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A COMPARATIVE STUDY OF MALE GONADAL ACTIVITY IN MANGIFERIN INDUCED RATS *VIS – A – VIS* CRUDE BARK EXTRACT OF MANGIFERA INDICA

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ABSTRACT

Mango tree (Mangifera sp.) is well known economic plant and its bark extract is mostly important in the field of pharmaceutical research and clinical applications. In the present study the ethanolic crude extract of mangifera bark (mother/tincture) at the dose of 0.1 ml/100gm b.wt and parallel the pure mangiferin at the dose of 1mg/0.1 ml/100gm b.wt./day has been studied in male rats for 14 days consicutively. The drugs were administrated i.p parallel with the vehicle (70% alcohol) control. The results showed no change in relative weights of testes, but the weights of ventral prostate and seminal vesicle were significantly reduced than control. The epididymal weight of pure treated group was increased significantly in comparison to extract treated group. The biochemical studies of ascorbic acid content in testes & liver was significantly reduced in all treated groups when compared to control but was increased in pure treated group but in epididymis it was significantly elevated in pure treated group in compare to control and extract treated group. The serum cholesterol level showed no change at all. The testicular cholesterol also followed the same trend but was increased in pure group in comparison to extract treatment. Again the fructose level in prostate was significantly reduced in all the treated groups, but in seminal vesicle, only a reductive change was found in pure treated group when compared to control. The SEM study supported also the above findings. As degeneration of spermatogenic cells in

testes (qualitative study) were predominant in extract treated group than pure drug treatment in comparison to control.

KEYWORDS: Mangiferin, *Mangifera indica*, Bark extract, Male Antifertility, Spermatogenesis.

INTRODUCTION

The world population explosion has pointed out the need for new, effective and safe contraceptive agents for maximum protection against fertilization. Side effects of existing synthetic contraceptives on the human body are increasingly aggressive and unpredictable at prolonged use. Research and family planning organizations have focused upon female methods of contraception for a long time because women bear a disproportionate portion of health and economic consequences of childbearing and rearing. The consequence of this long negligence for producing acceptable and reliable male contraceptives in developing countries results lower participation of males in family planning. The two most common male contraceptive methods are surgical (i.e., vasectomy) and physical barrier for sperm delivery (i.e., condom). Therefore, it is now time to think of an alternative in the field of male contraception. Accordingly, efforts are being made to explore the efficacy of plant products as a potential male contraception.[1]

One of such common herb is *Mangifera indica*, a species of mango belongs to the Anacardiaceae family, which is commonly found in our country.[2] The bark extract of *Mangifera indica* has been reported to possesses hepatoprotective[3], anticancer[4], analgesic & anti-inflammatory[5], antidiabetic[6,8], antioxidant[9,11], anti-tumor- anti- HIV[12], immunomodulatory[13], anti-microbial[14], diuretic[15] activities. But it's use in reproduction especially in male, are not reported. Mangiferin is a naturally occurring C- glycosyl xanthone extracted from the bark of Mangifera indica Linn. (Mango; Anacardiaceae).[16] Mangiferin is present in leaves[17], fruits, stem and roots[18] of M. indica. It has cardiotonic and diuretic properties.[19] Mangiferin rich plants are widely used medicinal plants in India for the treatment of immuno-deficiency diseases such as arthritis, diabetes, hepatitis, cardiac and mental disorders.[20] Being a polyphenolic antioxidant, mangiferin has strong antioxidant, antilipid peroxidative, immunomodulatory, cardiotonic, hypotensive, antidegenerative, antidiabetic activities[21] and also increases the rate of erythropoiesis.[22]

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 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.

Blood and tissue collection

After completion of the experimental schedule, all the animals were subjected to light ether anaesthesia after recording the body weight. Blood was collected from dorsal aorta of each animal just after anaesthesia and serum was separated by centrifugation at 3000g for 5min and stored at 20˚C for testosterone assay. Then animals were decapitated one by one using sharp knife. Reproductive organs i.e., testes, epididymis and seminal vesicles were dissected out. Fat and other connective tissues were removed from the surface of the organs and wet weights of these organs were recorded. Left testis of each rat was kept at 20˚C for enzymatic study and the right testis of each animal was placed in Bouin's fluid for histological study.

