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Spermatogenic Assessment in Mangiferin Induced Male Rats in Comparis0n to Crude Extract of Mangifera Indica (BARK)

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Abstract

Since ancient ages, mango tree is worshiped by the people of different counties like India .It has great economic as well as medical values in different fields of clinical treatment like fever, cough, hepatitis, diarrhoea, etc. But the medicinal value in different on its different parts like leaf, stem, bark etc .The bark has been reported vastly to have pharmacological importance. Through reports are available mostly on crude extract of bark but the report on pure mangiferin is very limited. Specifically few reports on reproduction by crude extract treatments are available but no such authentic report of pure mangiferin on male reproduction is found till date. So, we tried to study of pure mangiferin and crude extract of it as a trial study. The adult male rats were injected (i.p) extract and pure mangiferin at the dose of 0.1ml mother and 1mg/100gm b.wt/day accordingly for 14 days consecutively parallel to vehicle control.

The study of sperm motility showed significant reduction in all the treated groups, but is predominant in extract treatment. Again the total sperm count was significantly reduced in extract treated group but was increased significantly in pure drug treated group when compared to control .The abnormal sperm showed significantly rise in treated group but in abnormalities were comparatively lower in pure group in compare to extract group. Sperm viability by Eosin nigrosin stain study also showed the same nature alike the morphology study. The SEM study of Sperm morphology and epididymal also histology supported the above statement i.e. distortion of acrosomal cap in sperm head, abnormal tail and neck, which was also confirmed by Gimsa staining further.

Keywords: Mangiferin, *Mangifera indica*, Sperm count, Sperm motility, Sperm viability.

Introduction

The quest for the oral contraceptive agent that can control human fertility is continue since ancient times. Although a variety of synthetic contraceptive agents are available, but because of their side effects these cannot be used continuously. Scientists and researchers throughout the world are realising the importance of avoiding the harmful effects of synthetic compounds, and thus cooperating in efforts to design new and effective contraceptives from compounds of plant origin. Use of ethnobotanical information in medicinal plant research has gained considerable attention in recent years, especially in segments of the scientific community. The use of herbs is very common in developing countries, particularly in rural

communities. However, an increase in the use of plants has been observed in metropolitan areas of developed countries during the last decade [1]. The natural C-glucoside xanthone mangiferin [2-C-β Dgluco-pyranosyl-1,3,6,7-tetrahydroxyxanthone; C19H18O11; MW- 422.35; melting point- anhydrous 271°C has been reported to found in various parts of *Mangiferaindica*, i.e. leaves, fruits, stem bark, heartwood and roots, but which is predominant in stem bark [2]. Being a polyphenolic antioxidant, mangiferin has anti lipid peroxidative, immunomodulatory, cardiotonic, hypotensive, antidegenerative, antidiabetic activities [3], and also increases the rate of erythropoiesis [4]. The bark extract of *Mangifera indica* has been reported to possesses hepatoprotective [5], anticancer [6], analgesic & anti-inflammatory [7], antidiabetic [8-10], antioxidant [11-13], anti-tumor- anti-HIV [14], immunomodulatory [15], anti-microbial [16], diuretic [17] activities. But it's effect in male reproduction especially in spermatogenic activities yet not reported except few preliminary studies [18].

Materials and Methods

Plant material

Pure (98.2%) mangiferin (bark) was purchased from Sigma-Aldrich (USA) and crude mother of mangifera bark from Nityananda homeo hall,kolkata. Pure drug was prepared in 70% alcohol and was diuted by distilled water.

Animal selection and maintenance

18 Albino Wistar male rats of weighing 120–130g were housed in standard environmental condition. The rats were maintained under standard laboratory condition (temperature 25 ± 2˚C, 12/12hr dark and light, relative humidity 40-60%) with free access to standard normal diet, prescribed by ICMR, NIN, Hydrabad, India [24] and water *ad libitum* Animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) guided by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. ref.no. PU 796/03/ac/CPSEA.

Experimental design and drug administration

The experimental animals are divided into 3 groups and each group contained six animals. Treatment scheduled for 14 days and drug administered intraperitoneally (i.p). The groups were as follows:

Group I (Vehicle treated control) – 0.1ml 70% alcohol /100gm b.wt./day

Group II (*M indica* bark extract treated group) – 0.1ml mangifera bark mother/100gm b.wt./day.

