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Investigation on the Ability of Sulfonolipid from *Salinibacter ruber to* Ameliorate the Toxic Effect of Sodium Arsenite on the Sperm Count and Testoterone Level in Rat

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Authors' contributions

Author OOO carry out the experiment and perform the Statistical analysis. Author JKO designed the experiment. Author TOE prepare the manuscript for Publication. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: In this study, the ameliorative activity of sulfonolipid extracted from a local isolate of *Salinibacter ruber* was investigated against sodium arsenitetoxication on sperm quality, testosterone level and histological structure of rat testis. The treatment was applied for twenty-six days so as to be able to examine the effect of sodium arsenite and the activity of sulfonolipids on the sperm count. This is because it has been established that twenty-six days will be required for the maturation of albino rat's sperm
Study Design: An experimental layout involving the use of Wistar albino rat was employed for the study.
Place and Duration of Study: Sample: Department of Pure and Applied Biology (Animal Laboratory) Ladoke Akintola University of Technology Ogbomosho Oyo state Nigeria between June 2009 and July 2010.
Methodology: Sixty adult male Wistar Albino rats were divided into six groups of 10 animals each. Each group was administered sulfonolipid at a concentration of 0.2ml of

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0.16% for group A, 0.016% for group B, 0.0016% for group C with 0.2ml of 6mg/kg body weight of sodium arsenite, respectively. Group D served as negative control and, 0.2ml 0f 0.16% sulfonolipid was given only for positive control in group E, by gavage for 26 days. Group F were given water and feeds only ad libitum, also served as the normal control. The changes in sperm count, testosterone level and histological structure of testes were examined.

Results: A significant difference in epididymal sperm count of rat gavaged with sulfonolipid at a concentration of 0.2ml of 0.16%was observed ($37.8\pm5.54\times10^6$ m/ml) when compared with those treated to sodium arsenite ($23.76\pm7.50\times10^6$m/ml) only. No significant differences were observed in testosterone level among any of the groups. Sulfonolipid isolated from *Sanilibacter rubber* showed some significant activities on the severe effects of sodium arsenite on the histological structure of testis.

Conclusion: The administration of sulfonolipid extract at a concentration of 0.2ml of 0.16%alongside with sodium arsenite significantly reduced the toxic effect of the arsenic compound in the rats. However, effectiveness and potency of sulfonolipid can be better established through further investigations which may try to verify the fertilisation efficacy of the experimental animals.

Keywords: Sodium arsenite; sulfonolipids; salinibacter rubber; sperm count.