Estimation of serum and testicular cholesterol

Serum cholesterol level estimation was performed by Abell, Levy, Brodie and Kendall method.[23] Spectrophotometric estimation of testicular cholesterol level was performed using the protocol of the modified method of ZAK as described by Francy and Amador.[24]

Assessment of total Ascorbic acid in tissues

Spectrophotometric estimation of testicular, epididymal, liver ascorbic acid level was performed using the protocol of the method of Roe and Kuether.[25]

Estimation of fructose content in seminal vesicle and prostate

Spectrophotometric estimation of fructose content in seminal vesicle and prostate was performed using the protocol of the modified method of Roe.

Histological study

Testes 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 In Stat version 3 software. The value of $P<0.05$ was considered as the level of significance.

RESULT

Organ weight

The relative weights (gm) of the testes in all treated groups when compared to control showed no significant change (Table 1, Fig. 1). In relative weight (gm) of prostate, a significant ($P<0.05$) reduction has been found in extract and pure group in respect to control group, but no significant ($P>0.05$) change have been found between extract and pure group (Table 1, Fig. 1). In relative weight (gm) of epididymis, but a significant ($P<0.05$) rise have been found in pure group in comparison to extract group (Table 1, Fig. 1). In relative weight (gm) of Seminal vesicle, a significant reduction have been found in between control group

and extract group (P<0.001) and control and extract group (P<0.05), but a significant (P<0.05) rise have been found extract and pure group (Table 1, Fig. 1).

Serum and Testicular Cholesterol

Serum cholesterol level showed no significant change in treated groups when compared with control group (Table 2, Fig. 2). In testicular cholesterol level, no significant (P>0.05) change have been found in between control/extract, control/pure group, but a significant (P<0.01) rise occurs in pure group in comparison to extract group (Table 2, Fig. 3).

Ascorbic acid

In testicular ascorbic acid level in different groups, a significant reduction has been found in between control/extract (P<0.05), control/pure group (P<0.01), but no significant (P>0.05) change has been found in pure group in comparison to extract group (Table 2, Fig. 4). In epididymal ascorbic acid level, no significant (P>0.05) change have been found in extract group in comparison to control group, but a significant elevation have been found in between control/pure group (P<0.01), extract/pure group (P<0.05) (Table 2, Fig. 4). In liver ascorbic acid level, a significant reduction have been found in between control/extract group (P<0.05), control/pure group (P<0.05), but no significant (P>0.05) change have been found in between extract/pure group (Table 2, Fig. 4).

Fructose content

In fructose level of seminal vesicle, no significant (P>0.05) change have been found in sbetween control/extract group, extract/pure group, but a significant reduction have been found in pure group (P<0.01) in comparison to control group (Table 2, Fig. 5). In fructose level of prostate, a significant reduction have been found in between control/extract group (P<0.001), control/pure group (P<0.001), but no significant change have been found in pure (P>0.05) in comparison to extract group (Table 2, Fig. 5).

Histological studies

In the SEM study, degeneration of spermatogenic cells in testes (qualitative study) were predominant in extract treated group than pure drug treatment in comparison to control.

Table 1: Relative Weights of Reproductive Organs of Male Albino Rats Control vs. Treated With Crude Bark Extract of *Mangifera indica* and Mangiferin.

Relative weights of reproductive organs (gm/100)gm body wt)	Control	Extract	Pure
Testes	1.235± 0.046	1.245±0.029	1.420±0.070
Ventral Prostate	1.176±0.105	0.110±0.014*	0.113±0.018*
Epididymis	0.193±0.005	0.166±0.013	0.205±0.004*
Seminal Vesicle	0.546±0.026	0.278±0.024#	0.416±0.036*

*Values are expressed as mean ± SEM, n=6. P<0.05 is considered as significant. * P<0.05, # P<0.001.*