Group III (Pure Mangiferin treated group) – 1 mg in 0.1 ml 70% methanol/100gm b.wt./day.

Tissue collection

After completion of the experimental schedule, all the animals were subjected to light ether anaesthesia after recording the body weight. Then animals were decapitated one by one using sharp knife. Reproductive organs i.e. epididymis was dissected out. Fat and other connective tissues were removed from the surface of the organs.

Sperm count

The sperm concentration was determined by using the Haemocytometer method .Within a minute after anesthesia, the epididymis was dissected out and at definite length of the cauda and was taken in small beaker containing 2ml phosphate buffer saline (pH 7.4, 0.2M strength). The cauda was punctured with a fine hypodermic needle and spermatozoa were washed with the diluents. The beaker was shaken gently to have homogeneous concentrations of sperms. 40µl of sperm suspension was taken and mixed with 360µl dilutents (PBS). A

drop of the suspension (10µl) was placed on the haemocytometer and allowed to settle for 15 mins. The number of spermatozoa in the appropriate squares (4 small squares) were recorded at 10X magnification.

Sperm motility [20]

From the above preparation 40µl of sperm suspension (10µl) was placed on the haemocytometer and cover with a cover-glass and counted under light microscope as x150 magnification. Total number of motile sperm per 100 sperms was counted. The temperature of spermatozoa suspension was maintained at 37°C, throughout the process.

Sperm morphology

From the above preparation the sperm suspension was filtered through silken cloth to remove the debris and the filtrate was collected in a graduated tube, more saline was added to make the volume 10ml.The sperm suspension thus collected, was put in the centre of a clean slide over which 0.02 ml methanol was added .The material was allowed to dry. A drop of diluted Giemsa stock solution (6:1) was put on the material. The material was covered with a cover glass and sealed temporarily for observation as per the routine procedure.

Sperm viability count [21]

Sperm viability was studied by mixing 50 µl of sperm suspension with 50 µl of eosinnigrosin stain and incubated for 30 seconds at room temperature, then a thin smear was prepared. Two such smears were prepared from each sample. The smears were air dried and examined directly under microscope. At least 200 sperms were studied at magnification of 100X under oil immersion with a bright field objective. Unstained sperms were considered as live & pink or red coloured sperm as dead.

Histological study

Epididymis were embedded in paraffin block, sectioned at 5µm thickness and stained with hematoxylin-eosin. The stained slides were scanned under high power objective of computer attached trinocular microscope. A photograph of a particular field of the concerned section was snapped. Preparation was dehydrated by graded alcohol and air dried, coated with gold, and finally observed under S-530 Hitachi SEM.

Statistical analysis

Data were expressed as Mean ± SEM. 'Analysis of Variance (ANOVA)' followed by post hoc Tukey's multiple comparison test' was used for statistical analysis of data and performed by Graph Pad InStat version 3 software. The value of $P<0.05$ was considered as the level of significance.

Result

Epididymis weight

In epididymis weight, no significant ($P>0.05$) change has been found between control/extract and control/pure group, but a significant rise has been found in between extract and pure group (Fig. 1).

Sperm count

In sperm count, a significant ($P<0.05$) change has been found in between control/extract and extract/pure group, but no significant ($P>0.05$) change have been found between control and pure group (Fig. 2).

Sperm motility

In sperm motility, a significant reduction has been found in between control/extract ($P<0.0001$) and control/pure ($P<0.05$) group. A significant ($P<0.01$) change also found in pure group compared to the extract group (Fig. 3).

Sperm viability

In sperm viability, a significant reduction has been found in between control/extract ($P<0.0001$) and control/pure ($P<0.01$) group. A significant ($P<0.001$) change also found in pure group compared to the extract group (Fig. 3).

Normal and abnormal sperm

The no. of normal sperm was significantly reduced in both the extract (P<0.0001) and pure (P<0.0001) group and also a significant (P<0.01) reduction has been found in between extract and pure group. The no. of abnormal sperm was significantly increased in both the extract (P<0.0001) and pure (P<0.0001) group and also a significant (P<0.01) change has been found in between extract and pure group (Fig. 4).