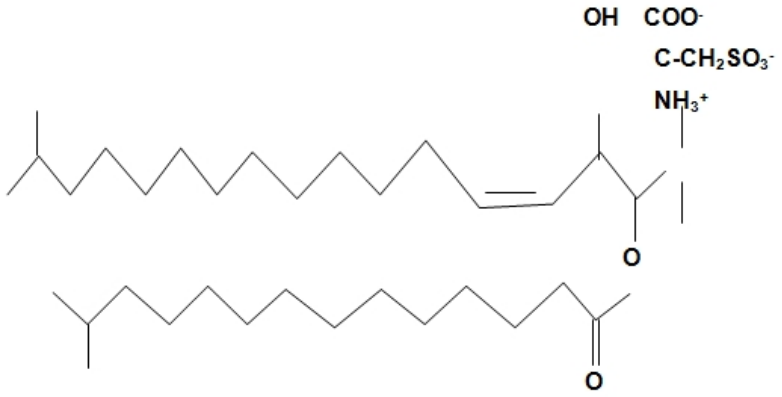
1. INTRODUCTION

Sulfonolipids are major component of cell envelope [1]. They are present in various organisms in amounts ranging from 2 to 10 µmol/g of cells (wet weight) and may constitute as much as 20% of the cellular lipids [2]. Sulfonolipid contains a sulfonic acid residue linked by a carbon-sulfur bond to 6-deoxyglucose as a moiety of a monoglycosyldiacylglycerol [2]. The best known and most abundant of the sulfonolipids is sulfoquinovosyl diacylglycerolor 2-di-0-acyl-3-0-(6'-deoxy-6'-sulfo-α-D-glucopyranosyl)-sn-glycerol, which is a key component of the photosynthetic mechanism of higher plants and other photosynthetic organisms (3). Sulfonolipids have been found in marine invertebrates [4,5,6], plants [7,8] and algae [9,10,11,12]. A group of unusual sulfonolipids have been found in bacteria of the genera Cytophaga, Capnocytophaga, Sporocytophaga and Flexibacter [2].These organisms are noted for their gliding motility on solid surfaces. One of such lipids is capnineand, it has been purified from the cells of a Capnocytophagasp. and evidence has been obtained that it is 2-amino-3hydroxyl-15-methylhexadecane-1-sulfonic acid (which could also be named as 1-deoxy-15-methylhexadecasphinganine-1-sulfonic-acid) [2] and occurs in the organism both in the free form and as N-acylated derivatives, though up to 20% of other homologues can occur, depending on species. The fatty acids are much more heterogeneous and vary from C_{14} to C_{16} in chain length, a high proportion with iso-or anteiso-methyl branches and hydroxyl groups in position 2 and 3 [2]. Sulfonolipid with some structural affinity to the capnoids has been isolated from a halophilic aerobic bacterium, *Salinibacter ruber* (Fig. 1). It has the structure of 2-carboxy-2-amino-3-o-(13-methyltetradecanoyl)-4-hydroxy-18-methylnonadec-5-ene-1-sufericacid, and represent about 10% of the total cellular lipids [13]. *Salinibacter ruber* is a red, extremely halophilic aerobic bacterium commonly found in crystallizer ponds of Alicante and Mallorca, Spain [14]. They were first identified using the 16S rRNA sequencing technique and the use of fluorescent oligonucleotides revealed that they are rod shaped bacteria but may be slightly curved. They initially called "*Candidatus salinibacter"* and several other species related to them had been isolated from other parts of Spain; hence these groups of bacteria had been classified to belong to the order Cytophagales.

Rhodothermus marinus, found in submarine hot springs is an example of the other members of the order Cytophagales [14].

1.1 Features of Sulfonolipids

It has been reported that sulfonolipids possess several functions in the cell growth control mechanism or regulation [9,10,11,15,16], which might be used as immunosuppressive drugs. Yoshitaka *et al.,2002* synthesized four sulfonolipds on the basis of the chemical structures of sulfoquinovosyl diacylglycerol from sea urchin and found that one of these demonstrated a strong inhibitory effect on the lymphocyte reaction (MLR) in a dose-dependent manner and significantly effective in suppressing the growth of solid tumour [16] Also, their work suggested that intramuscular administration of 5mg/rat also inhibited rat skin allograft rejection.



2-carboxy-2-amino-3-O-(13'-methyltetradecanoyl)-4-hydroxy-18- -5-ene-1-sulfonic acid methylnonadec

Fig. 1. Structure of sulfonolipids isolated from *Salinibacter ruber* (Corcelli et al*.,* 2004)

This work was designed to investigate the sulfonolipid ability of a local isolate of *Salinibacter ruber* to ameliorate the toxic effect of sodium arsenite on sperm count, testosterone level and histopathological structure of testis.

2. MATERIAL AND METHODS

2.1. Experimental Animals

Sixty adult male albino Wistar rats (10 weeks old, approximately 110±5 g body weight) were purchased from Ladoke Akintola University, Technology Teaching Hospital, Osogbo, Nigeria. They were housed in the experimental Animal House, Department of Pure and Applied Biology, LAUTECH Ogbomoso; Nigeria. They were fed with rat pellets and water ad libitum. The room temperature was 29 ± 7ºC with twelve hours light/dark cycle and relative humidity of 60 ± 5%.

2.2 Microorganism

The microorganism, *Salinibacter rube,* used in this study was isolated from water collected from abandoned borehole water at Agbabu Ondo State, Nigeria.

2.3 Sulfonolipids

Sulfonolipids used for this experiment were extracted from *Salinibacter ruber,* using the method of Bligh and Dyer as modified for extreme halophiles by Kates [17]. Fifteenml of methanol-chloroform (2:1, v,v;10ml methanol plus 5ml of chloroform) was added to cell suspension and the mixture was gently shaken for 20 minutes and centrifuged. After centrifugation, then the supernatant extract was decanted into a separating funnel, and the residue was re-suspended in 19ml of chloroform-methanol-water (1:2:0.8). The mixture was then shaken and centrifuged; 5ml each of chloroform and water were then added to the combined supernatant to obtain a two-phase system. After the completion of two-phase separation (requiring a few hours at room temperature) the chloroform phase, diluted with benzene. Dried lipids were re-suspended in a small volume of chloroform and stored at -20^0C. The Infra-Red spectrometry value of crude sulfonolipid produced was compared with standard available in literature [13].