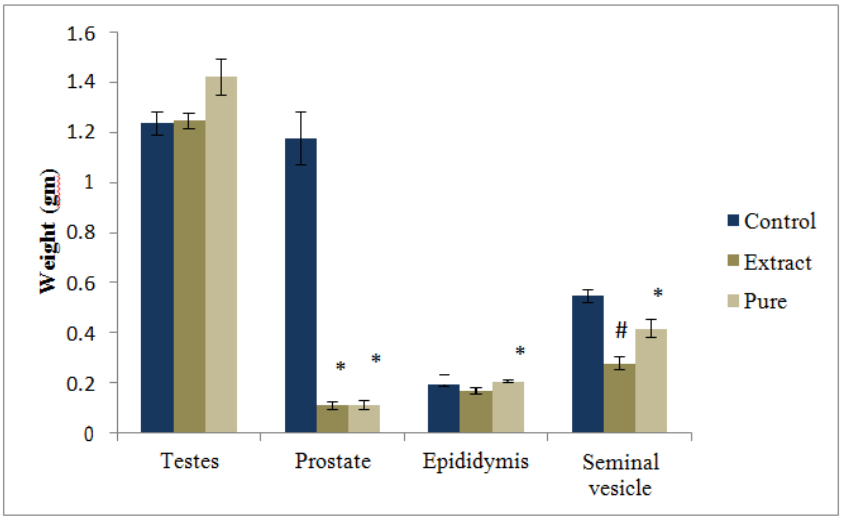

Fig. 1: Relative weights (gm) of different reproductive organs in different groups. (*P<0.05, # P<0.001).

Table 2: Changes in Biochemical Parameters in Male Albino Rats Control vs. Treated With Crude Bark Extract of *Mangifera indica* and Mangiferin.

Biochemical parameters	Control	Extract	Pure
Serum cholesterol	74.778±4.036	84.650±3.577	70.382±4.287
Testicular cholesterol	4.398±0.176	3.840±0.092	5.338±0.480**
Testicular ascorbic acid	0.170±0.023	0.111±0.013*	0.077±0.002**
Epididymal ascorbic acid	0.069±0.066	0.074±0.005	0.106±0.008**
Liver ascorbic acid	0.060±0.002	0.046±0.004*	0.044±0.001*
Fructose content in seminal vesicle	5.415±0.634	4.292±0.153	2.992±0.224**
Fructose content in Prostate	9.960±0.819	3.705±0.331#	4.705±0.371#

*Values are expressed as mean ± SEM, n=6. P<0.05 is considered as significant. * P<0.05, ** P<0.01, # P<0.001.*

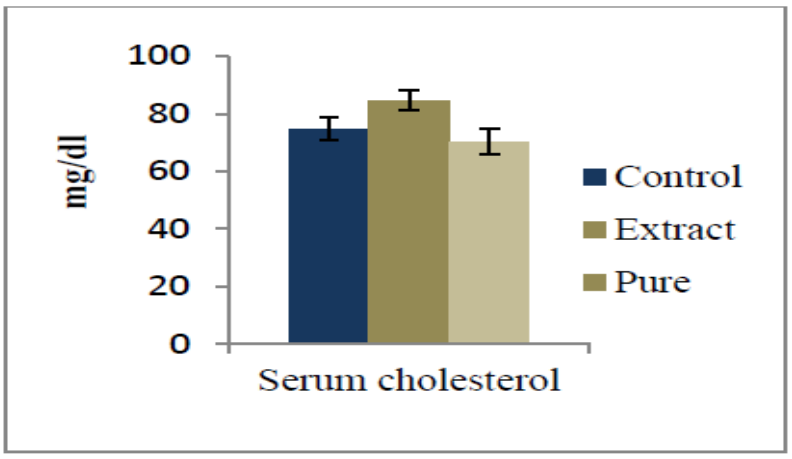

Fig. 2: Changes in serum cholesterol level cholesterol in different groups.

No significant (P>0.05) change has been found.

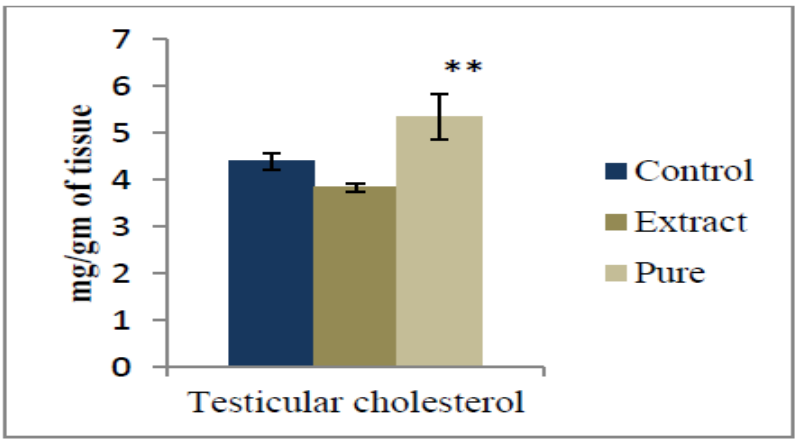

Fig. 3: Changes in testicular in different groups.