Histological studies

In the SEM study, degeneration of epididymis (qualitative study) was predominant in extract treated group than pure drug treatment in comparison to control (Fig. 5, 6, 7).

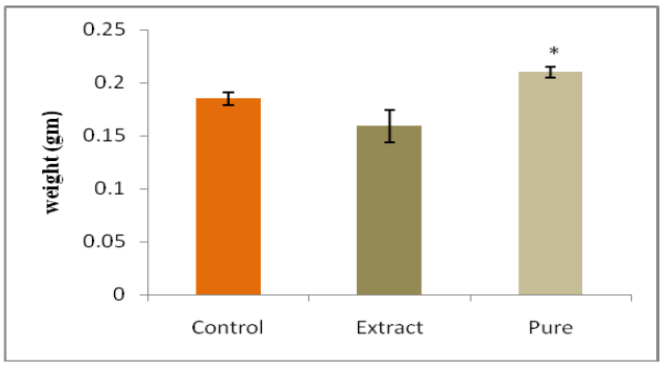


Fig. 1: Changes in percentage of epididymis weight in Male Albino Rats, Control vs. Treated With Crude Bark Extract of *Mangifera indica* and Mangiferin.

(*Values are expressed as mean ± SEM, n=6. P<0.05 is considered as significant. * P<0.05).*

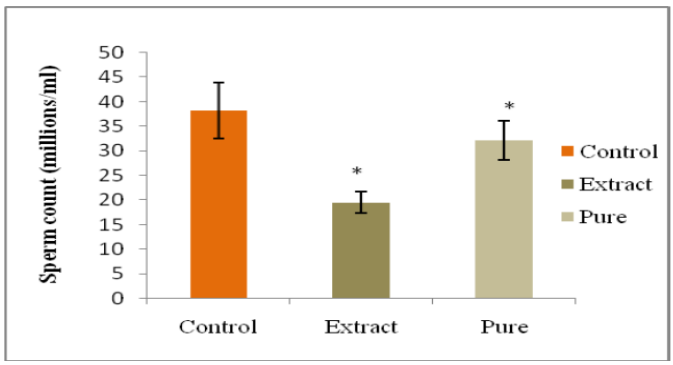


Fig. 2: Changes in Sperm count in Male Albino Rats, Control vs. Treated With Crude Bark Extract of *Mangifera indica* and Mangiferin.

*(Values are expressed as mean ± SEM, n=6. P<0.05 is considered as significant. * P<0.05, ** P<0.01, # P<0.0001).*

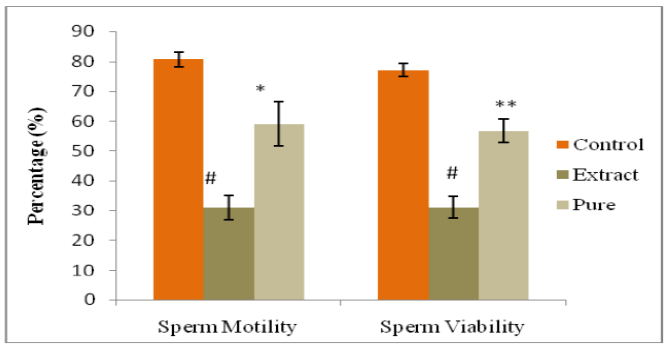


Fig. 3: Changes in sperm motility and sperm viability in Male Albino Rats, Control vs. Treated With Crude Bark Extract of *Mangifera indica* and Mangiferin.

(* P<0.05, ** P<0.01, #P<0.0001).

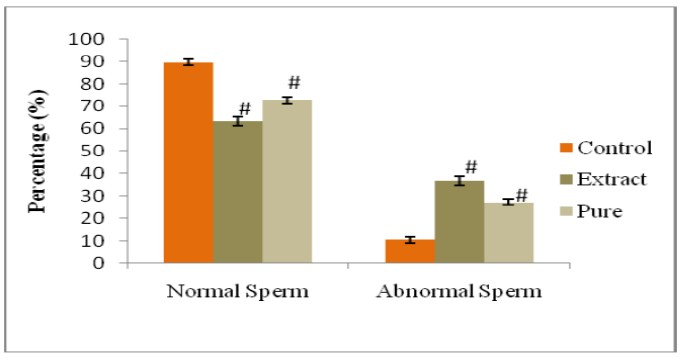


Fig. 4: Changes in percentage of normal and abnormal sperm in Male Albino Rats, Control vs. Treated With Crude Bark Extract of *Mangifera indica* and Mangiferin. (# P<0.0001).

