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FERTILITY POTENTIAL OF *LAWSONIA INERMIS* ON HORMONE PROFILE AND SEMEN ANALYSIS IN WISTAR RAT MODEL

[1]Christie E. Fischer, [2] Williams Ujah, [3]Victor A. Fischer*, [4]Eric A. Agaba

[1,3,4,]University of Calabar, [Department of Anatomical Sciences], Calabar, Cross River State, Nigeria.

[2,]Bingham University, [Department of Anatomy], Karu, Nigeria

* Corresponding author email address: vfischer2710@gmail.com

ABSTRACT

Some medicinal plants tested for their fertility properties caused increase in sperm counts and altered the motility of the sperm cells, others altered hormone levels and the histoarchitecture of the testis. *Lawasonia inermis* contains flavonoids and phenolics as its active phytochemicals responsible for its antioxidant properties. This study therefore seeks to scientifically evaluate the possible effects of this plant on the levels of reproductive hormones and semen parameters using Wistar rats. The animals were divided into four groups of seven rats (designated as A, B, C and D). Group A (Normal control) animals were given normal laboratory diet (growers mesh) and distilled water. Group B, C and D animals received 100mg/kgw, 200mg/kgw and 400mg/kgw of the extract respectively for 30 days. At the end of the administration, the animals were anaesthetized using chloroform inhalation method and the testes dissected. Semen was obtained for sperm analysis and serum extracted from blood for hormonal assay. Results showed a significant increase ($p<0.05$) in the level of follicle stimulating and luteinizing hormone in all experimental groups and significant increase ($p<0.05$) in testosterone level in the high dose group when compared to the control. Semen analysis revealed a significant increase ($p<0.05$) in sperm motility, viability, sperm count, percentage of sperm with rapid progressive movement was significantly higher in all experimental groups. This study showed that *Lawsonia inermis* has positive effects on hormone profile and spermatozoa and may have properties influencing the secretion of reproductive hormones and improving the quality of semen in the treatment of male infertility. *Lawsonia inermis* may be used as a booster to enhance fertility and general wellbeing of couples faced with male infertility.

keywords: *Lawsonia inermis*, FSH, LH, Testosterone, Semen, Fertility

Introduction

Herbal medicine is a major component of all indigenous people's traditional medicine and is so important that World Health Organization in assessing the health care systems in developing countries suggested that common medicinal plants could be utilized as substitutes for drugs to reduce overdependence on importation of allopathic drugs. However, there is need for proper scientific verification of their efficacy and systemic effects, particularly on reproduction. Herbal contraceptives are in popular demand because they are cost effective, readily available from local sources and have fewer side effects. However, herbal medicines may impair fertility in male animals. Whilst some medicinal plants tested for their anti-fertility properties caused reduction in sperm counts and altered the motility of the sperm cells, others altered hormone levels and the histoarchitecture of the testis. Many plants used as contraceptives or sterility agents decrease spermatogenesis, impair implantation or are spermicidal[1]. Fertility index can be influenced by genetic factors, environmental factors such as chemicals[2], socioeconomic parameters[3]. Male infertility affects approximately 7% of all men[4] and is commonly due to deficiencies in the semen. Semen quality is used as a measure of male fertility[5,6]. Traditional measures have been taken to improve fertility rate[7,8]. Herbs have been proven to increase the fertility in females due to strong antioxidant complex or phytochemicals contained in them[7]. *Lawsonia inermis* commonly known as henna belongs to the family Lythraceae and genus Lythrum. Henna a common name for a small shrub and for the dye that is obtained from its leaves. The shrub is also called alkanna mignonette tree, El-henna and Egyptian priest[9]. *Lawasonia inermis* plant is cultivated in Africa and Asia for both medicinal and industrial (dyeing) purpose[10]. Just as people use it for staining hair, nails and beard, it is pointed that *Lawasonia inermis* is used for various fields in medicine[11]. Henna has a common traditional usage throughout the world. Due to its palliative, curative and healing effects, it is a famous medicinal plant in most cultures. Recently, the studies about biological activities of henna have an increasing trend. *Lawsonia inermis* is seen to possesses various phytochemical such as tannins, anthraquinones, flavonoids, alkaloids, terpenoids cardiac glycosides, glycosides, reducing sugars phlobatanins, steroids, phenolic, aminoacids, protein and quinones which are responsible for its various medical properties targeting various organs at once and remedying various illnesses[11,12]. However, six compounds were identified in *Lawsonia inermis* leaves by GC-MS (Gas chromatography-mass spectrometry) analysis and the prevailing compounds were a-D-Glucopyranoside, methyl (51.73%) and 1, 4-Naphthalenedione, 2-hydroxy- [Synonyms: Henna] (19.19%)[13]. The immunomodulatory bioassay-guided fractionation of the methanolic extract of henna (Lawsonia inermis L.) leaves resulted in the isolation of seven compounds; three were been isolated for the first time from the genus, namely p-coumaric acid, 2-methoxy-3-methyl-1,4-naphthoquinone and apiin, along with the previously isolated compounds: lawsone, apigenin, luteolin and cosmosine. Structural elucidations of the isolated compounds were based upon their physical, chemical as well as spectroscopic properties. Their immuomodulatory profile was studied using an in vitro immunoassay, the lymphocyte transformation assay. The ABTS [2,2-azino-bis (3-ethyl benzthiazoline-6-sulfonic acid)], free radical scavenging assay depicted that all isolated compounds exhibited antioxidant activity comparable to that of ascorbic acid[14]. Hepato-protective and immune-modulatory effect, antimicrobial, anthelminthic, antifungal, anti-trypanosomal, abortifacient, antioxidant and anticancer activities of *lawasonia inermis* were reported from all over the world by previous studies[10]. The seeds of henna have been reported to possess deodorant action and are used in most cases of gynecological disorders such as menorrhagia, vaginal discharge and leucorrhoea[15]. Reports shows that methanolic root extracts of *Lawaonia inermis* is used in Nigeria for cosmetic purpose, anti-malarial as well as for abortifacient purpose. The powdered roasted seed is mixed with gingerly oil to make a paste which is used for treatment of ring worm. Decoction of the leaves is used by some individuals as blood tonic thus implying its multifaceted used[10]. *L. inermis* given to rats had no adverse effects on the morphology of

the kidneys, liver and spleen. Thus, indicating for safety and non-toxicity of *L. inermis*[16]. Similar results were observed when aqueous extract of *Lawsonia inermis* were administered at 200 mg/kg/day and 1000mg/kg/day[17]. Histological sections of the kidneys, liver and spleen derived from experimental rats treated with Henna leaves at dose 200 mg/kg/day for 42-days appeared normal when compared with the control group whereas changes were observed in sections from rats treated with a dose 1000 mg/kg/day[18].

Males are exposed continuously to compounds that affect their reproductive biology which makes them prone to infertility symptoms after a particular period of time. Infertility often creates one of the most distressing life crisis for couples and can create a deep feeling of loss. These can have devastating effects on the mental health, and general wellbeing of the couple[19]. With the rising trend in infertility and sexual dysfunction among males[20], and less attention being paid to male infertility[21], male infertility will continue to contribute largely to couple infertility. Attention is shifting to prevention, rather than cure of diseases, hence the abundance of supplements as health boosters. This has prompted the need to study herbs readily available, inexpensive with little or no side effects to treat fertility problems. The leaves of *Lawsonia innermis* (*L. inermis.*) have antibacterial activity with no toxicity symptoms on male rats based on their haematological and biochemical parameters[22]. In times past research has shown that Henna plant is used to treat infertility by the people of the western part of Nigeria[23]. This study therefore seeks to scientifically evaluate the possible effects of this plant on the levels of reproductive hormones and semen parameters using Wistar rats.

