Trends in
Natural Products Research

Effect of Aqueous Extract of *Agave sisalana* (Perr Syn) Root in Cadmium-Induced Infertility in Male Wistar Rat.

Omodamiro Olorunsola Dave[1], Omodamiro Temidayo Adebusola[2], Konyefom Nweze[2] and Ajah Obinna[1].

[1]Pharmacology Unit, Department of Biochemistry, Micheal Okpara University of Agriculture Umudike Abia State
[2]Department of Human Physiology, College of Basic Medical Sciences, Federal University Ikwo, Ebonyi State

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Abstract: The effect of aqueous root extract of *Agave sisalana* Perr. Synon on the semen parameters and serum testosterone level of cadmium-intoxicated male Wistar rats was investigated. Twenty five male rats were divided into five groups of five animals each (Groups I-V). Cadmium (0.2 mg/kg) was administered for seven days to induce reproductive toxicity. The animals were grouped and treated thus; Group 1 (normal control without cadmium), Group II (Cadmium control group) while Groups III-V were dosed orally with 50, 100 and 150 mg/kg of the aqueous root extract of *A. sisalana* for fourteen days after cadmium intoxication. The serum testosterone level and semen parameters were determined. There was a significant difference in the serum testosterone level between treated rats and control. The sperm count, sperm motility and sperm morphology were significantly ($p < 0.05$) increased in the extract-treated rats compared with the Cadmium control group. The result suggested that the *A. sisalana* root extract has the potential to improve male reproductive function and promote fertility which might be a consequence of both its potent antioxidant properties and androgenic activities.

*Corresponding author:
majekdamiro@yahoo.com;
+2347064868550

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INTRODUCTION

Plants are crucial part of health care system and they play major role in providing primary health care for people in the rural areas. Plants serve as raw materials for traditional and modern medicines. The importance of plants in medicine and economy are on the increase in both developing and industrialized nations (WHO, 1998). For most of the world's population medicinal plants continue to dominate the healthcare system, especially in developing countries, where herbal medicine has a long history of use (Dar, 2017).

Infertility is described as failure to conceive after one year of intercourse without any prevention, and approximately 8 to 12 % of couples worldwide are infertile (Kumar and Singh, 2015). Many factors are involved in the process of conception that affects both men and women. About 40 to 50 % of infertility cases are as the results of male infertility (Elahi *et al*., 2013). Studies by Sreedhar *et al* (2016) showed that male fertility has been adversely affected by environmental and occupational exposure to various chemicals, heavy metals, heat and radiation. In addition, it has been reported that lifestyle risk factors such as cigarette smoking, alcohol consumption, chronic stress and nutritional deficiencies could adversely affect the spermatogenesis (Alizadeh *et al.*, 2015). The role of oxidative stress has been suggested in the pathogenesis of male infertility (Alizadeh *et al.*, 2015, Sreedhar *et al.*, 2016). The first factor to be assessed in male infertility is the semen quality, which is commonly evaluated by sperm concentration, morphology, and motility via semen analysis (Fekrazad *et al*., 2014). The ability of sperm to move properly toward an oocyte is described as sperm motility. It is known that sperm motility is an important factor in evaluation of semen quality. Insufficient sperm motility is considered as one of the most important causes of infertility (Remya *et al*., 2009). The fertilization capacity of sperm is not only dependent on motility but also on other parameters such as viability (Mukhopadhyay and Kumar, 2002) and sperm DNA fragmentation (Lopez *et al.*, 2013).

The use of spices and herbs as medicine has been gradually increasing in developing countries (Ganguly *et al.*, 2003). Plants have a long history of use in aiding fertility, including fertility-enhancing properties and aphrodisiacal qualities. For male infertility, numerous medicinal plants have been investigated (Okon and Etim, 2014, Sreedher *et al*., 2016) ;), but only a few are traditionally used. *Agave sisalana* Perr. Syn (Agavaceae) also known as Sisal is an herbaceous plant that is indigenous to tropical and sub-tropical America and is also found from South America northwards to Mexico. It is widely known for the production of fiber which is economically very important. It is also known by several other vernacular names like Ram bans (Indians), Garingboom (Africans), Ngwengwe (Angola:umbundu).