2.4. Antimicrobial Assay

The antimicrobial activity of the sulfonolipid was evaluated by using Agar diffusion method. The organism used for the sensitivity test was pre-cultured overnight at 37˚C in Nutrient broth for 18hrs to resuscitate and revitalize the cell. One ml of the test isolates was then used to flood a sterile Petri-dish containing nutrient agar that has set with the aid of sterile syringe and swirled gently for homogeneity.

2.5 Treatment Protocol

Sixty adult male Wistaralbino rats were divided into 6 groups of 10 rats per group as shown below. The animals were housed in wire mesh cages under standard environmental conditions with the provision of 12 hour light and 12 hour darkness. Feeds were purchased from Azeez Feeds, Nig. Ltd Ogbomoso, Oyo State and water was provided. The rats were acclimatized to laboratory condition for 2 weeks before the commencement of the experiment as stated below.

Group A were given 0.2ml of 0.16% sulfonolipid by gavage daily, 0.2ml of 6mg/kg body weight of sodium arsenite by gavage daily allowed freeaccess to feeds.
Group B were given 0.2ml of 0.016% sulfonolipid by gavage daily, 0.2ml of 6mg/kg body weight of sodium arsenite by gavage daily, also allowed free accessto feeds.
Group C were given 0.2ml of 0.0016% sulfonolipid by gavage daily, 0.2ml of 6mg/kg body weight of sodium arsenite by gavage daily, also allowed free accessto feeds.
Group D was given 0.2ml of 6mg/kg body weight of sodium arsenite by gavage daily and allowed free access to feeds and it served as negative control.
Group E was given 0.2ml of 0.16% of sulfonolipid daily and it served as positive control.
Group F was given water and feeds only ad libitum, also served as normal control.
The duration of the administration was 26days.

On the 27th day of the experiment, all the animals were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture. The scrotal sacs of rats were immediately opened and their epididymides were separated from right testis and placed in a beaker containing 1ml of physiological saline solution. The epididymides were then lacerated at many spots so that their spermatozoa would swim out into the solution within a few minutes.

2.6 Sperm Count

The sperm count was determined using Neubauer improved haemocytometer. To minimize error the count was repeated at least five times for each rat which was done as follows;

(1) After complete liquefaction of semen, the specimen was mixed thoroughly.
(2) 1:10 semen dilution was prepared using micro pipette; 0.1 ml semen was added to 0.9ml of water.
(3) Both sides of the counting chamber was filled and allowed to settle for several minutes.
(4) All spermatozoa were counted within five of the red blood cell squares ($\times 10^6$).

2.7. Hormonal Assay

Each blood sample was centrifuged at 1.500 rpm for 10min to obtain serum. Enzyme immunoassay (EIA) method was used for the testosterone analysis in the serum samples. For this process, the commercial EIA test kits were used.

2.8. Histopathology Studies

Histopathological studies of testes were carried out.The organs were placed in already prepared 10% fermol-saline, a fixative of volume preparation with constituent of 10ml formalin to 90ml of distilled water and 0.9g of sodium chloride and the method used for staining was H and E (Haematoxyline and Eosin).

2.9. Data Analysis

Results are expressed as mean $\pm$ standard deviation. The means of all the parameters were subjected to statistical analysis using the one-way analysis of variance (ANOVA) according to the procedure of Steal and Torrie (1960) and further analyses using least significant difference (LSD) and student t test.

3. Results

3.1 Isolated Microorganism

In the course of this study, the organism isolated had bright pigment on the minimal medium. The organism is Gram negative, motile, straight or slightly curved rod when viewed under the microscope. The characteristics of the microorganism were in irregular in shape, flat colony elevation, papillate surface and striated in differentiation (Table 1). The organism was rubber-like and very difficult to streak on agar medium.