(** P<0.01).

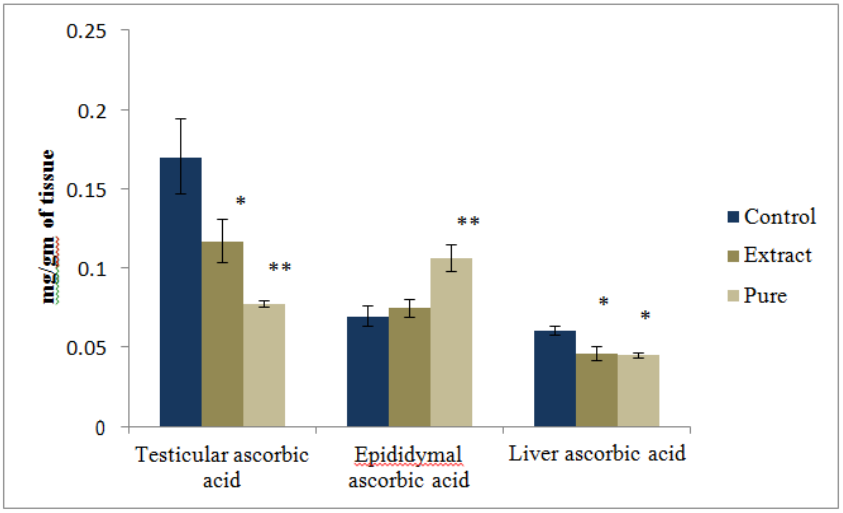

Fig. 4: Ascorbic acid level in testes, epididymis and liver in different groups.

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

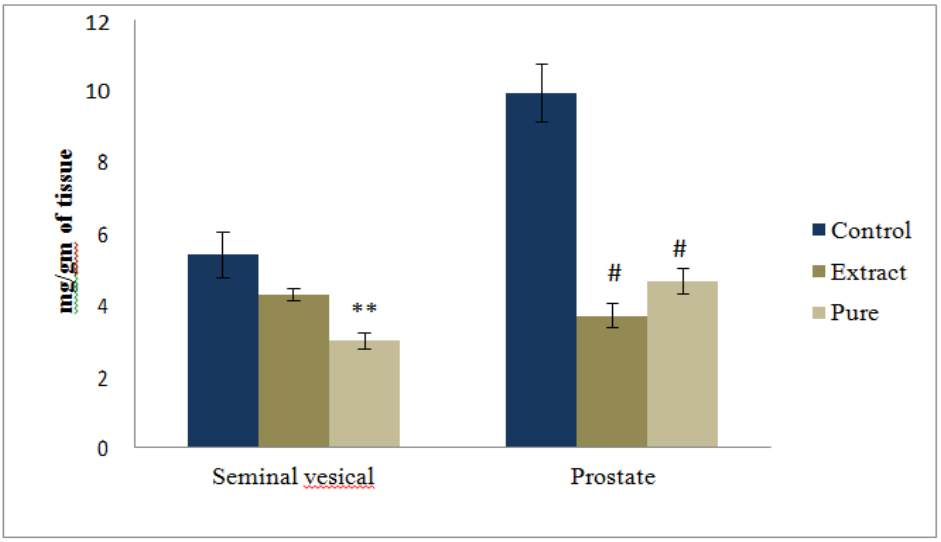

Fig. 5: Fructose content in seminal vesicle and prostate in different groups.

(** P<0.01, # P<0.001).

