depending on the body weight of each animal.

Preparation of Stock Solution of the Extract

To prepare the stock solution of extract, 4 g of the extract was dispensed into sterile specimen bottle, 20 ml of distilled water was added to give a solution containing 0.2 g/ml (200 mg/ml). The

resultant solution was thoroughly shaken. Fresh solution was prepared daily. Ethanol extract of *Secamone afzelii* (EESA) was administered orally using oral cannula.

Experimental Design:

The rats were divided into six equal groups of five rats each.

Group I was the control (received 1 ml distilled water orally once daily)

Group II was positive control group (treated with 35 mg/kg bw $AlCl_3$)

Group III was co-administered with 35 mg/kg $AlCl_3$ and100 mg/kg EESA

Group IV was co-administered with 35 mg/kg $AlCl_3$ and 200 mg/kg EESA

Groups V and VI were given 100 and 200 mg/kg of EESA respectively.

Doses of EESA were given once daily via oral route for 14 consecutive days.

Blood Sampling:

Blood samples were collected by cardiac puncture, transferred into separate cryovial plain bottles using 2 ml syringes. Blood samples were centrifuged for 10 minutes at 3000 rpm using a cold centrifuge to get the serum.

Sex Organs Weight:

Testes and, epididymis and seminal vesicles were dissected out, trimmed off the attached fatty tissues and weighed. The relative weight of the organ was calculated by

$$\text{Relative weight} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

Semen Characteristics:

Seminal content of epididymis was obtained by mincing cauda epididymis using surgical blades and squeezed into a sterile specimen bottle. This content was diluted 10 times with 2.9% sodium citrate dehydrate solution and thoroughly mixed to estimate the progressive motility and sperm concentration. One drop of the suspension was smeared on a glass slide and stained by Eosin-nigrosin stain to determine the percentage of sperm cell viability and morphological abnormalities [8-9].

Hormonal and Antioxidant Assay:

Serum Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and testosterone were quantified using Enzyme-linked Immunosorbent Assay (ELISA) method. Manufacturer's instructions were followed. Glutathione was quantified by the method of Beutler and Kelly [10].

Histopathological Examination:

Sections were taken from testis and epididymal tissues from different animals in each group immediately after sacrifice. The tissues were washed with normal saline solution to remove blood, fixed in Bouin's fluid for a period of at least 24 hour, dehydrated in different grades of alcohol, and processed for paraffin embedding. Sections of 5 μm thickness were cut using a rotary microtome. The sections were processed and passed through graded alcohol series, stained with Haematoxylin and Eosin, cleared in xylene and examined microscopically and photomicrographs were taken using Leica DM750 Camera Microscope.

Statistical Analysis:

Data were analyzed using One-Way Analysis of Variance (ANOVA) and post *hoc* analysis was carried out using Student Neuman-Keuls test on Graph Pad 5.03 (Graph Pad Software Inc., CA, USA). P values less than 0.05 were considered to be significant.

Results:

Body and Sex Organs Weight:

The effect of EESA on body weight and relative organ weight change in $AlCl_3$ toxicity of male rats is represented in Table 1. There was significant decrease in final body weight and relative organ weight ($p < 0.05$) in $AlCl_3$ group as compared with control. This was, however, significantly reversed in the group treated with EESA.

Sperm Characteristics:

The effect of EESA on $AlCl_3$-induced toxicity on epididymal sperm motility, viability and abnormal sperms and total sperm count is represented in Table 2. $AlCl_3$ significantly decreased ($p < 0.05$) sperm motility, sperm viability and total sperm count, compared with control group. Meanwhile, in evaluating total number of sperm cells with morphological abnormalities (head and tail), $AlCl_3$ group had significantly the highest level of abnormalities as compared with control group. Treatment with EESA in combination with $AlCl_3$ significantly modulated the effect of $AlCl_3$ on total sperm count, motility, and viability. Also, there was a highly significant decrease in the percentage of dead and abnormal sperm cells relative to $AlCl_3$ untreated group. This translates to an obvious finding that EESA has an anti-toxicity effect on $AlCl_3$.

Hormonal Profile and Antioxidative Stress Assay:

Table 3 shows the effect of EESA on reproductive hormone and GSH on $AlCl_3$-induced reproductive toxicity in male rats. There was a sharp significant decline ($p < 0.05$) in FSH, LH testosterone and GSH of group II relative to control. Also, there was a significant decrease in LH of groups III and IV compared to the control. However, there was a significant increase in FSH and GSH of EESA treated group. The concentration of testosterone in the EESA groups (III and IV) showed a significant increase (in a dose dependent manner) when compared with both control group and the $AlCl_3$ only group. This is indicative that EESA initiated a negative feedback mechanism between LH and testosterone.