Traditionally, *Agave sisalana* is used in lowering blood pressure and as an antiseptic. The roots are used orally for the treatment of blackleg by Zay people in Ethiopia. Phytochemical studies have indicated the presence of steroidal saponins in the Agave genus (Blunden *et al* 1980; Blunden *et al* 1986; Ding *et al* 1989; Ding *et al* 1993). Several studies have documented some pharmacological properties of *A. sisalana* (Peana *et al*., 1997, Santos *et al.*, 2009, Cerquerira *et al.*, 2012) however, its effects on some male reproductive parameters have not been reported. This study aimed at investigating the effects of the aqueous roots extract of *Agave sisalana* on semen parameters and testosterone level in adult male wistar rats.

MATERIALS AND METHODS

Plant collection and identification

Fresh roots of *Agave sisalana* were harvested from a farm at National Roots Crops Research Institute (N.R.C.R.I), Umudike, Abia State, Nigeria. The plant was authenticated by a botanist in the Department of Botany, Michael Okpara University of Agriculture, Umudike, Abia State. The Voucher specimen number PSB/2019/68 was deposited in the Herbarium. The roots were air dried at room temperature (25-28 ◦C) for two weeks and blended into a powdery form using a hand mill to afford 650 g of the sample.

Preparation of plant extract

The powdered plant material (200 g) was macerated in 1.5 L of distilled water for 48 h with intermittent shaking. The extract was then filtered using Whatman No. 1 filter paper and concentrated *in vacuo* using rotary evaporator at 40 ^{o}C. The extract was stored in the refrigerator at 4 ^{o}C until the time of use.

Experimental animals

Twenty five (25) male Wistar rats (120-180 g) were purchased from the Animal House of the Department of Physiology, Federal University Ndufu-Alike Ikwo (FUNAI). Care of the animals was as per the guidelines of NIH for care and use of Laboratory animals (pub. No. 85-23, revised 1985) and the protocol was approved by Animal use Ethical Committee of the Federal University Ndufu-Alike Ikwo with Ethical number DOP/EC/05/143. The animals were allowed to acclimatize for 14 days in the Animal House of the Department of Physiology, FUNAI, in ventilated cages (25 ± 2.0

◦C). The animals were fed with standard rat pellets and allowed free access to water *ad libitum* throughout the period of the experiment.

Experimental design

The rats were randomly selected and divided into five (5) groups as follows:
Group 1 (normal control), received distilled water only for 21 days
Group 2 (cadmium control) was given cadmium (0.2 mg/kg) for 7 days.
Group 3: received cadmium (0.2 mg/kg) for 7 days followed by extract (150 mg/kg) for 14 days
Group 4: received cadmium (0.2 mg/kg) for 7 days followed by extract (100 mg/kg) for 14 days
Group 5 received cadmium (0.2 mg/kg) followed by extract (50 mg/kg) for 14 days.
The body weight of the rats was recorded at the termination of the experiment. After the administration of the last treatment, the animals were fasted overnight and sacrificed under light anaesthesia.

Collection of semen

The region around the penile shaft was opened, the bladder was located and the prostate removed, and the loop of the epididymis was excised and macerated in 5 ml of normal saline.

Blood collection

Blood sample were collected from the animals via cardiac puncture using heparinized capillary tubes for the determination of testosterone.

Determination of Semen Parameters

Sperm Morphology

Using the Ash and Wells technique (WHO, 1999, Dong *et al.*, 2008)). The following parameters were used to identify the morphological appearance of the sperm-head shape (pin head and headless tail), tail shape (coiled tail and looped tail) and midpiece (bent midpiece, coiled midpiece).

Sperm Motility

The epidermal sperm content was obtained by maceration of the tail of the epididymis in 5 ml of physiological saline. An aliquot of this solution was place on the slide and percentage of motility was evaluated microscopically at a magnification of 400 x. Motility estimates were performed from the three different field in each sample. The mean of the three estimations was used as the final motility score and the percentage was calculated.

Sperm Count

The epididymis was minced in 5 ml of physiological saline (pH 7.2) to obtain a suspension. The suspension was filtered through nylon mesh (80 µm). The sperm was counted in the filtrate using Neubaurer's chamber. Briefly, an aliquot from the suspension (up to 0.5) was taken in leukocyte haemocytometer and diluted with Phosphate Buffer saline up to the 11th mark. The suspension was well-mixed and charged in to Neubauer's counting chamber. The total sperm count in eight squares (except the central erythrocyte area) of 1mm^2 each was determined and multiplied by 5×10^4 to express the number of spermatozoa/epididymis.