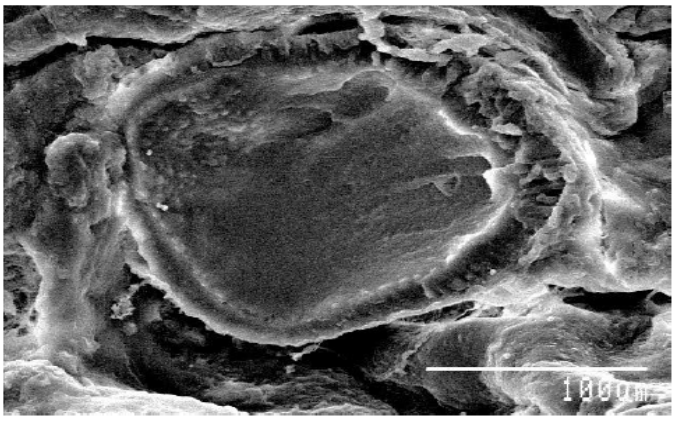


Fig 5: SEM photograph of epididymis showing

compact tubule and distinct wide epithelial lining – in rats of control group.

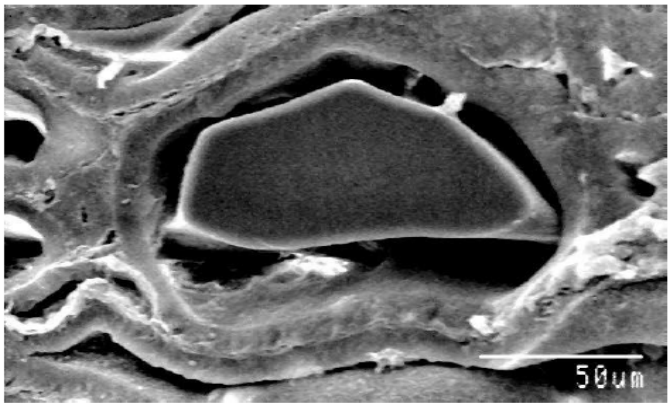


Fig 6: SEM photograph of epididymis showing tubular necrosis and indistinct epithelial lining – in rats of extract treated group.

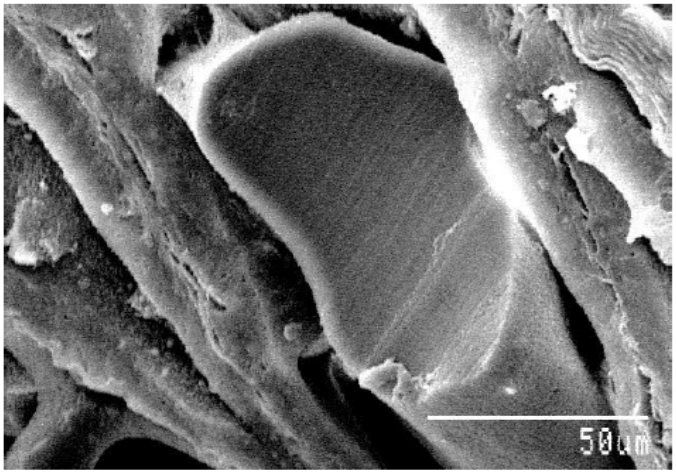
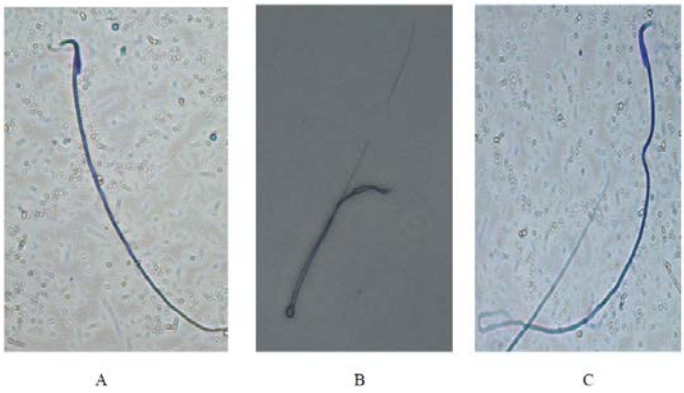


Fig 7: SEM photograph of epididymis showing less tubular necrosis and indistinct epithelial lining – in rats of pure mangiferin treated group.