Table 1. The colonial morphology of *Salinibacter ruber* on nutrient agar plates

Colony	Feautures
Shape	Irregular
Elevation	Flat
Surface	Papillate
Gram staining	Negative
Differentiation	Straited

2. CHARACTERISTICS OF SULFONOLIPID EXTRACT

The sulfonolipid produced had a characteristic smell. IR spectrum showed a complex intense bands in the sulfonate region at 1,260cm^{-1} along with characteristic peaks for NH_2 at 3,335cm^{-1} and at 1,650cm^{-1} (Fig. 2). This spectrum was similar to that obtained for the novel sulfonolipid of *Salinibacter ruber* described by Corcelli et al. [13].

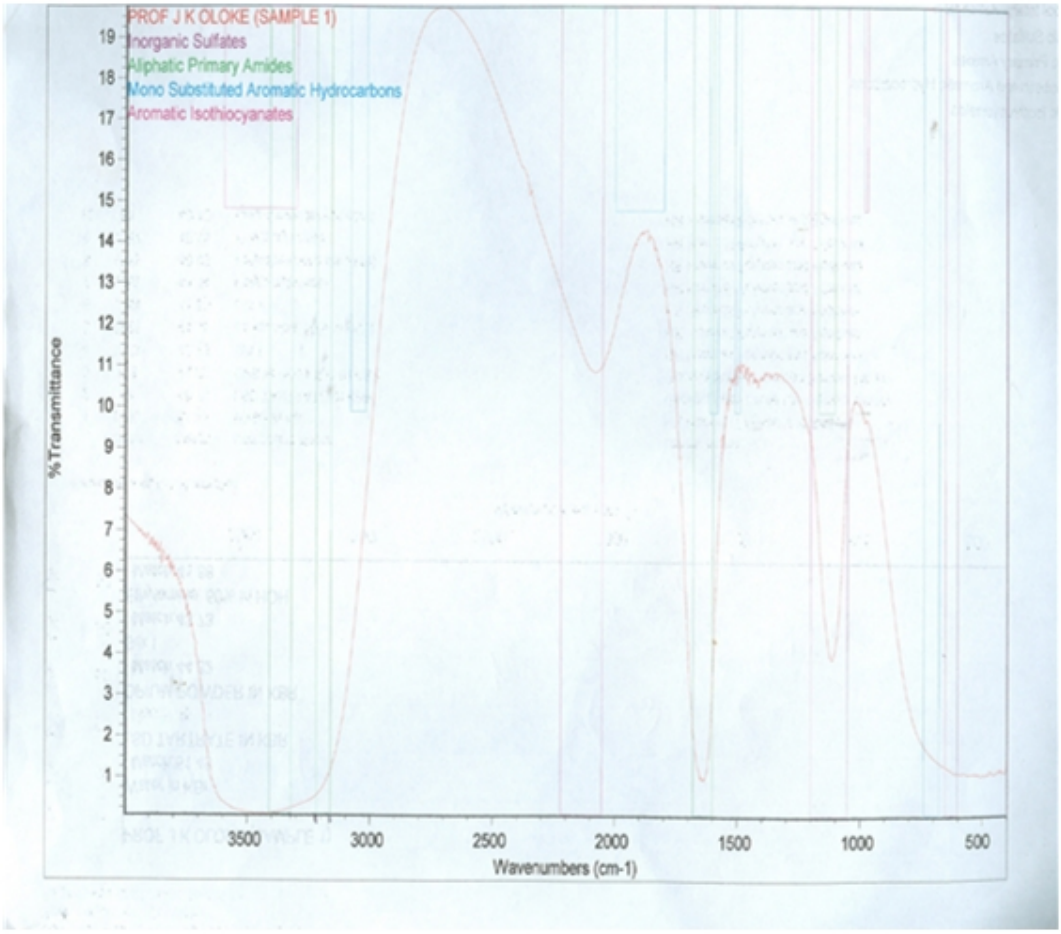


Fig. 2. IR spectrum of sulfonolipid extract

3.3 Antimicrobial Activity

The sulfono lipid extract was sensitive against some of the test organisms with zone of inhibition; *E.coli* (9mm),*Streptococcus pyogenes* (10mm), *Bacillus cereus* (8mm), *Pseudomonas aeruginosa*(19mm),*Proteus vulgaris*(7mm), *Staphylococcus aureus*(11mm) ,*Micrococcus acidophilus* (5mm) (Table 2).