Hormonal assay

Blood samples were collected by retro orbital puncture. Blood was centrifuged at 3000 rpm to separate the serum for the measurement of testosterone. The quantitative determination of the hormones was done using Enzyme Immuno Assay method (EIA).

Statistical analysis

Results are expressed as mean ± SEM. Means were statistically analyzed by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) software, version 20.0 and values of $p < 0.05$ were considered statistically significant

RESULTS

Effect on sperm motility

The extract significantly ($p < 0.05$) increased the number of active motile sperm cells. The percentage motility increased from 18 to 44 % (150 mg/kg) and from 29 to 46 % (100 mg/kg). The increase by 50 mg/kg) was not significant ($p > 0.05$) compared to the cadmium control group (Figure 1).
There was a significant ($p < 0.05$) decrease in the number of sluggish sperm cells in the high and average dose treatment groups when compared to the Cadmium control group (Figure 2).
The number of non-motile sperm cells was significantly decreased ($p < 0.05$) in the groups treated with 100 and 150 mg/kg of the extract (Figure 3).

Effect on sperm morphology

A significant decrease ($p < 0.05$) in the number of sperm cells with head abnormalities was evident in all the treatment groups when compared to the Cadmium control group (Figure 4).

There was lesser tail abnormalities in the 150 mg/kg treated group than the Cadmium control group (Figure 5)

Effect on sperm count

The extract at higher doses significantly ($p < 0.05$) increased the number sperm cell counts when compared to the Cadmium control group (Figure 6).

Effect on serum testosterone level

Cadmium intoxication did not significantly ($p > 0.05$) affect the testosterone serum level (Figure 8). The effect of the extract on serum testosterone level was non-dose dependent as significant elevation occurred only at the 100 mg/kg dose (Figure 7).

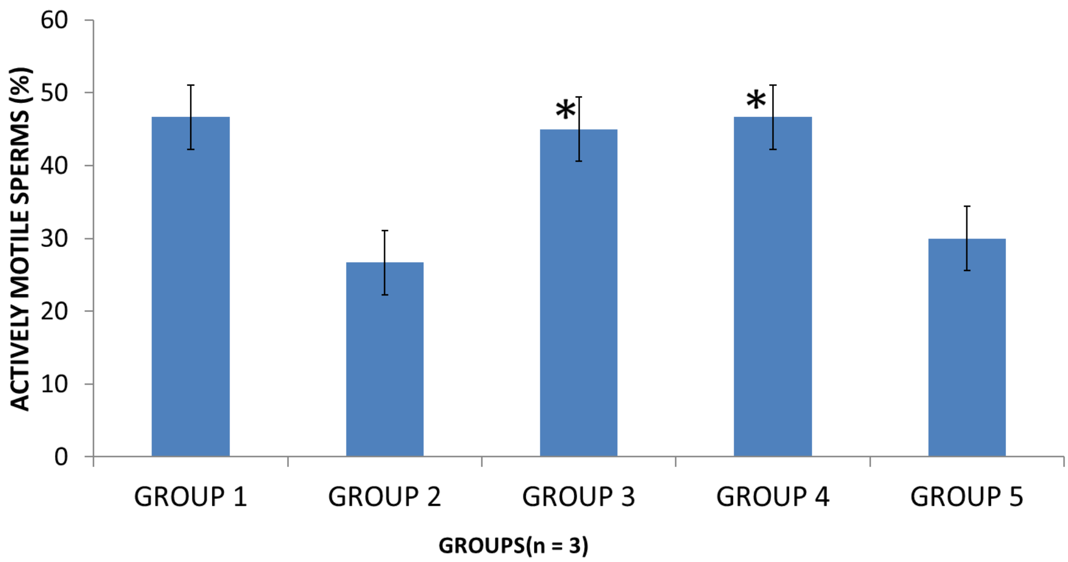

Figure 1: Effect of the extract on active sperm motility. Group 1(normal control group), Group2 (Cadmium induced non-treated group), Group 3 (150 mg/kg), Group 4 (100mg/kg), Group 5 (50mg/kg) of the extract * ($p < 0.05$)