Methods

Twenty-eight adult Male Wistar rats procured from National Veterinary Research Institution (NVRI) Vom, was used for the experiment. The rats were kept in the animal house of University of Calabar and allow to acclimatize for two weeks before the experiment commenced. The rats were fed on standard diet (vital feeds) and water. The animal house was well ventilated under a 12-12h photoperiodicity. The weight of the rats was taken prior to the commencement of treatment and 24 hours after the last day of administration before the animal were sacrificed. Fresh Henna leaves were harvested and authenticated by a botanist in Ahmadu Bello University, Zaria with voucher specimen number (900270). The henna leaves were air- dried and blended to fine powdered form. The extract of Henna Leaves was extracted using the method described by[24]. 1800mg of the blended henna leaf was measured and soaked in 18mls of distilled water for 48hrs then sieved and stored in a clean cool environment. Extract administration was done daily for a period of 30days using oropharyngeal cannula. The animals were divided into four groups (designated as A, B, C and D) of seven (7) rats in each. Group A (Normal control) animals were given normal laboratory diet (growers mesh) and distilled water. Group B (Low dose) animals received 100mg/kgw of the extract. Group C (medium dose) animals received 200mg/kgw, while Group D (High dose) animals received 400mg/kgw of the extract (Table 1). At the end of the administration, the animals were anaesthetized using chloroform inhalation method and the testes dissected. The right testis was fixed in 10% formal saline fluid for histological analysis while the left testis was homogenized in 10% formal saline for enzymes assay. Spermatozoa was obtained from the caudal part of the epididymis for sperm analysis. Cardiac puncture was done and blood collected, centrifuged and serum extracted for hormonal assay for Testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH)

Results

The results of the hormonal analysis showed a significant increase ($p<0.05$) in the level of follicle stimulating hormone (FSH) in all experimental groups when compared to the control. A non-significant ($p>0.05$) increase in the level of FSH in the group treated with the medium dose of the extract when compared with the group treated with low dose of the extract was also revealed (Fig 1). A significant increase ($p<0.05$) in the level of luteinizing hormone (LH) was also observed in the low dose, medium dose and high dose extract treated group when compared to the control group

(Fig 2). Hormonal analysis results also showed a significant increase ($p<0.05$) in testosterone level in the group given high dose of extract compared to the control group and the low dose and medium dose experimental groups. There was no significant ($p>0.05$) increase in testosterone level in medium dose group when compare to the low dose group (Fig 3). Result from the semen analysis test revealed a significant increase ($p<0.05$) in sperm motility in all experimental groups administered extract of *Lawsonia inermis* when compared to the control group (Fig 4). There was also a significant decrease ($p<0.05$) in non-motile sperm in the experimental groups administered extract of *Lawsonia inermis* when compared to the control group (Fig 5). A significant increase in viable sperm cells ($p<0.05$) was observed in experimental groups when compared to the control group (Fig 6). However, non-viable sperm where significantly lower ($p>0.05$) in all experimental groups when compared to the control (Fig 7). Percentage of sperm with Rapid Progressive Movement (RPM) was significantly higher ($p<0.05$) in all experimental groups when compared to control (Fig 8). Significant decrease ($p<0.05$) in the percentage of sperm with Slow Progressive Movement (SPM) was also observed in experimental groups administered *Lawsonia inermis* (Fig 9). Percentage of sperm with Residual Movement (RM) also decreased in all experimental group when compared with control group significantly ($p<0.05$) (Fig 10). There was also a significant increase in the sperm count ($p<0.05$) in experimental groups when compared to control group (Fig 11). Sperm analysis also revealed no difference ($p>0.05$) in the number of sperms with head and tail defects in experimental groups when compared to control group (Fig 12). There was also no difference ($p>0.05$) in the number of sperms with head and tail defects between experimental groups (Fig 13).

Discussion

Globally, infertility affects about 50-80 million couples at some point of their reproductive lives with a variety of biological and behavioural determinants. Male infertility is commonly due to deficiencies in the semen and affects approximately 7% of all men[4]. Herbs have been proven to increase the fertility due to strong antioxidant complex or phytochemicals present in them[7]. In many parts of the world, efforts are now being aimed at investigating therapeutic efficacy of locally available medicinal herbal plants[26]. The results of the hormonal analysis showed a significant increase in the level of follicle stimulating hormone (FSH) and Luteinizing hormones in all experimental groups when compared to the control. The increase observed in the level of FSH and LH may be due to the ability of *Lawsonia inermis* to cause increase in the expression of kisspeptin receptor on GnRH neurons[27] in the hypothalamus thus increasing the release of GnRH hormone which leads to a resultant increase in secretion of FSH and LH by the anterior pituitary gland. The significant increase ($p<0.05$) in testosterone level in the group given high dose of extract compared to the control group and the low and medium dose experimental groups may have been as a result of the increased secretion of luteinizing hormones which bind to leydig cells thus causing increase in the production of testosterone which in turn stimulates spermatogenesis. A non-significant increase in the level of FSH and testosterone was observed in the group treated with the medium dose of the extract when compared with the group treated with low dose of the extract. This result suggest that the activity of *Lawsonia inermis* in production of sex hormones may not necessarily be dose dependent. So that *Lawsonia inermis* is capable of stimulating spermatogenesis even at low doses. This is similar to the work of Olawuyi[25] who reported a significant increase in the level of FSH, LH and testosterone after administration of 60mg/kg of *Lawsonia inermis* to albino Wistar rats for three weeks. He also reported that administration of increasing doses of *Lawsonia inermis* was able to alleviate the negative effects caused by administration of 0.5mg/kg of aluminium chloride to albino Wistar rats for three weeks. This is dissimilar to the work of Nuha[28] who reported a decrease in the level of LH and FSH in female albino Wistar rats after administration of *Lawsonia inermis* at 0.5 g/kg and 1.0 g/kg orally for two weeks. Dissimilarity in the result obtained in these studies could be due to the dosage used, the duration as well as differences in the sex of the animals used. In this study, a dose dependent significant increase in sperm motility with rapid