Table 1: Effects of EESA on Percentage Weight Change and Relative Weights of Reproductive Organs of Male Wistar Rats following $AlCl_3$ Toxicity

Groups	% Weight change	Testis	Epididymis	Seminal Vesicle
Control	143.5 ± 6.92	0.69 ± 0.03	0.23 ± 0.01	0.31 ± 0.02
$AlCl_3$	97.1 ± 2.06*	0.41 ± 0.06*	0.21 ± 0.01*	0.13 ± 0.03*
$AlCl_3$+100 mg/kg EESA	135.9 ± 4.36$^{\#}$	0.73 ± 0.02$^{\#}$	0.25 ± 0.03$^{\#}$	0.33 ± 0.02$^{\#}$
$AlCl_3$+200 mg/kg EESA	154.4 ± 8.11$^{\#a}$	0.68 ± 0.03$^{\#}$	0.21 ± 0.02	0.26 ± 0.04$^{\#}$

*Results are presented as Mean ± SEM, n = 5, # = significantly different from control * =significantly different from group II, a = significantly different from group III*

Table 2: Effects of EEESA on Sperm Parameters of Rats following $AlCl_3$-induced Toxicity

Groups	Motility (%)	Viability (%)	Morphology (%)	Sperm Count (million/ml)
Control	93 ± 1.23	85.00 ± 1.70	11.18 ± 0.31	138.60 ± 5.58
$AlCl_3$	57.50 ± 4.79*	57.20 ± 5.51*	15.22 ± 0.14*	76.50 ± 4.66*
$AlCl_3$+100 mg/kg EESA	76.00 ± 2.45$^{\#}$	72.80 ± 1.88$^{\#}$	13.01 ± 0.52$^{\#}$	122.40 ± 4.65$^{\#}$
$AlCl_3$+200 mg/kg EESA	78.20 ± 2.00$^{\#}$	78.00 ± 2.55$^{\#}$	12.75 ± 0.53$^{\#}$	131.20 ± 10.77$^{\#a}$

*Results are presented as Mean ± SEM, n = 5, # = significantly different from control * =significantly different from group II, a = significantly different from group III*

Table 3: Effects of EEESA on Hormonal Parameters of Rats following $AlCl_3$-induced Toxicity

Groups	LH (mIu/ml)	Testosterone (ng/ml)	FSH (mIu/ml)	GSH (mmol/mL)
Control	0.31 ± 0.01	0.51 ± 0.01	0.26 ± 0.01	16.18 ± 1.46
$AlCl_3$	0.09 ± 0.03*	0.20 ± 0.01*	0.13 ± 0.01*	8.48 ± 1.10*
$AlCl_3$+100 mg/kg EESA	0.10 ± 0.02*	0.650 ± 0.05$^{\#}$	0.14 ± 0.02	19.65 ± 3.14$^{\#}$
$AlCl_3$+200 mg/kg EESA	0.06 ± 0.04*^{a}	0.96 ± 0.04*$^{\#a}$	0.19 ± 0.03$^{\#}$	19.69 ± 3.14$^{\#}$

*Results are presented as Mean ± SEM, n = 5, # = significantly different from control * =significantly different from group II, a = significantly different from group III.*
LH - luteinizing hormone, FSH - follicle stimulating hormone, GSH - glutathione

Testicular Histology:

Groups I and IV are characterized by normal histoarchitecture. The seminiferous tubules appear normal showing regular interstitial spaces. In group II, the interstitial space (red arrow) reveals testicular atrophy. Also noted were abortive seminiferous tubules (yellow arrow) and erosion of the germinal epithelium. However, groups III and IV revealed improved histoarchitecture in a dose dependent manner relative to group II save for widened interstitial space readily observable in group III (represented in Figs. 1 and 2).

Epididymal Histology:

The epididymis of Groups I and IV revealed normal histoarchitecture. The lumen is filled with matured sperms (black arrow). Groups II revealed thickened septa resulting from increased cellularity (blue arrow) (represented in Figs. 3 and 4).