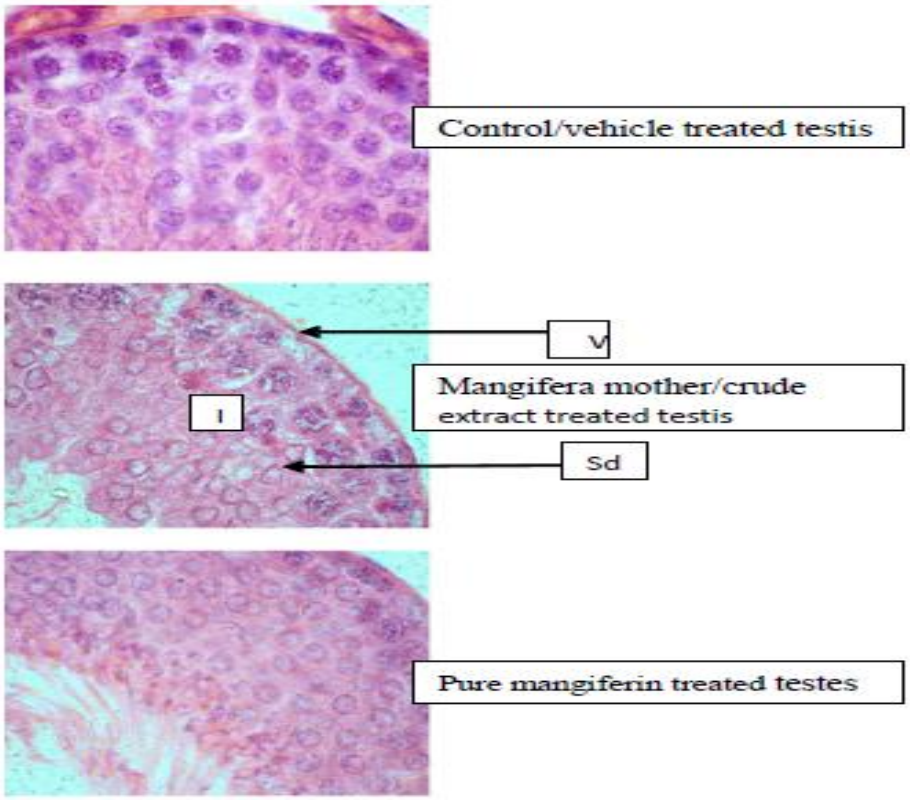

Fig. 6: Eosin and Hematoxylene stained testes (TS view). I = large intracellular space, V = Vaculation, Sd = reduced no. of spermatids.

SEM photograph of TS of testis showing Seminiferous tubules and Leydig cell's space.

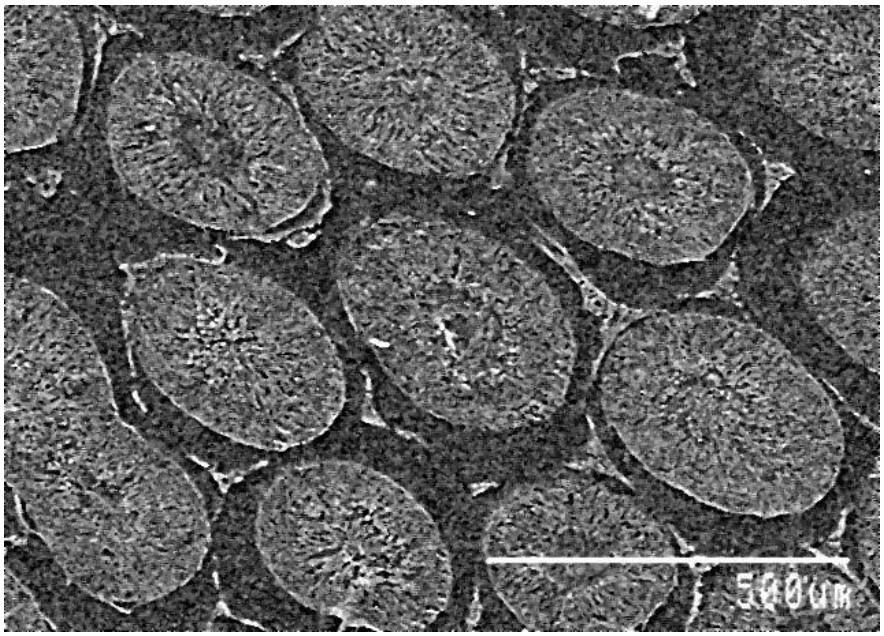

Fig. 7: SEM photograph of TS of testis with seminiferous tubules of control/vehicle treated rat, showing the normal spermatogenic pattern with intact tunica albugenia and compactness of cell mass with normal Leydig cell's space.