forward progression movement, sperm count, and viable sperm cells was observed when compared to the control and a dose dependent decrease in the number of non-motile sperm with slow forward rapid progression movement, non-viable sperms. Sperm motility of 50% and above consisting of rapid forward progression movement is essential for conception in human[29]. Sperm motility less than 50% is known to result in failure of conception. The calcium (Ca^{2+}) pathway and the cyclic adenosine monophosphate (cAMP)-dependent protein kinase or protein kinase A (PKA) pathway are two important metabolic pathways involved in the regulation of sperm motility[30]. These pathways involve calcium ions, adenylyl cyclase, bicarbonate ions, different membrane channels, and phosphorylation events. The increase in sperm motility with rapid forward progression movement observed may be due to the ability of *Lawsonia inermis* to increase bicarbonate and calcium ions which leads to the activation of a soluble adenylyl cyclase (SAC or SACY), that increases the production of cAMP and brings to the activation of PKA, a protein kinase that phosphorylates several tyrosin kinases and leads to a phosphorylation cascade that ends with the phosphorylation of the axonemal dynein and the start of flagellar movement[31]. This is dissimilar to the work of Abu[32] who reported a significant decrease in spermatozoa motility in a concentration-dependent manner after *in vitro* administration of 10 mg/ml, 20 mg/ml, 40 mg/ml, 80 mg/ml and 160 mg/ml of ethanol extract of *Lawsonia inermis* leaves on adult male white albino rats. The dissimilarity observed could be due to the differences in the extracting medium used because appreciable amounts of phytochemicals have been reported to be extracted by alcohol derivatives than its aqueous counterpart. Hence additional phytochemicals extracted by the ethanol extract may be responsible for the deleterious effect observed by Abu[32]. This could also be due to the route and form of administration. Increase in the number of viable sperms observed in experimental groups could be due to the ability of aqueous extract of *Lawsonia inermis* to increase glucose metabolism leading to the production of pyruvate which is known to be the preferred substrate essential for the activity and survival of sperm cells[33]. Within seminiferous tubules, appropriate Sertoli cell function is highly dependent on testicular androgen levels and is essential to initiate and maintain spermatogenesis. The increase in sperm cell count in experimental groups when compared to the control corroborates the use of *Lawsonia inermis* in traditional medicine for fertility issues. The increase in sperm cell count observed may be due to the antioxidant components of *Lawsonia inermis* since crude extract of *Lawasonia inermis* has been reported to contain flavonoids and phenolic compounds[11] and recent studies have shown that treatment with antioxidants improves steroidogenesis by enhancing the primary effect on Leydig cell endocrine function along with increased circulatory testosterone production and stimulation of spermatogenesis[34,] as seen in the increase in FSH, LH and testosterone observed in this study. The resultant decrease in non-motile sperm with slow forward rapid progression movement and non-viable sperms when compared with the control observed shows that *Lawsonia inermis* not only increases spermatogenesis but also maintains the activity of the sperm.

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Table 1.

Administration of the extract showing animal weights, dosage and duration

Experimental Groups	Dosage	Duration (mg/kg)	Animal weight
Group A	Nil	30 days	……
Group B	100mg	30 days	……
Group C	200mg	30 days	……
Group D	400mg	30 days	……

Dosage of administration was determined from LD50 750mg/kg body weight according to Oluwuyi[25]

Hormonal profile result

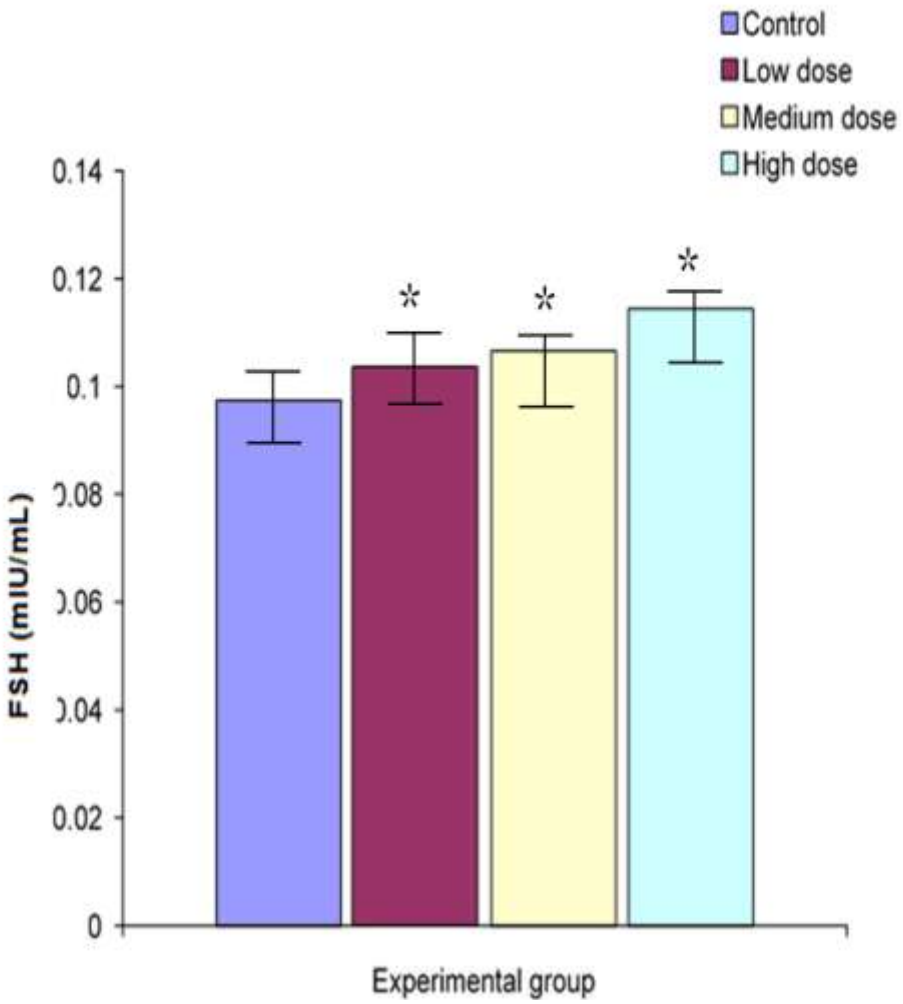

FIG 1: Comparison of follicle stimulating hormone concentration in control and *L. inermis* treated groups.Values expressed as mean ± SEM. * = significantly different from control at $p<0.05$. FSH - follicle stimulating hormone.

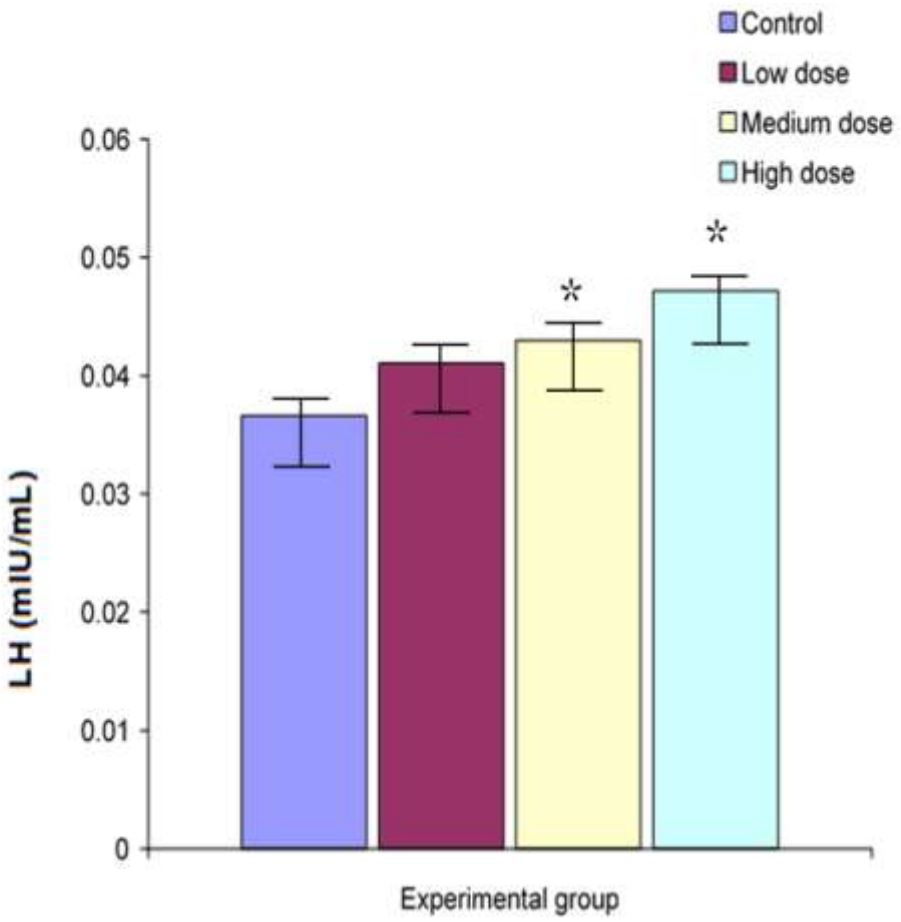

FIG 2: Comparison of luteinizing hormone concentration in control and *L. inermis* treated groups.Values expressed as mean ± SEM. * = significantly different from control at $p<0.05$. LH = luteinizing hormone