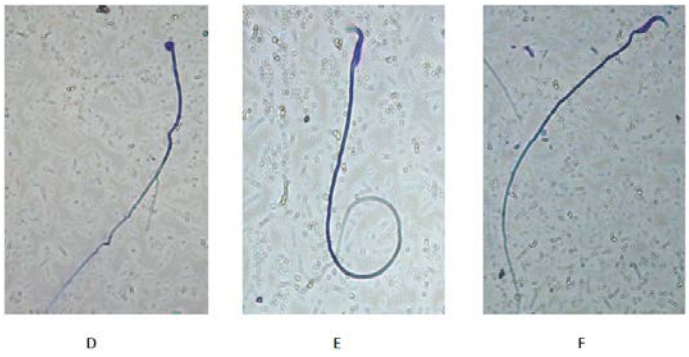


Fig 8: Gimsa staining showing normal and different abnormalities in sperm morphology.

A - Normal, B – Looped tail with bent neck, C – Bent mid piece, D – Pin head, E – Loop tail, F – Bent neck.

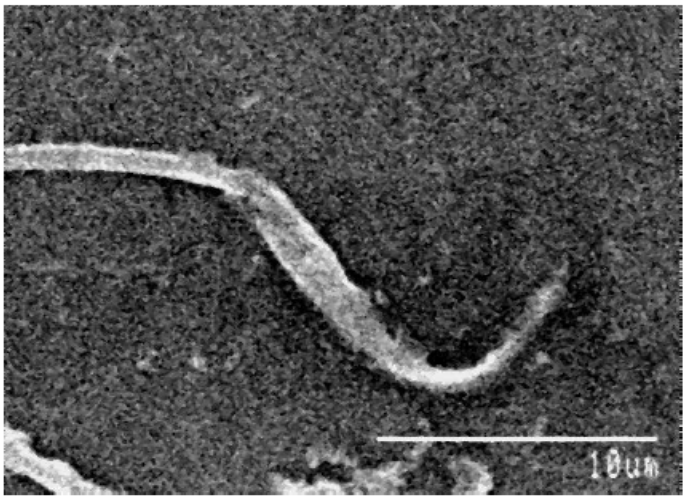


Fig 9: SEM photograph of spermatozoa showing normal gesture of head with intact acrosome.

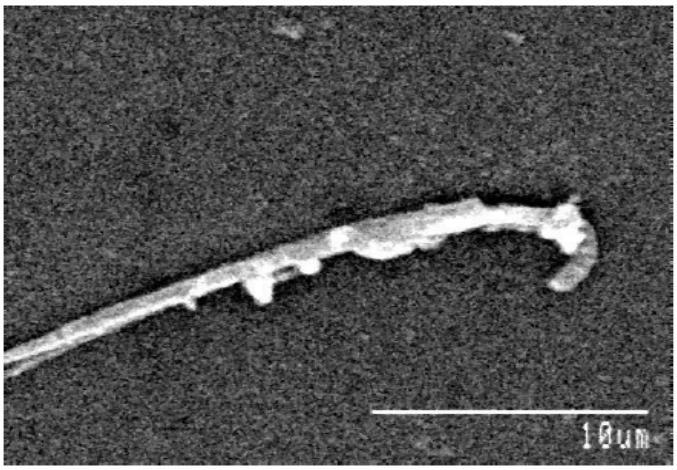


Fig 10: SEM photograph of spermatozoa showing abnormal gesture of head with partial distorted acrosome.