Table 2. Zones of inhibition (in diameter) of the sulfonolipid extract on the test organisms

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Test organisms	Zone of inhibition(mm)
Escherichia coli	9
Streptococcus pyogenes	10
Bacillus cereus	8
Pseudomonas aeruginosa	19
Proteus vulgaris	7
Staphylococcus aureus	11

3.4 The Behaviour and Body Weight Changes, and Mortality Rates

Morphological changes such as swollen upper limb and a skinny lower limb were observed in rats during the experimental period. Three rats from treatment groups died during the course of the experiment as follows; growth was observed on rat in group A between the 4th and 8th day which later died on the 11th day. One rat died in group B, others were observed to be sluggish in their behaviour. Also C group were active until 26th day which is the last day of the experiment. One rat died in group D before 26th day and no death was observedin the positive control till the last day of the experiment.

No differences in food consumption were seen in any of the group of animals throughout the experimental schedule. The initial body weight of the rat in groups A, B, C given sulfonolipids following sodium arsenite administration, that of the group given sulfonolipid only, group given only feed and water only, that of sodium arsenite only were 150±4.50, 147±2.35, 149±5.25, 145±5.88, 144±18.30, 150±10.30g respectively. The final body weight at the end of the experiment before sacrificing the animals (after 26 days) were 181±10.39, 187.30±5, 189.2±4.32, 192.6±6.50, 195.7±5.60, 190.5±5.32 (g) respectively (Table 3).The initial and final body weights in all the groups studied were not significantly different.

Table 3. Changes in body weight of rats treated with sodium arsenite and in combination with different dilutions of sulfonolipid

Group	Initial body weight	Final body weight
A	150±4.50	181.8±10.39
B	147.20±2.35	187.30±5
C	149.50±5.25	189.2±4.32
D	150±10.30	190.5±5.32
E	145±5.88	192.6±6.50
F	144±18.30	195.7±5.60

3.5 Changes Inepididymal Sperm Count

The effect of 0.2ml of 6mg/kg body weight of sodium arsenite administered daily for 26 days on sperm count for all the groups was not significantly different(Table 4).The sperm count of the group D administered with only sodium arsenite was low (23.8±2.71 m/ml) and about 90% of the sperms were dead when compared to the sperm count of group A (36.55±1.90 m/ml) group B(28.76±1.90 m/ml), group C(25.8±2.71 m/ml), group E (37.8±5.54 m/ml) and group F (40.1±8.03 m/ml) (Table 4). Moreover, the group A treated with 0.2ml of 0.16%of sulfonolipid successfully alleviate the effect of sodium arsenite when compared with the negative control (Table 4).

Table 4. Epididimal sperm count in different experimental groups of rat.

Group	Total sperm count m/ml(10^6)	Remark
A	36.55±38.5	Immature spermatozoa, round headed and acrosomeless
B	28.76±1.90	50% mature and immature, poor motility
C	25.8±2.71	Head and tail defect
D	23.76±7.50	90% dead, mature spermatozoa
E	37.8±5.54	None died ,matured and motile spermatozoa
F	40.1±8.03	Matured spermatozoa which consist of a head, body and tail.

3.6 Serum Testosterone Level

The testosterone level of both treated group A, B, C (0.22±0.00, 0.50±0.70, 0.31±0.10) of animals with sulfonolipid alongside sodium arsenite, sodium arsenite only (0.31±0.04) and sulfonolipid only (0.36±0.01) were not significantly different from each other (Table 5).

Table 5. Effect of sodium arsenite on testosterone with different concentration of sulfonolipid in rats

Group	Testosterone level ng/ml
A	0.22±0.00
B	0.50±0.70
C	0.31±0.10
D	0.31±0.04
E	0.36±0.01
F	0.49±0.37

3.7 Changes in Histologic Structure of Testis

The studies showed that rat (in group A) administered sodium arsenite with 0.2ml of 0.16% of sulfonolipid, the testes showed spermatogenic degeneration and necrotic. Group B administered sodium arsenite with 0.2ml of 0.016% of sulfonolipid showed degeneration in the testis. Group C administered sodium arsenite with 0.2ml of 0.0016% of sulfonolipid showed moderate spermatogenic degeneration occur in testis. Group D given sodium

arsenite only showed spermatogenic degeneration of testis and histopathological section of testis had mild spermatozoon degeneration with tubular vacuolation. Groups Eand F showed normal histological structure (Fig. 3).