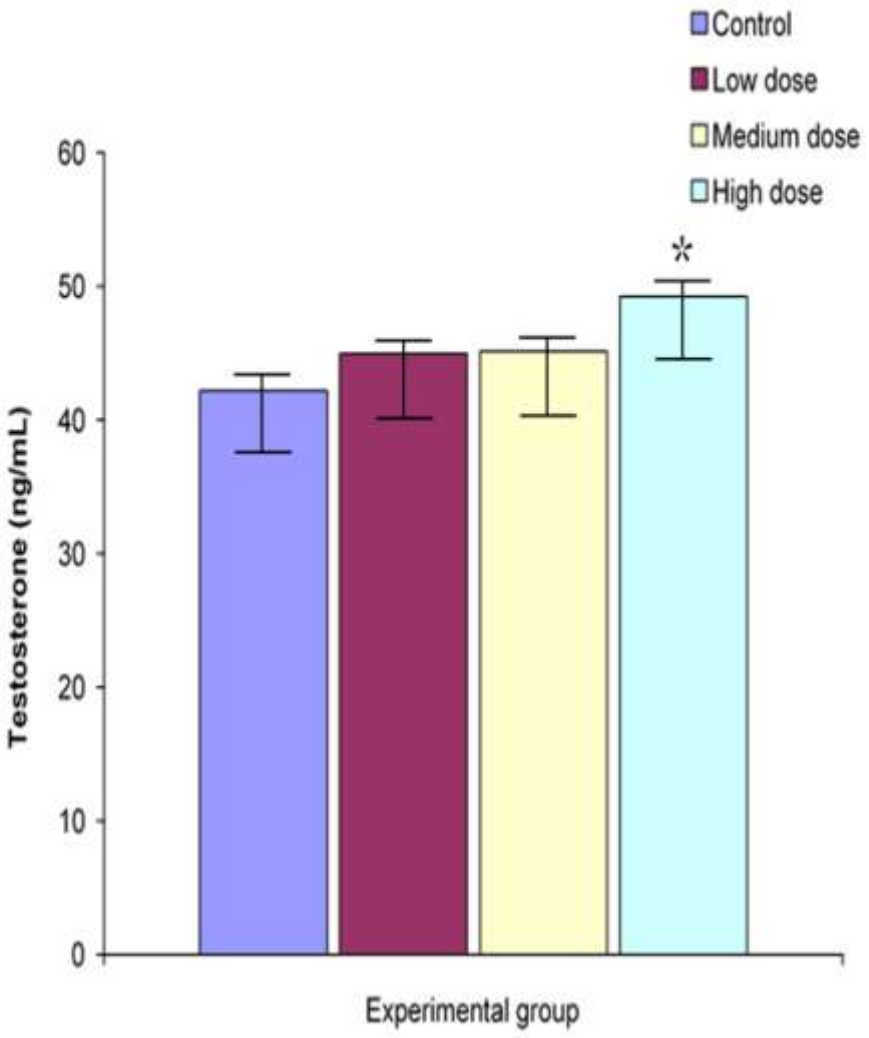

FIG 3: Comparison of testosterone concentration in control and *L. inermis* treated groups.Values expressed as mean ± SEM. * = significantly different from control and other groups at $p<0.05$.

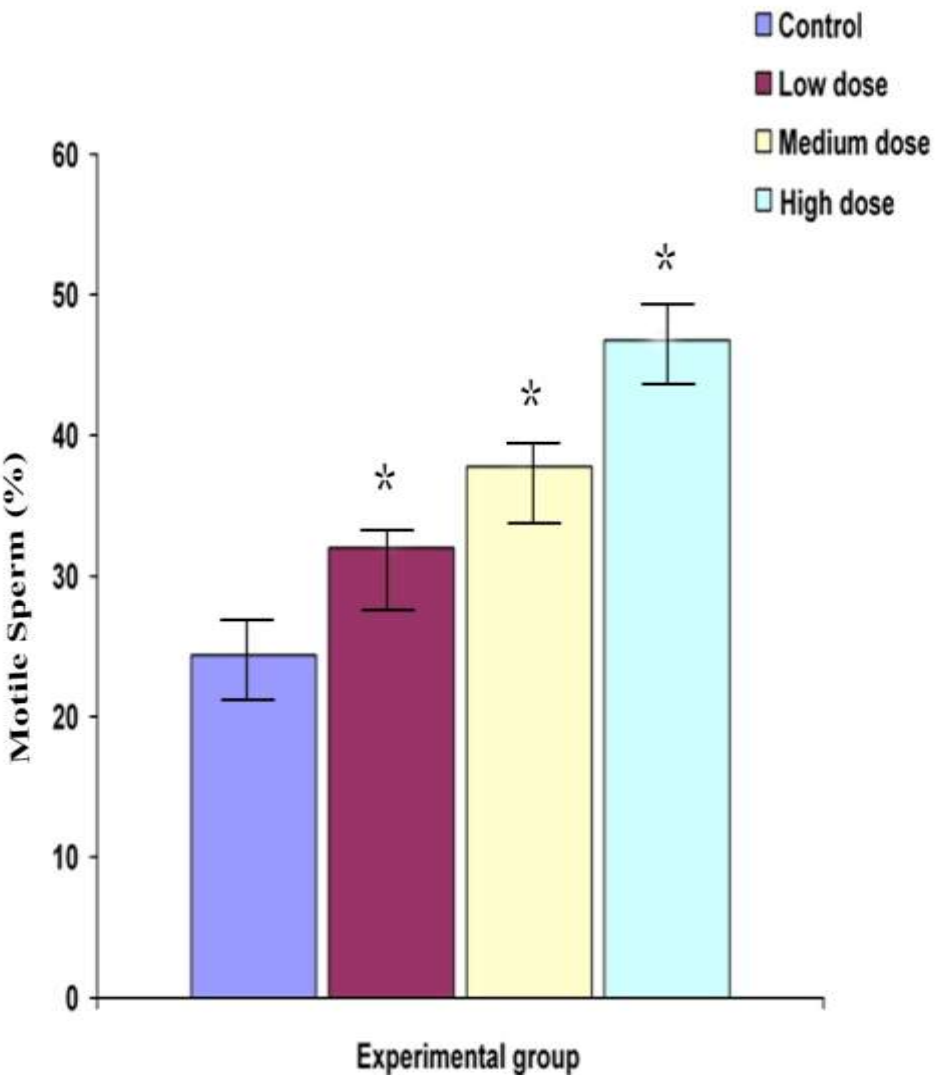

FIG 4: Comparison of percentage of motile sperm in control and *L. inermis* treated groups. Values expressed as mean ± SEM. * = significantly different from control at $p<0.05$.