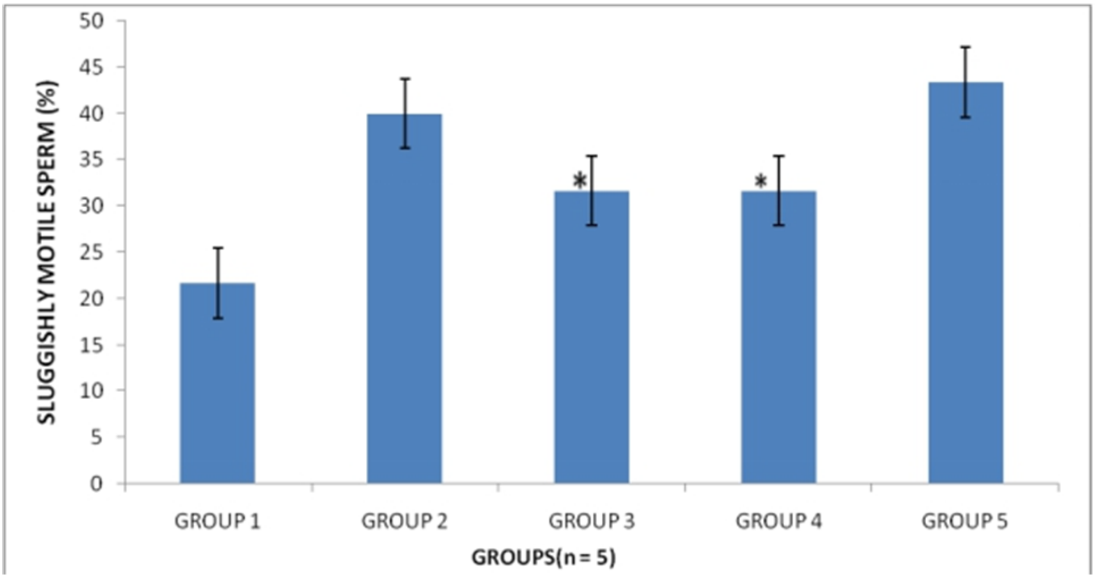

Figure 2: Effect of the extract on sluggish sperm cells *$p < 0.05$

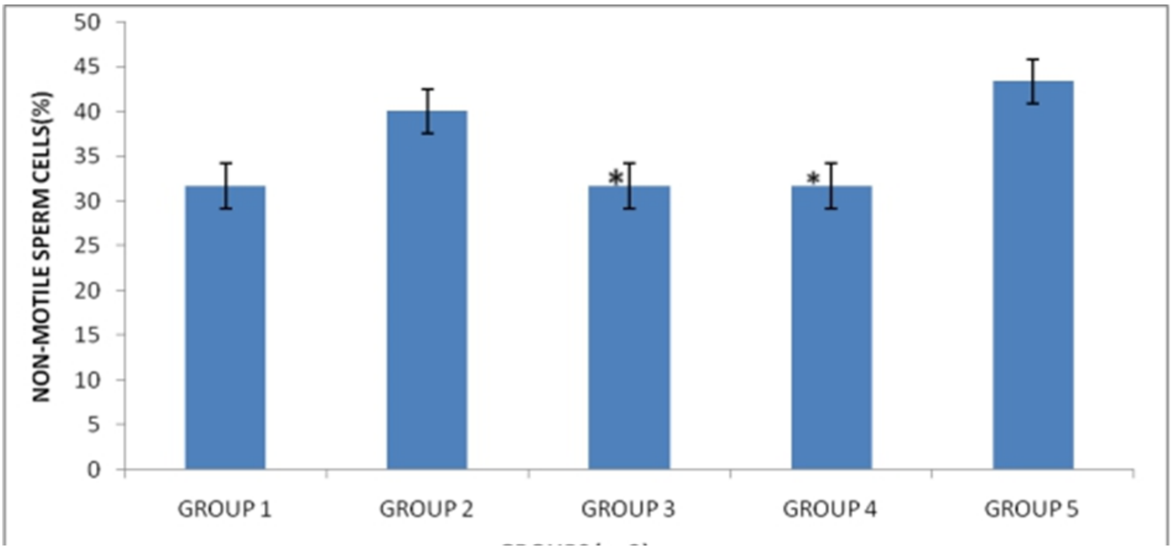

Figure 3 Effect of the extract on non-motile sperm cells * *p* < 0.05)