DISCUSSION

Salinibacter ruber appears as pigmented colonies with a bright red coloration on plates containing the culture of the selected water samples. The bright red pigmentation had been described as a mechanism through which the bacterium protects itself from high sunlight intensity to which itis exposed [18]. In this study, they were seen as reddish motile straight-slightly curved rods and the colonies on plate were about 1mm in diameter with an entire margin. The *Salinibacter ruber was* rubber- like; and very difficult to streak on an agar medium. *S. ruber* was found containing significant amount of novel sulfonolipd. The novel lipid appears to be an interesting structural variant of the sulfonolipids found as main component of the cell envelope of bacteria genus Cytophaga and closely related general (1) and of diatoms [3]. The sulfonolipid produced had a characteristic smell with IR spectrum showing characteristic peaks for NH_2 at 335cm^{-1} and at 1,650 cm^{-1} (fig3).This spectrum was similar to that obtained for the novel sulfonolipid of *Salinibacter ruber* described by Corcelli et al, (2004) [13].

The antibacterial activity of sulfonolipid extract on the test organisms was more pronounced in *Pseudomonas aeruginosa*(19mm) zone of inhibition followed by *Staphylococcus aureus*(11mm),*Streptococcus pyogenes*(10mm), *E.coli*(9mm)in (Table 2) compared to *Proteus vulgaris* which had (7mm). However *Salinibacter ruber*proved sensitive to Penicillin G, Ampicillin, Chloramphenicol, Streptomycin, Novobiocin, Rifampicin and Ciprofloxacin [14]
The animals used in this study were challenged with 0.2ml of 6mg/kg body weight of sodium arsenite daily, a known carcinogen. The US Environmental Protection Agency (US/EPA) standard for arsenic in drinking water is 50mg/L (US: EPA, 1993; WHO 1993). However, WHO recommended 10kg arsenic per litre of drinking water as a guideline because at 50mg/l the risk of dying from cancer of the liver, lung, and kidney bladder is about 13 per 1000 person.

There was no significant difference in body weight of the animals in the entire group studied. This implies that despite the challenge with 0.2ml/6mg/kg body weight of sodium arsenite daily, they were able to metabolize it for normal body growth.

Morphologically, the animals administered sulfonolipid and sodium arsenite had swollen limb observed in line with the work of Rodriguez et al.,[19] where development alteration were noticed in laboratory animals.

Also arsenic causes a characteristic set of malformation mainly neural tube defect, anophthalmia and exopthalmia, renal and gonadal agenesis, fused ribs and developmental with behavioural alteration in laboratory animals (20, 21,22) .Arsenic clinical manifestations such as skin lesion, gangrene of the limbs and alignment neoplasms were commonly observed daily [23].

The deleterious effect of sodium arsenite on the sperm cell and sperm count was alleviated significantly in group A administered 0.2ml of 0.16% of sulfonolipid with 0.2 ml 6mg/kg body weight of sodium arsenite (Table 4). It has been shown in this study that 0.2ml of 0.16% of sulfonolipid successfully alleviate the toxic effect of 0.2ml of 6mg/kg body weight of sodium arsenite on sperm count as the sperm count when sodium arsenite alone was administrated

to the rats was not only low but also about 90% of the sperm were dead (Table 4). It has been shown that when 6mg/kg of sodium arsenite was administered consecutively for 26 days, about 50% reduction in sperm count over untreated control was obtained [24].