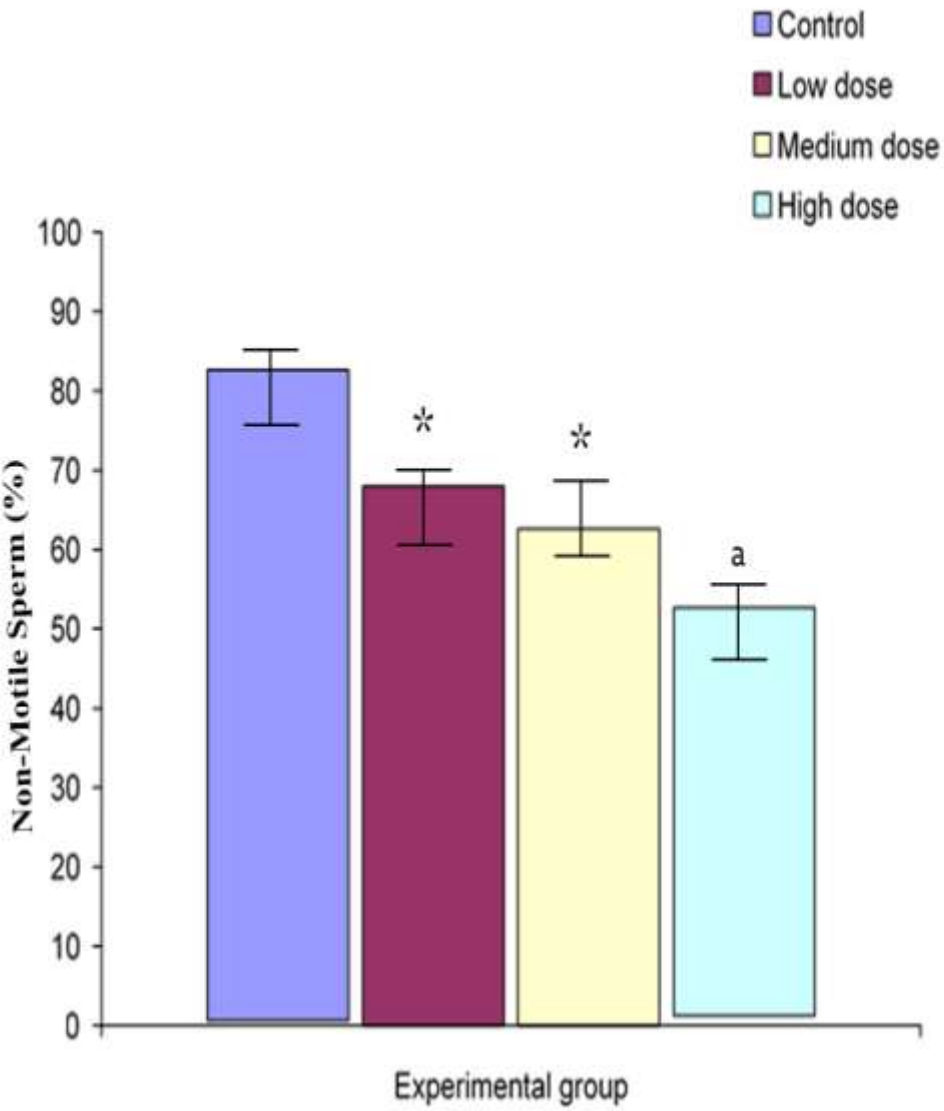

FIG 5: Comparison of percentage of Non-motile sperm in control and *L. inermis* treated groups.Values expressed as mean ± SEM. * = significantly different from control, a = significantly different from control and low dose groups at $p<0.05$.

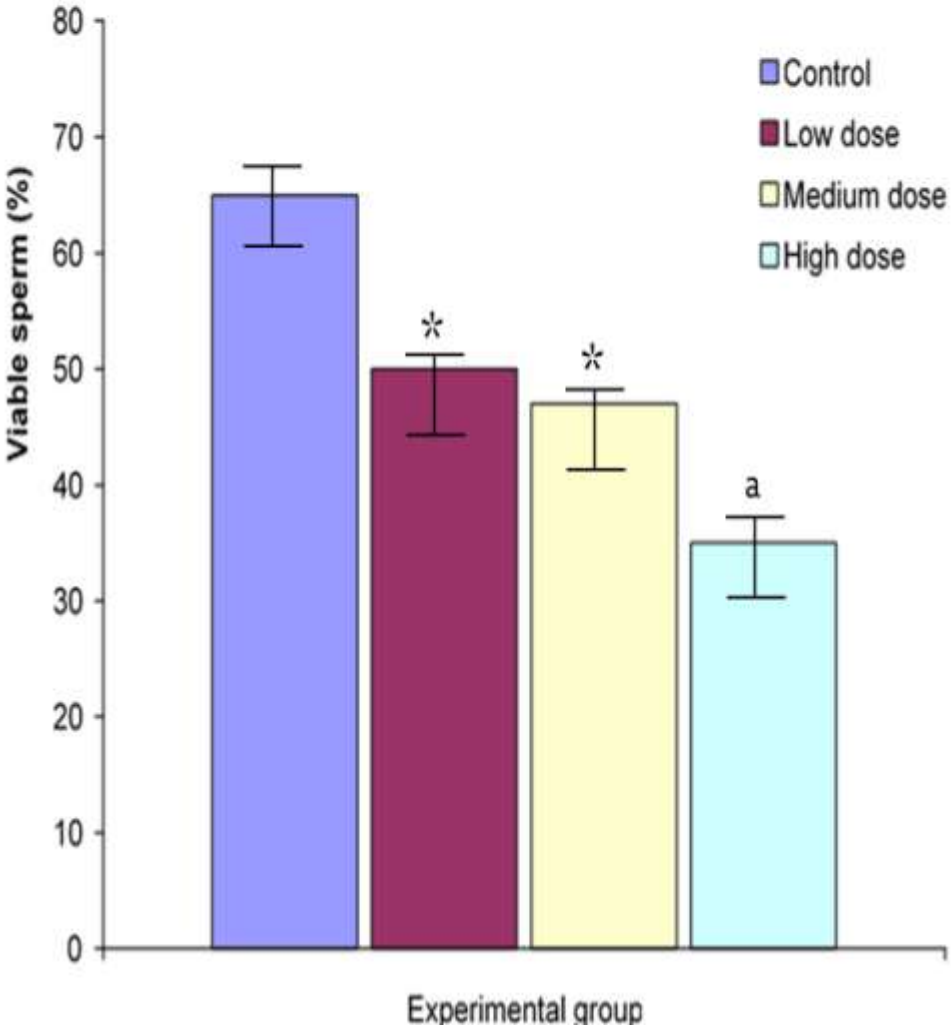

FIG 6: Comparison of percentage of viable sperm in control and *L. inermis* treated groups.Values expressed as mean ± SEM. * = significantly different from control, a = significantly different from control and low dose groups at $p<0.05$.