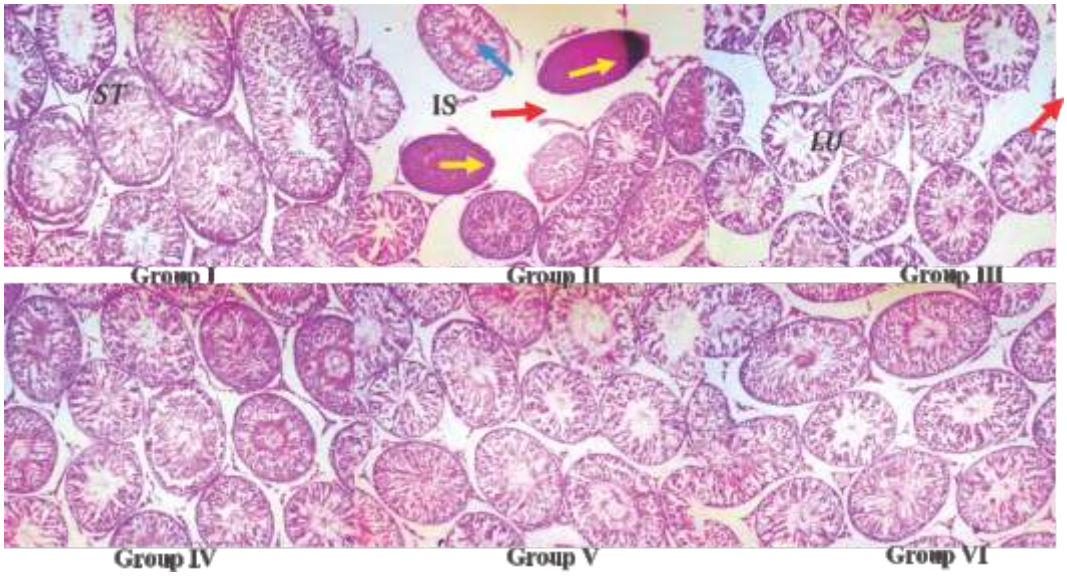

Fig. 1: Showing Photomicrographs of Testes of Control Group I, and Groups II (35 mg/kg bw $AlCl_3$), III (35 mg/kg bw $AlCl_3$ +100 mg/kg EESA), IV (35 mg/kg bw $AlCl_3$ + 200 mg/kg EESA), V (100 mg/kg EESA) and VI (200 mg/kg EESA). Widened interstitial space (red arrow) reveals testicular atrophy. Abortive seminiferous tubules (yellow arrow) observed in group II. (H & E × 100 Magnification).LU=Lumen, IS= Interstitium, ST= seminiferous tubule