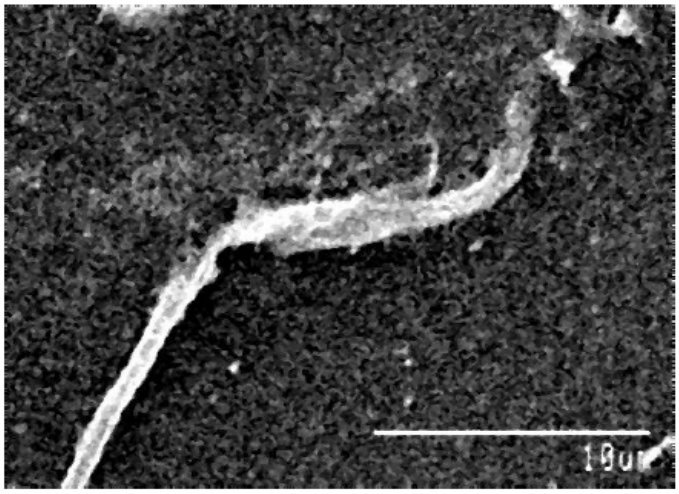


Fig 11: SEM photograph of spermatozoa showing abnormal gesture of head with distorted acrosome in head.

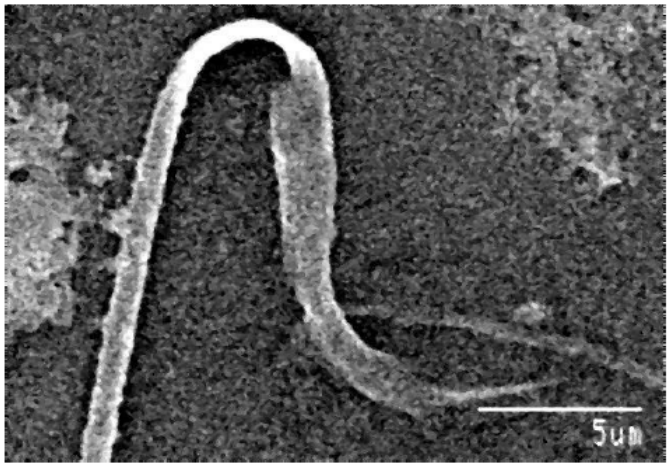


Fig 12: SEM photograph of spermatozoa showing bent neck.

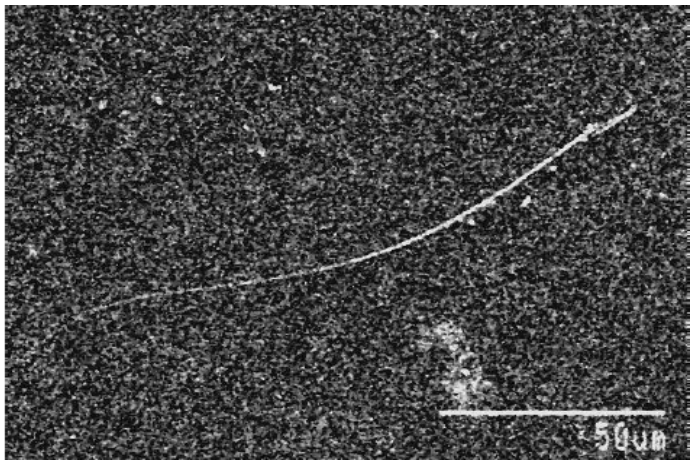


Fig 13: SEM photograph of headless spermatozoa.

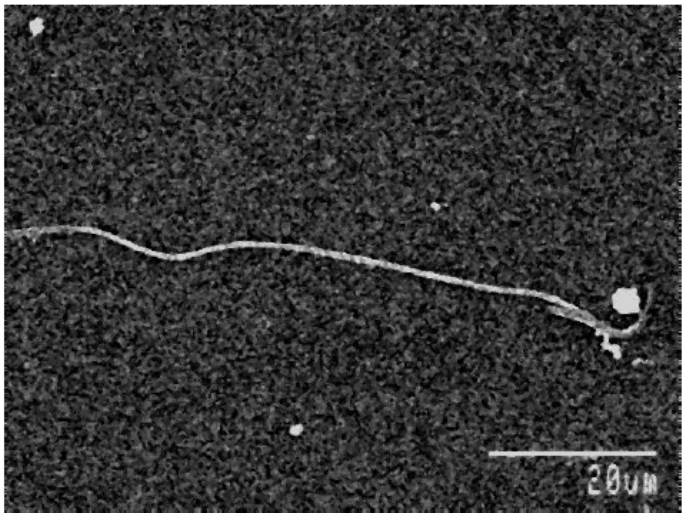


Fig 14: SEM photograph of looped tail spermatozoa.