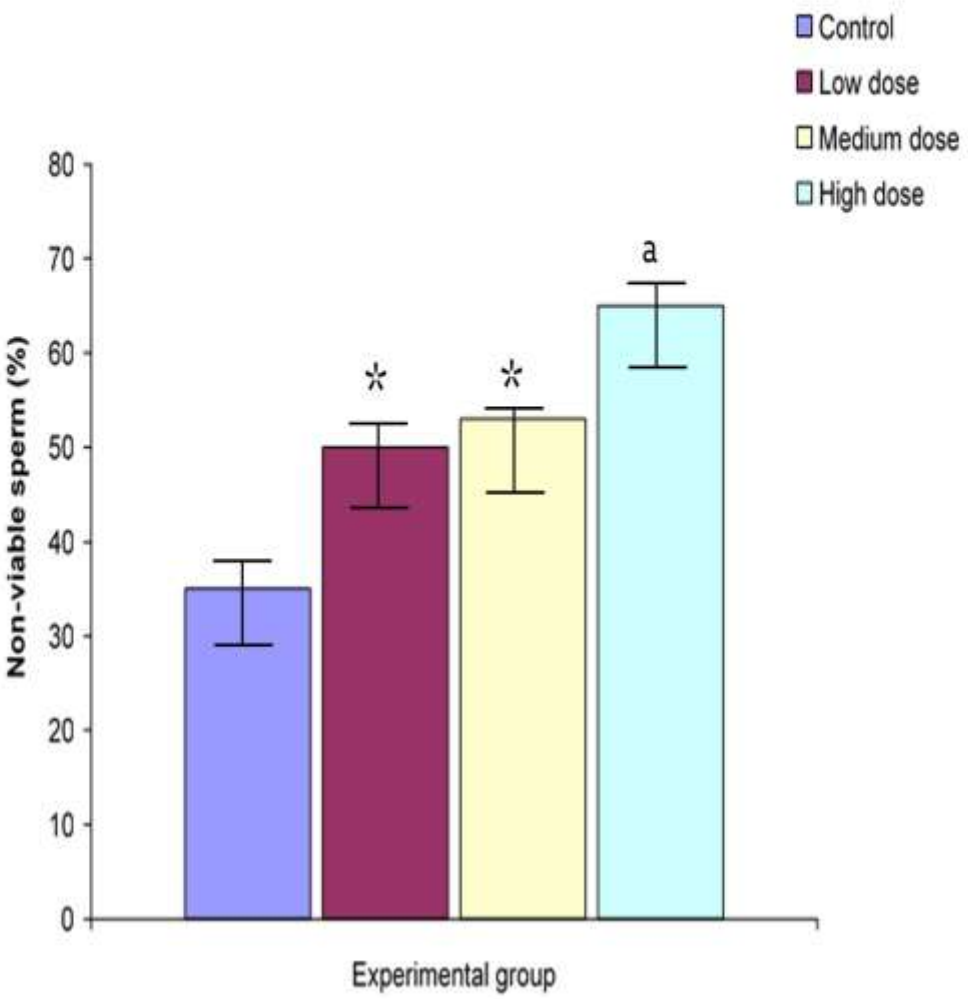

FIG 7: Comparison of percentage of non-viable sperm in control and *L. inermis* treated groups.Values expressed as mean ± SEM. * = significantly different from control, a = significantly different from control and low dose groups at $p<0.05$.

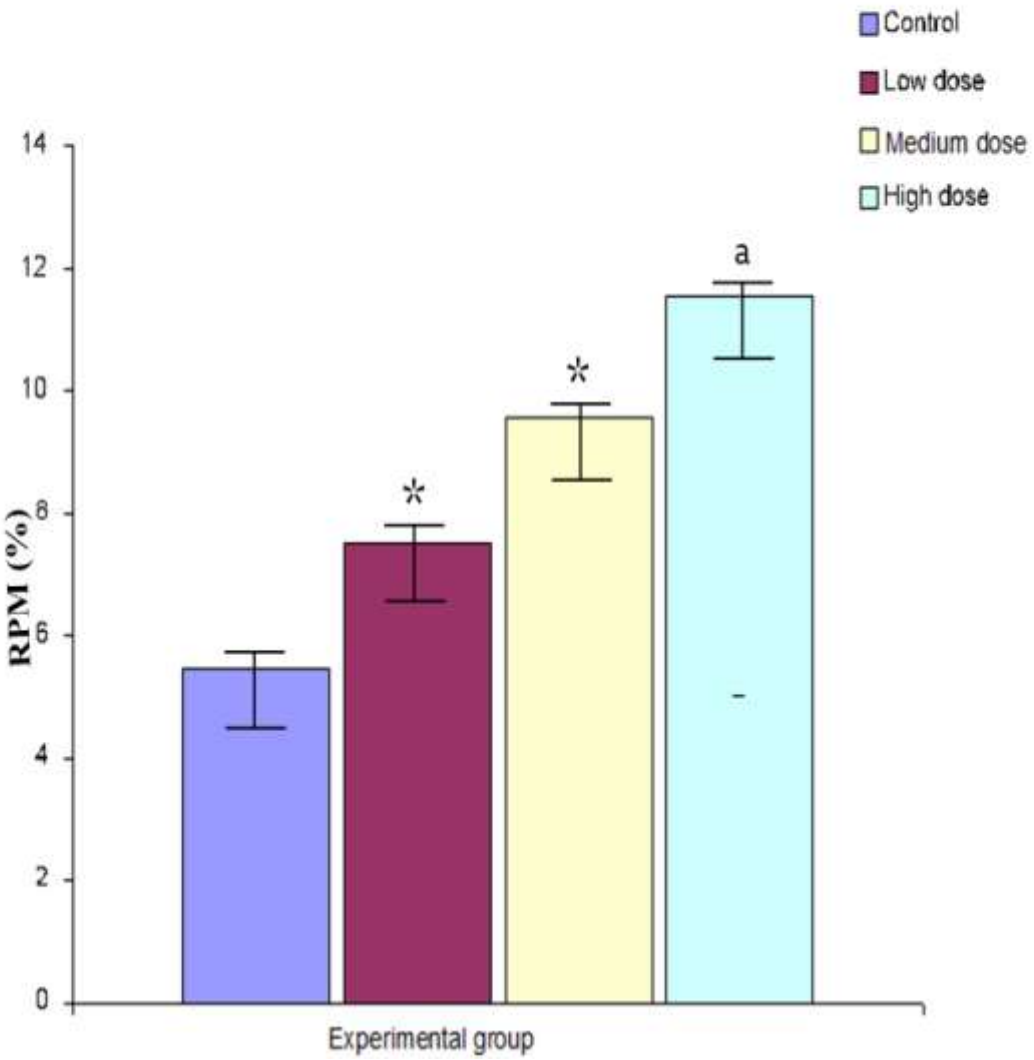

FIG 8: Comparison of percentage of rapid progressive movement in sperm in control and *L. inermis* treated groups. Values expressed as mean ± SEM. * = significantly different from control, a = significantly different from control and other experimental groups at $p<0.05$. RPM - rapid progressive movement.