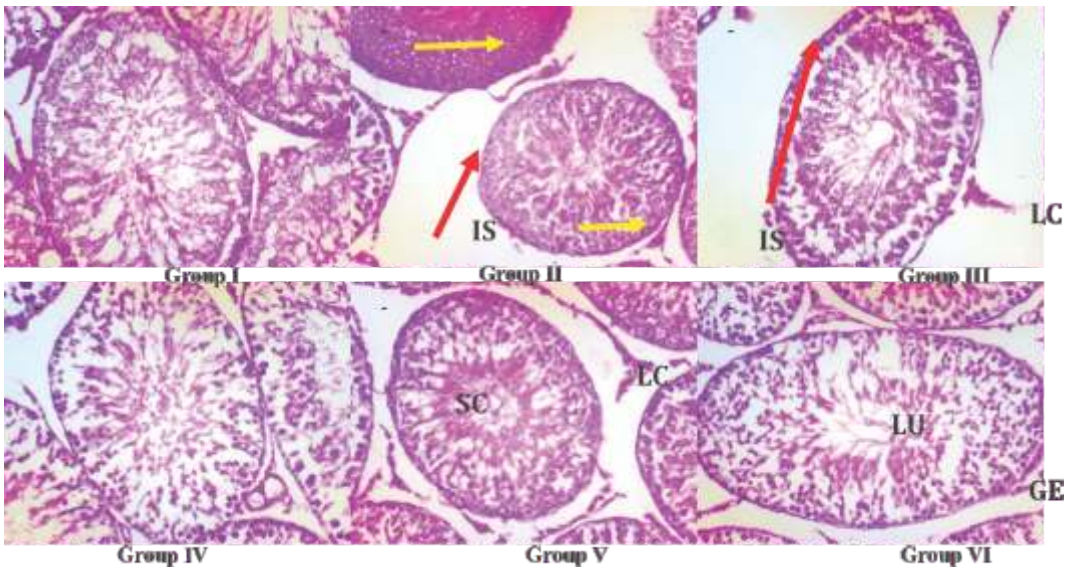

Fig. 2: Showing Photomicrographs of Control Group I, and Groups II (35 mg/kg bw $AlCl_3$), III (35 mg/kg bw $AlCl_3$ +100 mg/kg EESA), IV (35 mg/kg bw $AlCl_3$ + 200 mg/kg EESA), V (100 mg/kg EESA) and VI (200 mg/kg EESA). Widened interstitial space (red arrow) reveals testicular atrophy. Abortive seminiferous tubule (yellow arrow) seen in group II. (H & E × 400 Magnification). LU=Lumen, IS=Interstitium, SC= sperm cells, LC= Leyig cells, ST= seminiferous tubule

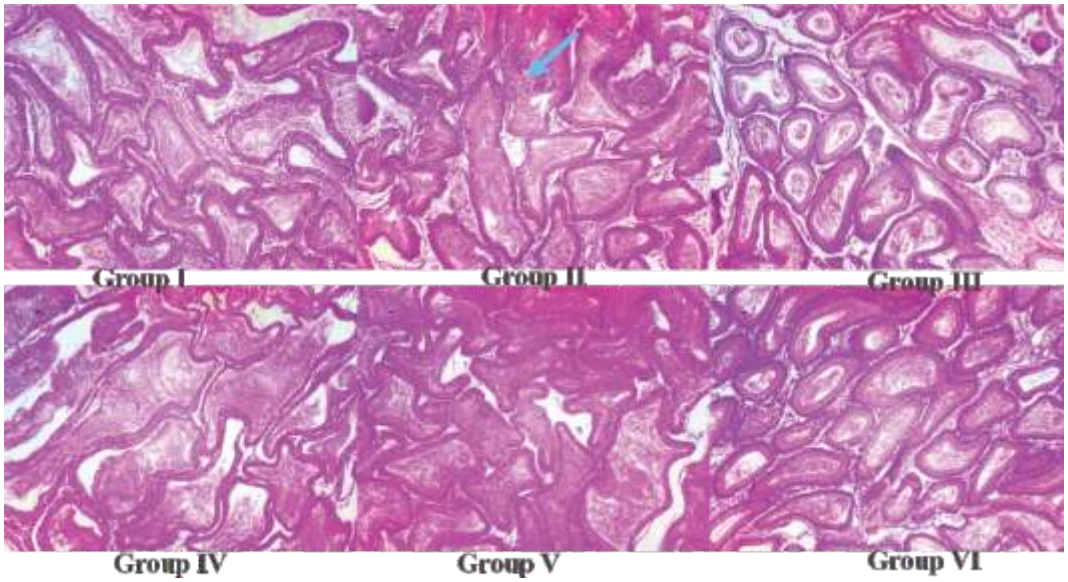

Fig. 3: Showing Photomicrographs of Epididymis of Control Group I, and Groups II (35 mg/kg bw $AlCl_3$), III (35 mg/kg bw $AlCl_3$ +100 mg/kg EESA), IV (35 mg/kg bw $AlCl_3$ + 200 mg/kg EESA), V (100 mg/kg EESA) and VI (200 mg/kg EESA). Thickened septa resulting from increased cellularity (blue arrow) were observed in group II. (H & E × 100 Magnification).