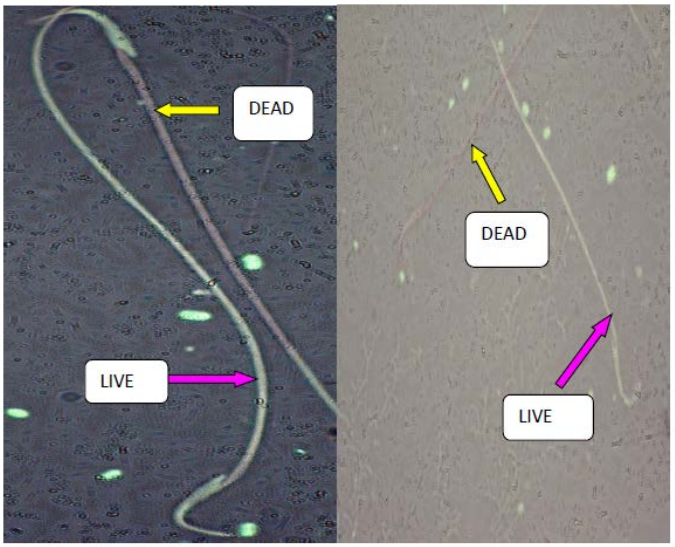


Fig 15: Eosin – Nigrosin staining shows the sperm viability.

Discussion

The present study, deals with the treatment of Mangifera bark extract (mother) and pure mangiferin at the dose of 1ml and 10mg/kg body weight/day accordingly for consecutive 14 days showed no physical sign of toxicity i.e. death, changes in the nature of stool, urine, and eye color. Animals did not express diarrhoea, hematuria, restlessness, uncoordinated muscle movements and respiratory or cardiovascular distress during treatment. The non toxic nature of both the mangifera bark and pure mangiferin [18] at present dose and duration also support

this study. The significant reduction in epididymal weight in extract treated groups of present study indicates the possible inhibition of androgen synthesis, as different investigators [22-25] reported the change in circulating androgens would affect the internal microenvironment of epididymis and thereby lead the alteration in sperm motility and metabolism. It was reported that, the reduction in sperm motility is directly related to androgen deficiency [26]. Again, here, the reduction in progressive motility of epididymal sperms may be responsible for decreased fertility , which may be due to presence of some spermatogenic inhibitory agent (mostly flavonoids) [27-30] as supported by many investigators [31-33]. It is also claimed that low level of androgen is not enough to maintain the motility, weights of gonads and accessories, which are directly androgen dependent target organs. The reduction in epididymal sperm count, viability and rise of abnormal sperm morphology in drug treated rats may be due to inhibitory activity of the extract towards spermatogenesis and steroidogenesis. It was reported that animal having more than 10% abnormal sperms are considered as infertile [34]. The androgen depletion causes reduction in sperm count, sperm motility and maturation of sperm which is highly correlative with our findings [35].

It has been proposed that sialic acid moities on sperm membrane increased negative surface charge on sperm during the epididymal transit [36]. An earlier study, supporting the same demonstrated that PCB exposure caused decreased sperm motility and count in rats [37]. Sialic acid is a sialo mucoprotein essential for the maintenance of the structural integrity of the sperm membrane and for sperm maturation [32]. Epididymal plasma contains high concentration of proteins and sialic acid which helps in sperm maturation and sperm fertilizing capacity. Therefore, reductions in sialic acid in the epididymis could be responsible for morphological abnormalities observed in cauda epididymal spermatozoa in this study.

Conclusion:

The results of the present study show that crude extract of Mangifera bark is relatively more effective in spermatogenic inhibitory activities in male rats than pure form of mangiferin. In crude bark extract, there may be other different antispermatogenic agents including mangiferin, possibly due to synergistic effect of those agents, express more inhibitory effect on male reproduction. Alone, pure mangiferin, may exerts antioxidant property, as why it shows mild stimulatory effect. But it needs further detailed studies in respect to various doses and durations of treatment.

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