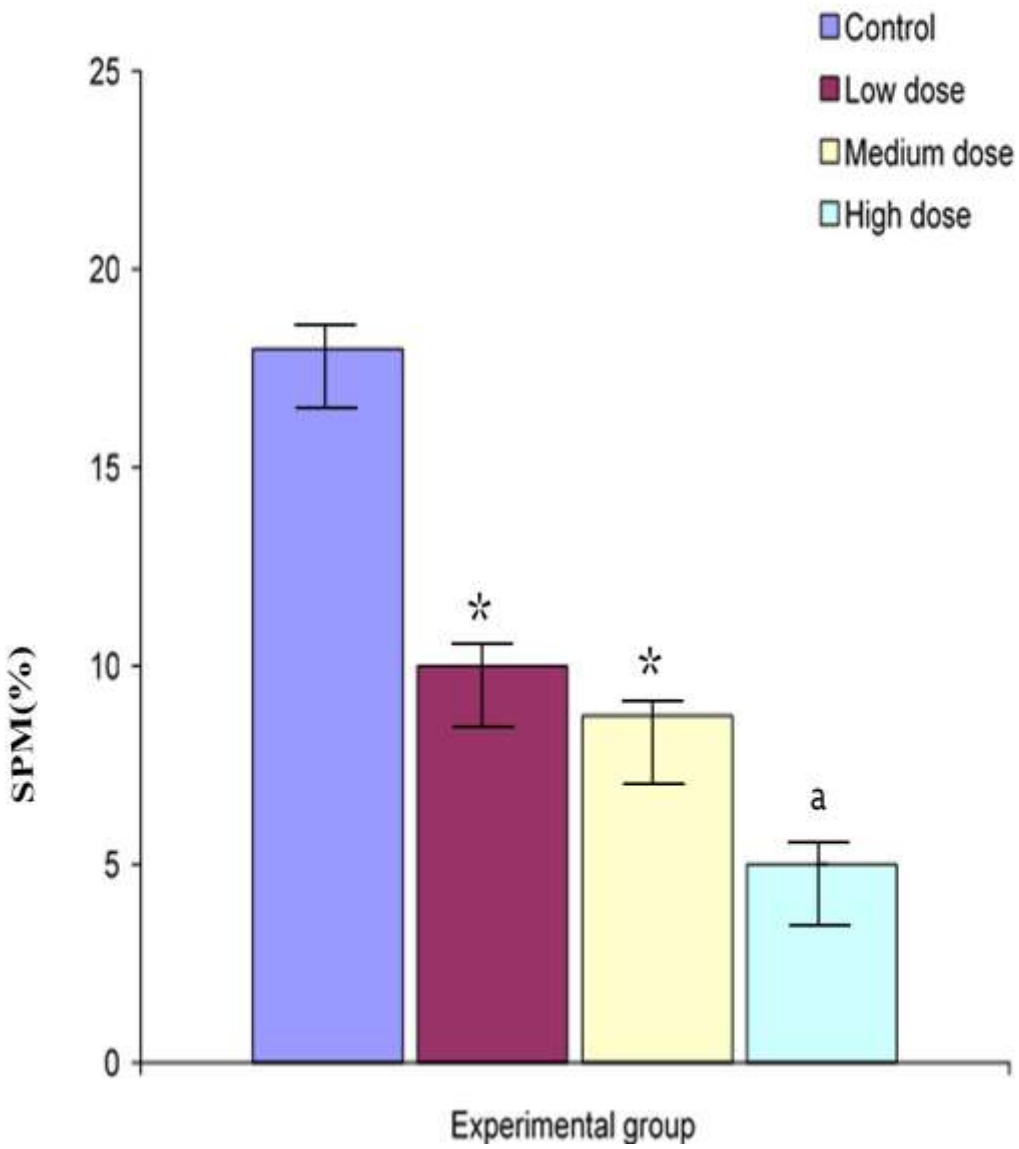

FIG 9: Comparison of percentage of slow progressive movement in sperm in control and *L. inermis* treated groups.Values expressed as mean ± SEM. * = significantly different from control, a = significantly different from control and other experimental groups at $p<0.05$. SPM – slow progressive movement

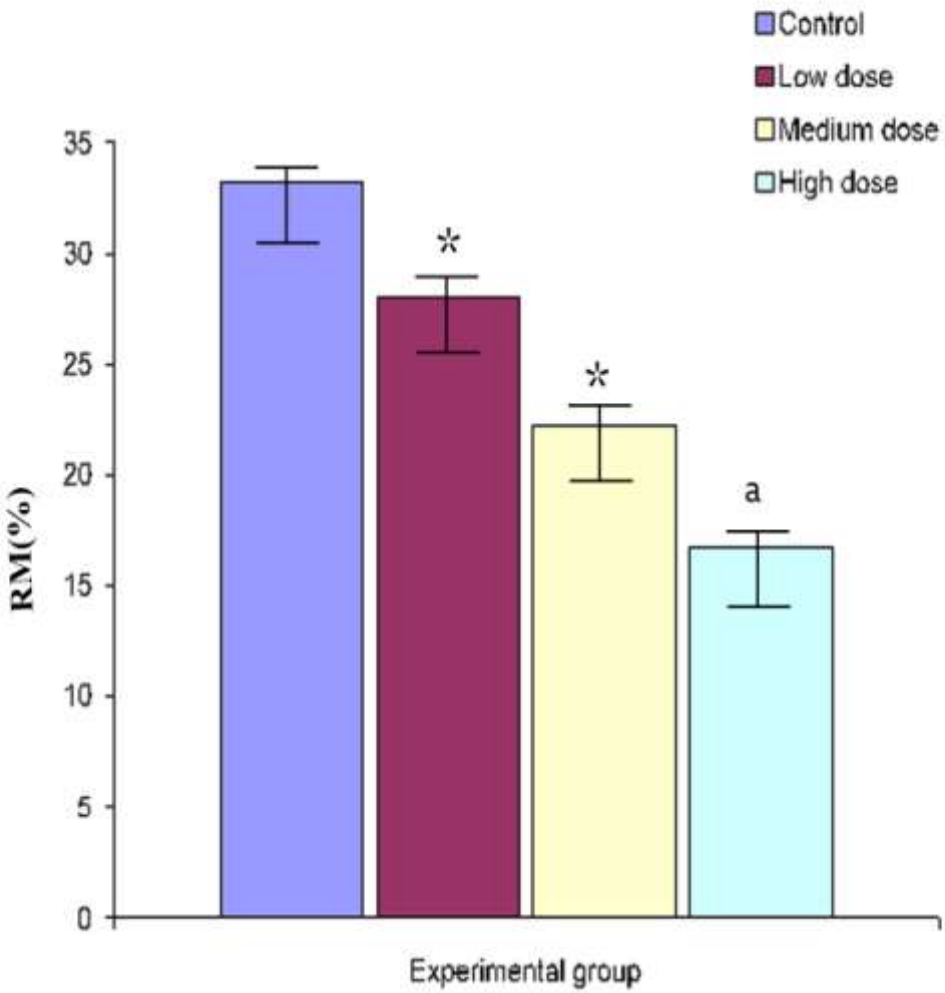

FIG 10: Comparison of percentage residual movement in sperm in control and *L. inermis* treated groups.Values expressed as mean ± SEM. * = significantly different from control, a = significantly different from control and other experimental group at $p<0.05$. RM – residual movement.