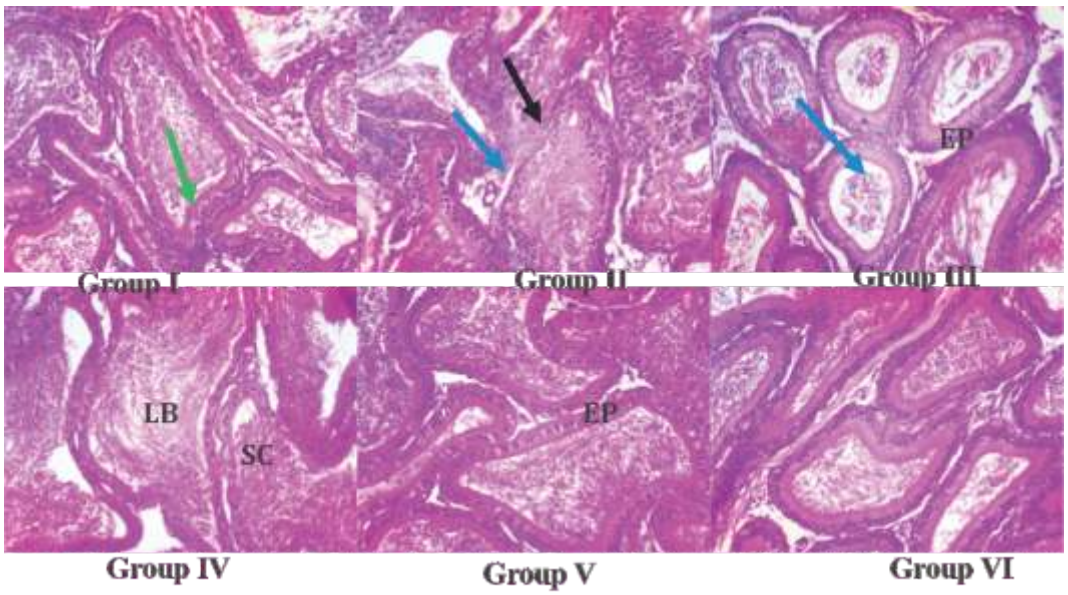

Fig. 4: Showing Photomicrographs of Epididymis of Control Group I, and Groups II (35 mg/kg bw $AlCl_3$), III (35 mg/kg bw $AlCl_3$ +100 mg/kg EESA), IV (35 mg/kg bw $AlCl_3$ + 200 mg/kg EESA), V (100 mg/kg EESA) and VI (200 mg/kg EESA). Thickened septa resulting from increased cellularity (blue arrow)were observed in group II. (H & E × 400 Magnification). LB= Lobule, EP = Epithelia

Discussion:

Secamone afzelii is a popular plant commonly used in the sub-Saharan African region for the management of diverse diseases ranging from pain, abscess, boils, stomach problems, diabetes, colic, dysentery to kidney diseases among others. Common among its uses in the traditional tenet is as a fertility enhancer and an aphrodisiac. In this work, the protective property of the leaf extract of *S. afzelii* was investigated in order to justify its reported traditional use as fertility enhancer scientifically. A simple qualitative phytochemical analysis of *S. afzelii* revealed that it is a rich source of alkaloids, tannins, resins phenols and flavonoids. It is however free from anthracene derivatives, cyanogenetic and cardiac glycosides. This is in tandem with the report of Abere and Onwukaeme [11]. These, coupled with high oral LD_{50} value of more than 5000 mg/kg imply that ethanol extract of *S. afzelii* is relatively safe. Al and its compounds like some other reprotoxic metals (like lead [12-13]) are known to easily accumulate in the bone, liver, testes, kidney and brain [14]. Al accumulation in tissues and organs results in their dysfunction and toxicity. This study corroborates such finding as seen in the structural and functional perturbations observed in the testes and epididymis of the rats treated with $AlCl_3$ only in this study. $AlCl_3$ caused a significant decrease in the final body weight and the relative weight of the reproductive organs (testes, epididymis and seminal vesicle) as shown in Table 1. The reduction in the relative weight of sex organs may also have contributed in no little way to the decrease in total body weight. However, the groups treated with EESA showed significant increase in body weight and relative sex organ weight.

$AlCl_3$ enhances generation of free radicals and alterations in antioxidant enzymes [15]. Oxidative stress usually has been attributed to the accumulation of $AlCl_3$ (and other heavy metals) in soft tissues which results in structural distortion and functional impairment [16]. These detrimental changes are the evidence of $AlCl_3$ ability to induce oxidative stress reported in previous studies [17-19]. From this study, the group treated with $AlCl_3$ showed significant decrease in the level of GSH whereas, groups treated with EESA showed improved GSH serum concentration. This could not be unrelated to the presence of antioxidant phytochemicals present in the extract such as flavonoids which are known to have free radical scavenging activities. Another probable mechanism (although not established in this study) is the chelation of Al from the tissues thereby limiting its toxicity and generation of free radicals.

The observed structural changes in this current study are characteristics that have been associated with Al toxicity in many studies [20-22]. Histopathological results showed that oral administration of $AlCl_3$ caused marked degeneration and necrosis of germ cells lining seminiferous tubules, as well as erosion of the interstitial spaces which accompanied atrophy (Figs. 1 and 2). Similar findings were reported by Khattab [20]. Guo and colleagues reported the deleterious effects and histopathological changes in testicular tissues after two weeks of aluminum treatment, as well as noticeable germ cell loss via necrosis in the spermatids [23]. The histological alterations may be explained by oxidative stress resulting from an increased production of reactive oxygen species

(oxygen radicals) in excess of the antioxidant capacity of the testicular and epididymal tissues(Figs.3 and 4). Most conditions associated with male infertility are inducers of oxidative stress, which leads to an increase in germ cell apoptosis and subsequent hypospermatogenesis, which results in changes in the dynamics of testicular microvascular blood flow, endocrine signaling, and germ cell apoptosis [24]. The structural changes (as seen in Figs. 1-4) will no doubt alter the physiological integrity of the testes and epididymis.