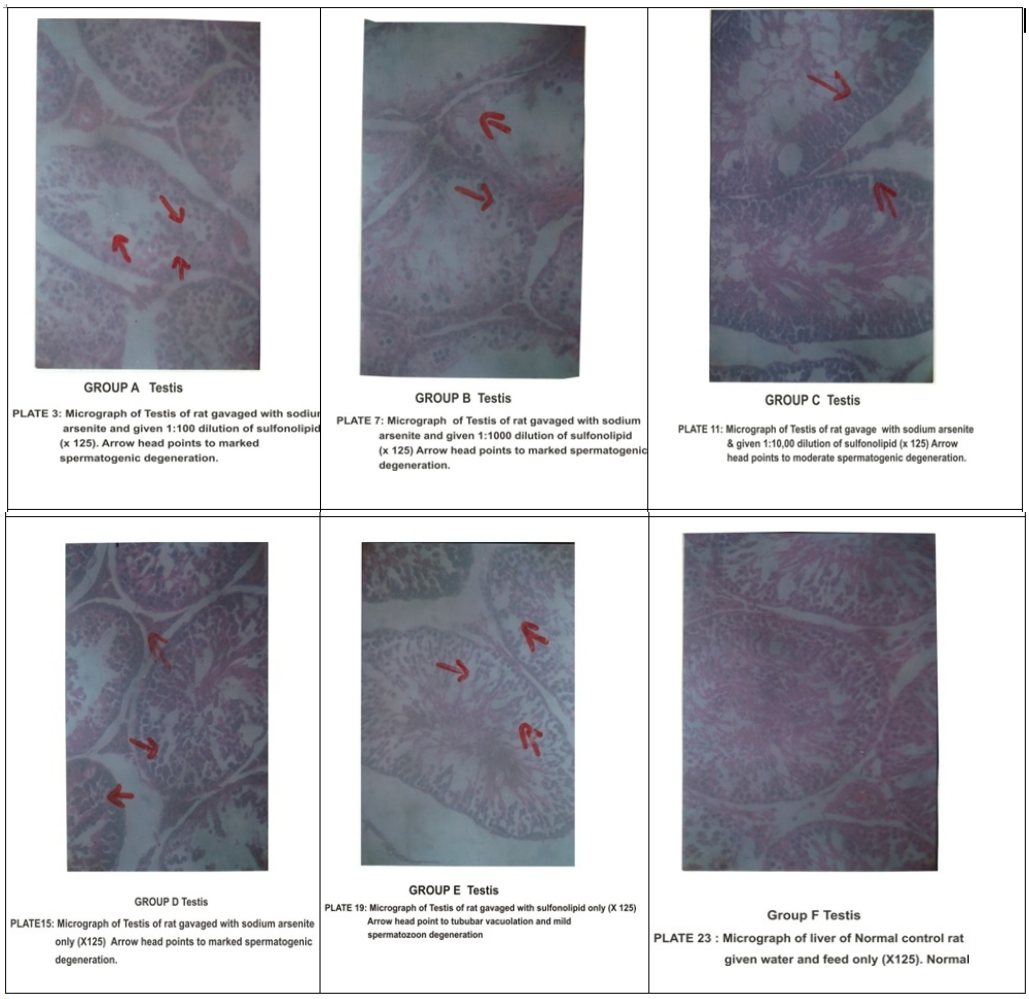

Fig. 3. Changes in histological structure of testis in different treatment groups

It is evident from this study that sulfonolipid alleviated part of the deleterious effect of sodium arsenite on the sperm count; since the mean values were not significantly different from the control group F but significantly different from that of the group given arsenite alone (Table 4). Spermatogenesis is a highly specialized and efficient process resulting in the production of large number of spermatozoa [25]. Hormonal control of spermatogenesis is also mediated through Sertoli or nurse cells. Follicle stimulating hormone in sexually mature males acts on spermatogonia stimulating (with the aid of testosterone) the production of sperm. Luteinizing hormone acts on interstitial cells (Leydig cells of the testes) stimulating them to synthesize and secrete the male sex hormone, testosterone, which is essential for the production of sperm. It has been reported that sodium arsenite administration has a deleterious effect on the Leydig cell [26] which affects the production of testosterone.

In all the groups, group A(0.22±0.00) gavaged with soduimarsenite and 0.2ml of 0.16% of sulfonolipid had the least mean value for testosterone level, group B(0.50±0.70) had the highest mean value (Table 5).The mean values of the other groups C,D,E were not significantly different from the others while control group F was 0.49±0.35.From this result one would expect the groups gavaged with sulfonolipid to have high mean values for testosterone level ,since testosterone is essential for sperm production. Mahitosh et al. [27] showed that rats treated with 6mg/kg body weight of sodium arsenite showed a significant decrease in testosterone levels and the degenerative changes.

In all, the cell growth control mechanism of activities of the sulfonolipid used in this work as indicated by the amelioration of the deleterious effects of sodium arsenite was higher in groups treated with Sulfonolipids.

5. CONTRIBUTION TO KNOWLEDGE

From this work it has been shown that 0.16% of sulfonolipid can ameliorate the deleterious effect of very high concentration of sodium arsenite on Sperm count and Testoterone level.

6. CONCLUSION

Since sulfonolipid at a very small concentration reduce the deleterious effect of sodium arsenite. Further work needs to be done to establish this fact which may be useful for the treatment of patients who are accidentally exposed to toxic metal, especially arsenite.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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