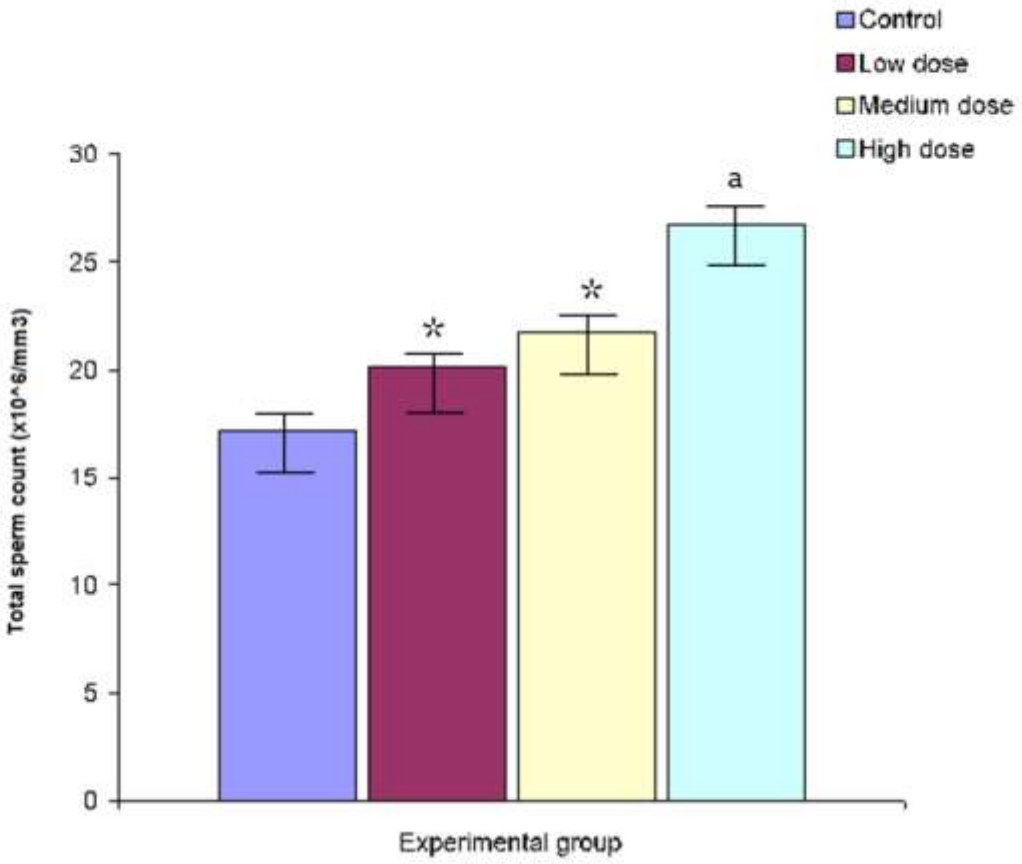

FIG 11: Comparison of total sperm count in control and *L. inermis* treated groups. Values expressed as mean ± SEM. * = significantly different from control, a = significantly different from control and other experimental groups at $p<0.05$.

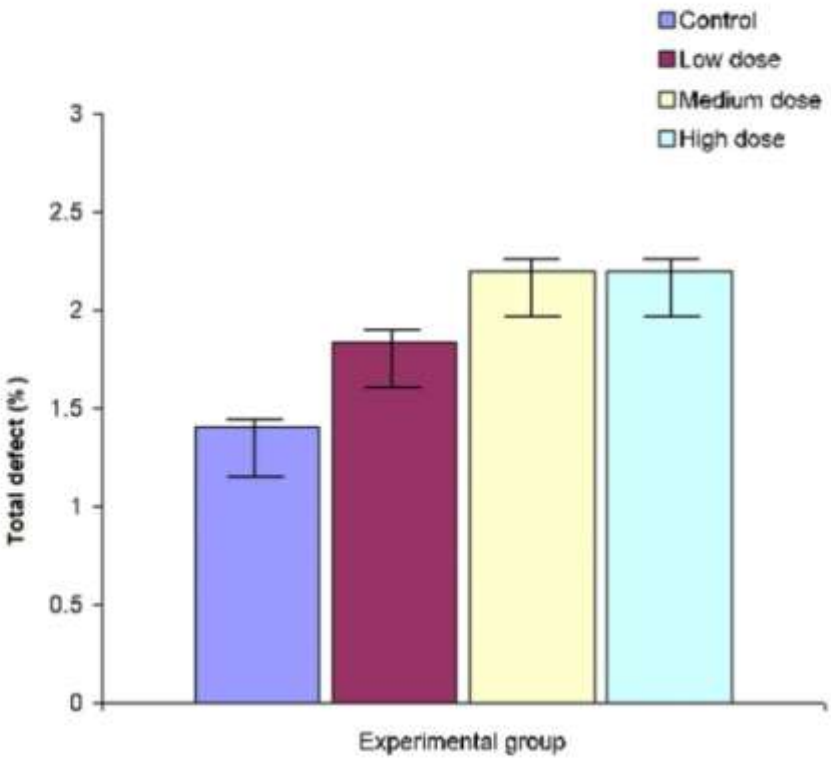

FIG. 12: Comparsion of percentage of total sperms with defect in control and *L. innocmis* extract treated groups.

Values are expressed as mean +SEM, n = 5.
No significant differences among groups

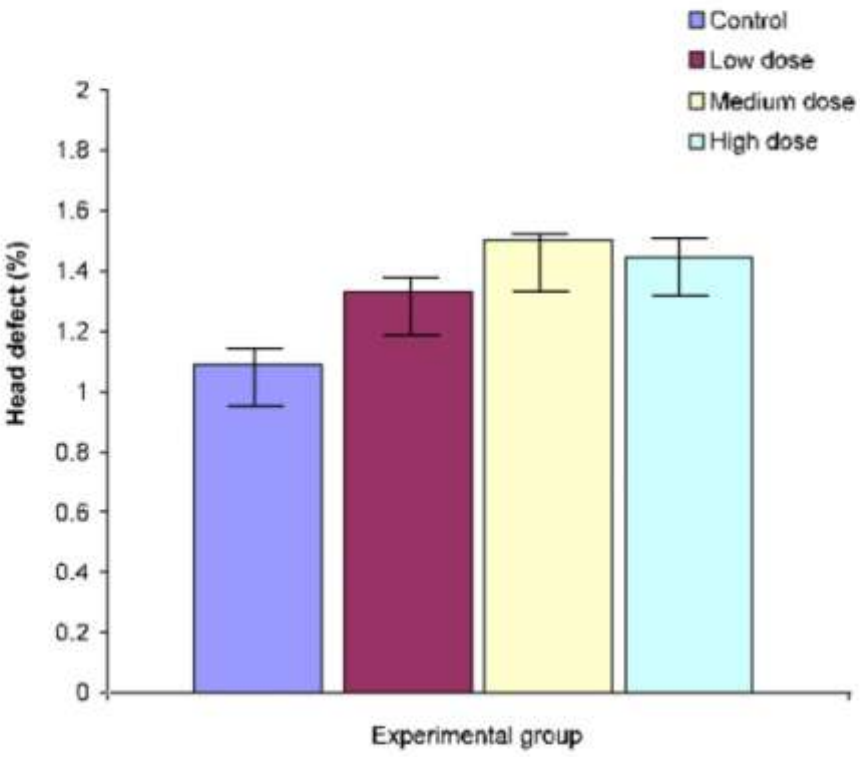

FIG. 13: Comparsion of percentage of sperms with head defect in control and *L. innermis* extract treated groups.

Values are expressed as mean +SEM, n = 5.
No significant differences among groups