From this study, $AlCl_3$ caused significant reduction in sperm motility, viability and total sperm count (Table 2). The number of sperm cells with abnormal morphology was also significantly increased in rats exposed to Al (Table 2). This may be traced to damaged testicular and epididymal tissues which are responsible for spermatogenesis. This supports the research of Geeta and Gyan who reported that inhalation, oral, or dermal exposure to aluminum in excessive amount resulted in accumulation in target organs causing damage to testicular tissues in both humans and animals with accompanying alterations in the histology of testis [25], deterioration in spermatogenesis and sperm quality; enhancement of free radicals and alterations in antioxidant enzymes [25]; interruption in sex hormone secretion [26-27]; and biochemical changes in testis and other accessory reproductive organs [28-29] most of which are also reported in this current study. Increased ROS and oxidative damage may contribute to male reproductive toxicity by reducing sperm function [20].

However, treatment of $AlCl_3$ group with EESA revealed noticeable improvement in $AlCl_3$-induced histopathological disruptions in the testes and epididymis. This implies that EESA has protective and modulatory effects on the hazards induced by $AlCl_3$. EESA could have achieved such feat probably because of the flavonoid and alkaloids it contains, which have been known to possess the ability to increase intercellular antioxidant levels, scavenge oxidants and free radicals decrease capillary permeability and fragility and exert many other health-promoting effects [31].

GSH which is the most important antioxidant found in the human body (because it is capable of interacting directly with hydroxyl radical (ROS) to detoxify them) was assayed in this study. $AlCl_3$ significantly reduced GSH in group II (Table 3). Meanwhile, treatment with EESA reversed the reduction in a dose dependent manner. This must have helped in eliminating ROS generated by oxidative stress thereby improving the testicular health. This corroborates with the findings of Mensah [32].

Serum FSH, LH and testosterone were significantly depleted in the group that received $AlCl_3$ only as expressed in Table 3. These hormones are responsible for various stages of spermatogenesis. The reduction in the hormonal levels could have been an additional cause for the decrease in total sperm count and increased teratozoospermia recorded in this study. EESA significantly increased the serum content of FSH and testosterone. This could result from the ability of the extract to improve the overall health of the testis, thus sending right signal to the pituitary gland and hypothalamus. However, LH was significantly reduced in groups treated with EESA. LH is a hormone that regulates the secretion of

testosterone in males. Whenever there is an increase or excess concentration of testosterone in the circulation, a negative feedback is sent to the brain (specifically the anterior pituitary) which reduces secretion of LH and in turn causes a reduction in testosterone. In this study, the serum testosterone in EESA treated groups was significantly higher than that of the control group in a dose dependent manner. This surge in testosterone must have sent a negative feedback to the brain thereby reducing the serum LH. Since the extract was administered continuously for 14 days, resulting in high testosterone level, it may be suggested that EESA aphrodisiac propensities.

An aphrodisiac is any agent (food, drug, scent or device) that can arouse or increase sexual drive or libido. They can therefore, be described as substances that enhance sexual drive or libido [32]. Testosterone has been reported to stimulate sexual desire and help maintain the tissues of the penis that enables erection [33-34]. The significant increase serum testosterone reported in rats treated with EESA could be further explored in conjunction with other empirical parameter to ascertain if the extract has aphrodisiac properties.

Conclusion:

The results of this study further provide evidence for adverse effects of aluminum chloride on reproductive indices such as sperm motility, viability and total sperm count, histology of testis and epididymis and reproductive hormonal levels. It also showed that $AlCl_3$ has the capacity to induce oxidative stress. This study further concludes that ethanol extract of *Secamone afzelii* has a protective effect on $AlCl_3$-induced reproductive toxicity. It could also function well as an aphrodisiac. The antioxidant and free radical scavenging activities of the extract was noticeable. However, further studies need to be carried out to elucidate the mechanism(s) of action of EESA in its protective effect on reproductive toxicity.

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