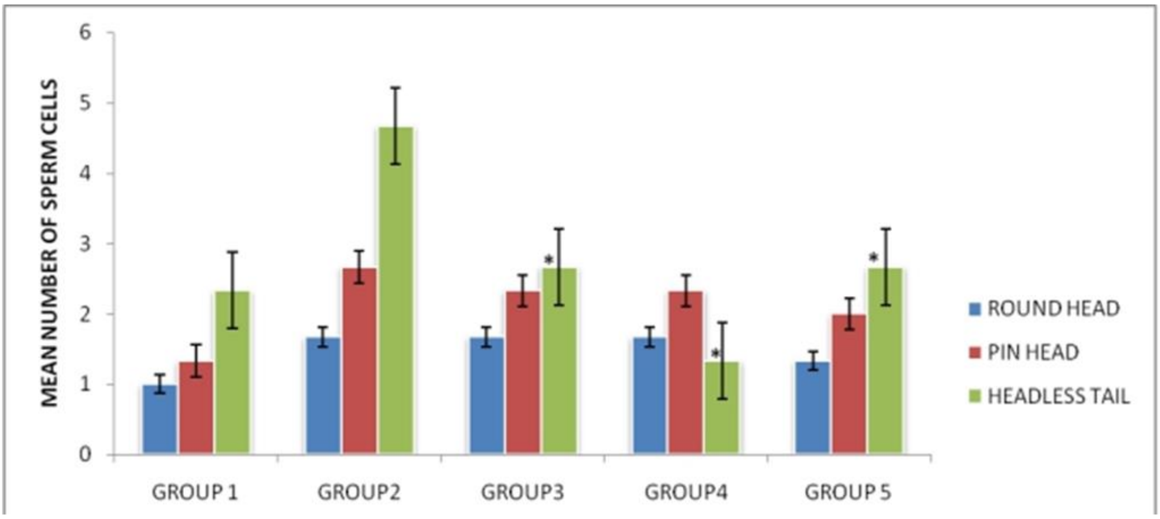

Figure 4: Effect of the extract on sperm cells heads * *p* < 0.05

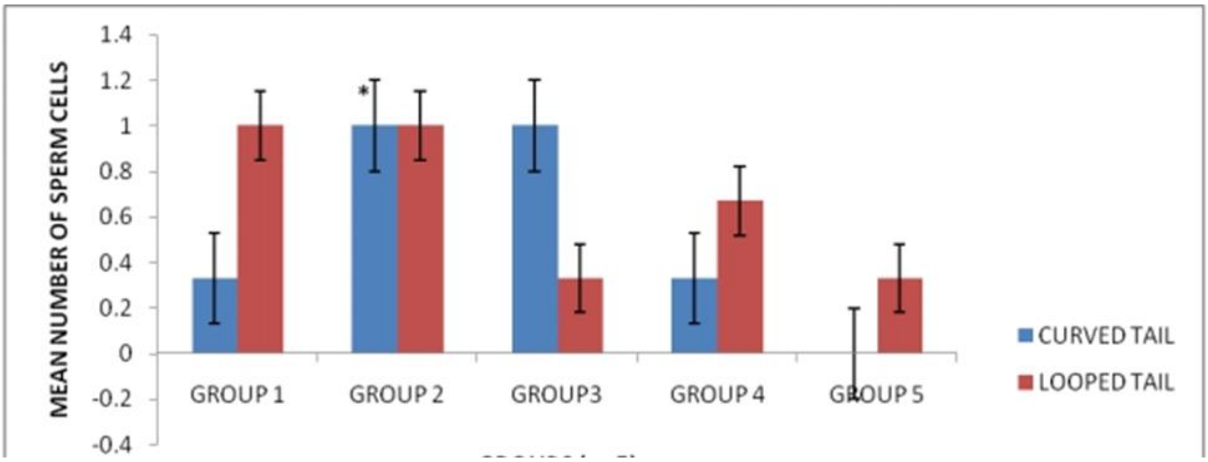

Figure 5: Effect of the extract on sperm cells tail. *$p < 0.05$.

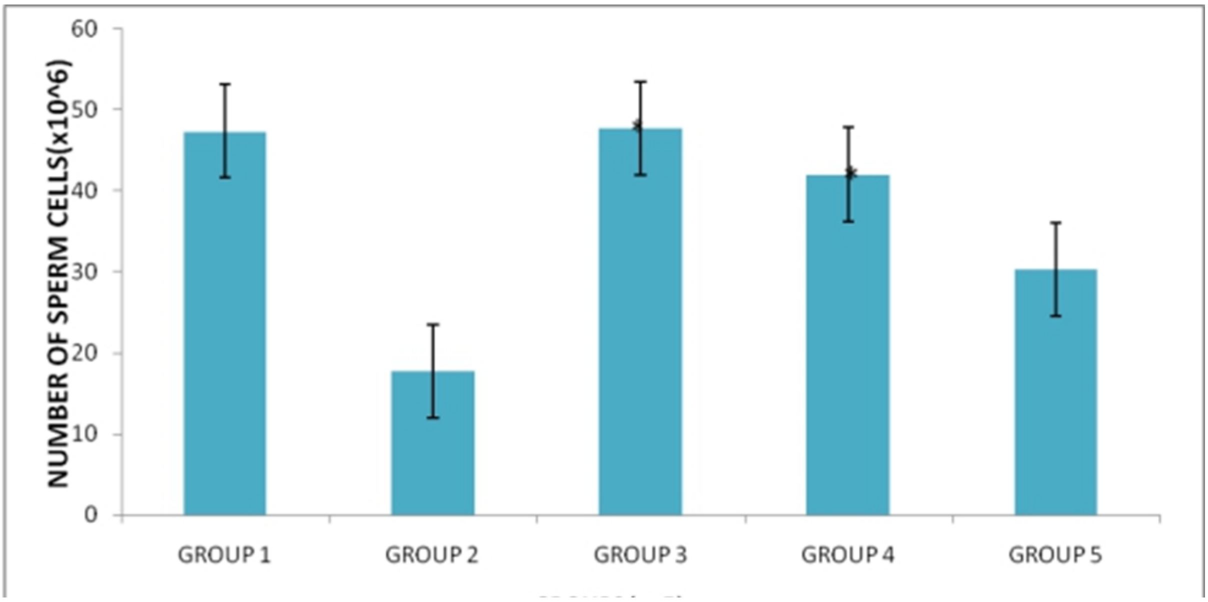

Figure 6: Effect of the extract on sperm cell count. * $P < 0.05$.

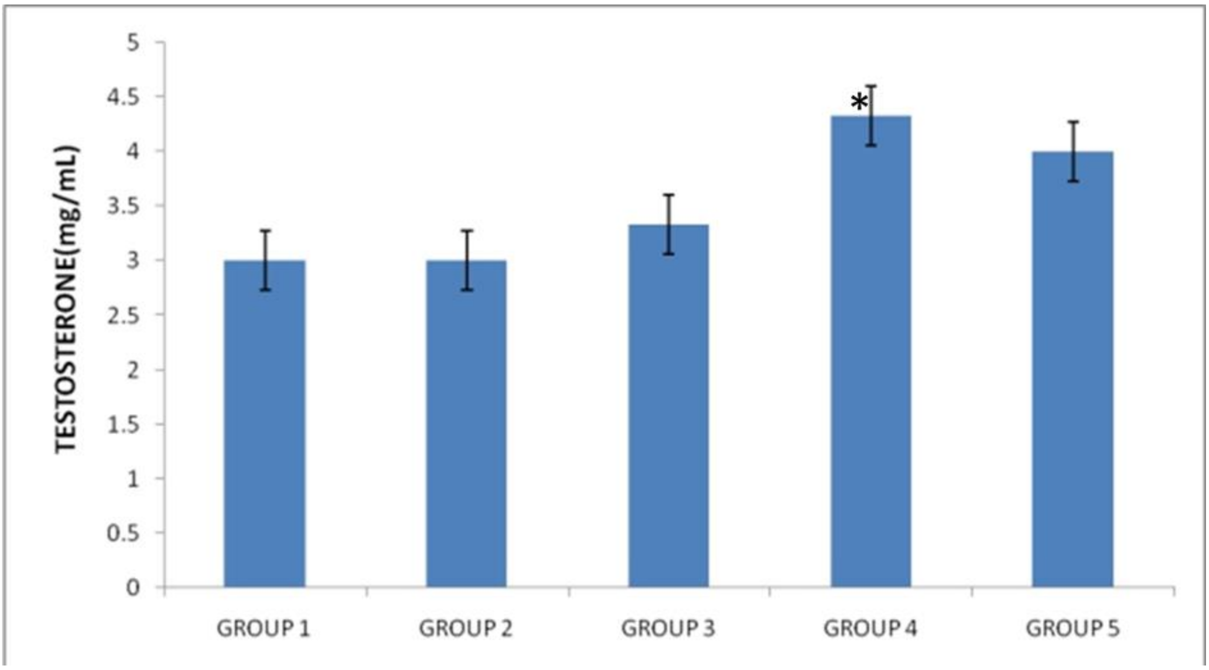

Figure 7: Comparison of testosterone serum level, *p < 0.05.

DISSCUSION

A. *sisalana* has pharmacological properties of interest due to its main phytochemicals. The plant is reported to contain steroidal saponins, flavonoids and homoisoflavonoids (Ding *et al.*, 1989; Botura, 2010), Studies (Santos *et al.*, 2009, Cerquerira *et al.*, 2012;) have documented the different pharmacological properties of *A. sisalana*, but its effects on semen parameters have not been documented. According to Alaee *et al.*, (2014), Cadmium (Cd) has adverse effects on the male reproductive system and the testes are the main target of cadmium. Cadmium enters the body through contaminated air, water and food. It then circulates in the blood and reaches tissues such as testis, where it accumulates. Cadmium disrupts the blood-testis barrier, comes into close contact with different cells of testis and, by increasing the production of ROS and decreasing various antioxidants' levels, enhances the lipid peroxidation of cell membranes, causes apoptosis and necrosis of all testicular tissue leading to disturbance of spermatogenesis, reduces sperm's motility and finally leads to infertility (Alaee *et al.*,2014).

Tam and Liu (1985) reported that in rats, cadmium caused diminished sperm count and impeded movement of sperm cells, resulting in a weakened typical case of infertility. This agrees with the significant reduction in semen parameters recorded in this study. Testosterone is the major male sex hormone created by Leydig cells, situated in interstitial tissue of testis. Nearness and capacity of this hormone is important for spermatogenesis and assessment of the plasma testosterone level is viewed as a helpful tool of testicular capacity (Gorbel *et al*., 2002; Lafuente *et al*., 2004). Investigations have reported that cadmium builds testosterone level (Zeng *et* al., 2003, Haouem *et al*., 2008)), others demonstrated that cadmium weakens it (Amara *et al.*, 2008; Monsefi *et al.*, 2010; Jahan *et* al., 2014). Our result however revealed no significant change in serum testosterone levels of cadmium treated animals when compared to the normal control group.

The results of this study indicated that *A. sisalana* extract may have a beneficial effect in the treatment of infertility as it positively influenced sperm motility and count, although sperm morphology was not significantly affected. The mechanisms through which the extract of *A. sisalana* improves sperm motility, sperm counts and serum testosterone levels are unknown, but the plant contains various organic compounds of which saponins are outstanding (Ding *et al*., 1989; Botura, 2010). The effects of saponins appear to be mediated via interactions with steroid receptors since the basic structures of saponins are

similar to steroidal hormones that are important for reproductive function (Francis *et al*., 2002).
The presence of the steroidal saponins may reflect the ability of that plant to affect male reproductive parameters positively (Ding *et al*., 1989; Francis *et al*. 2002, Botura, 2010). Free radicals play important role in male infertility, and antioxidants could prevent their harmful effects on the sperm. It has been reported that antioxidants have effect on sperm motility (Alizadeh *et al.*, 2015), and *A. sisalana* extract contains phloba tannins, tannins, glycosides and saponins which exhibit antioxidant activity. (Manach*et al.*, 2005, Giovanelli and Buratti, 2009)

CONCLUSION

The root extract of *A. sisalana* has the potential to improve male reproductive function and consequently promote fertility due to its potent antioxidant properties and androgenic activities.

Conflict of interest

There was no conflict of interest as declared by the authors

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