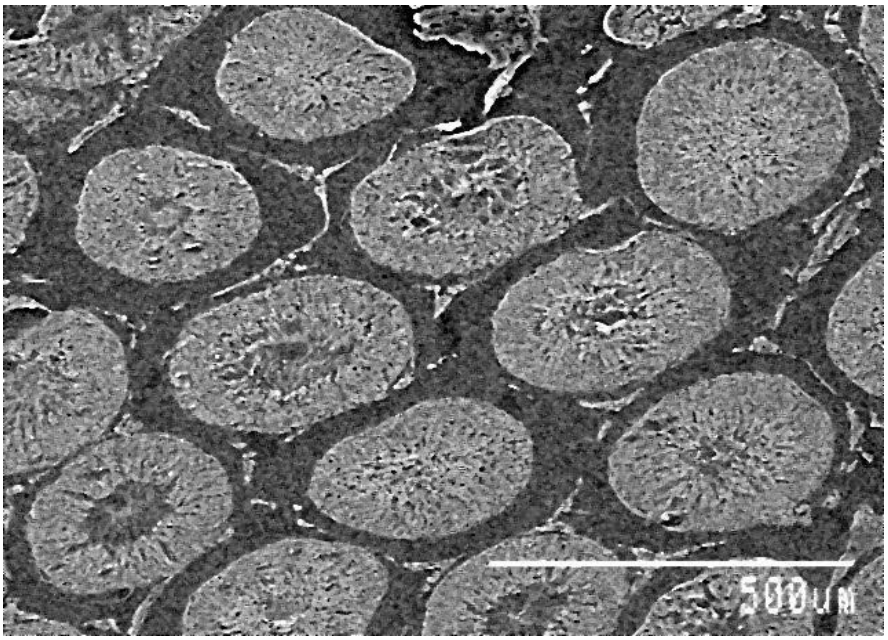

Fig. 8: SEM photograph of TS of testis with seminiferous tubules of treated (crude extract/mother of mangifera bark) rat, showing the reduced spermatogenic pattern with necropsiesed tunica albugenia and loose compactness of cell mass. Leydig cell's spaces are mildly increased.

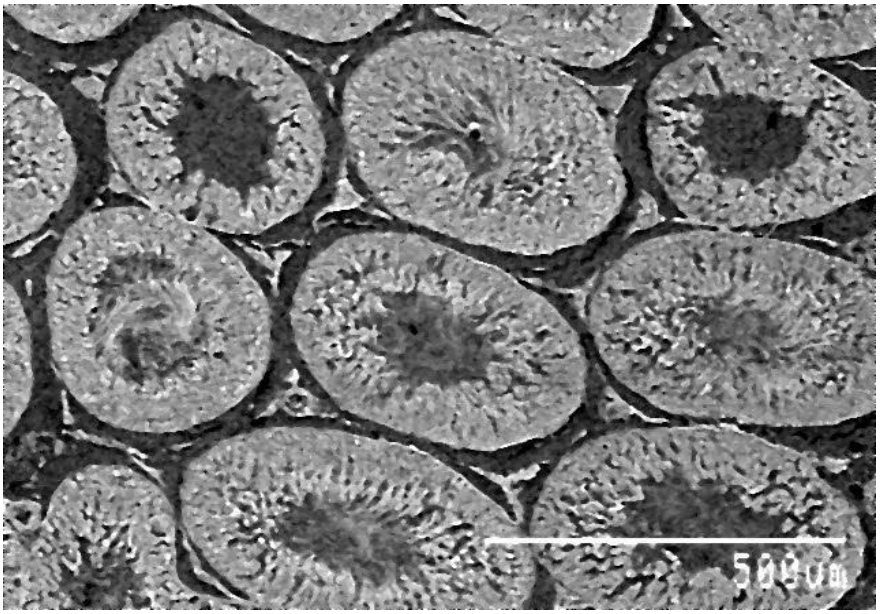

Fig. 9: SEM photograph of TS of testis with large seminiferous tubules of treated (pere mangiferin from mangifera bark) rat, showing large luminal space. Compactness of spermatogenic cells are near to normal. Leydig cell's spaces are reduced than extract treated rat.

SEM photograph of TS of a single Seminiferous tubule – enlarged view.

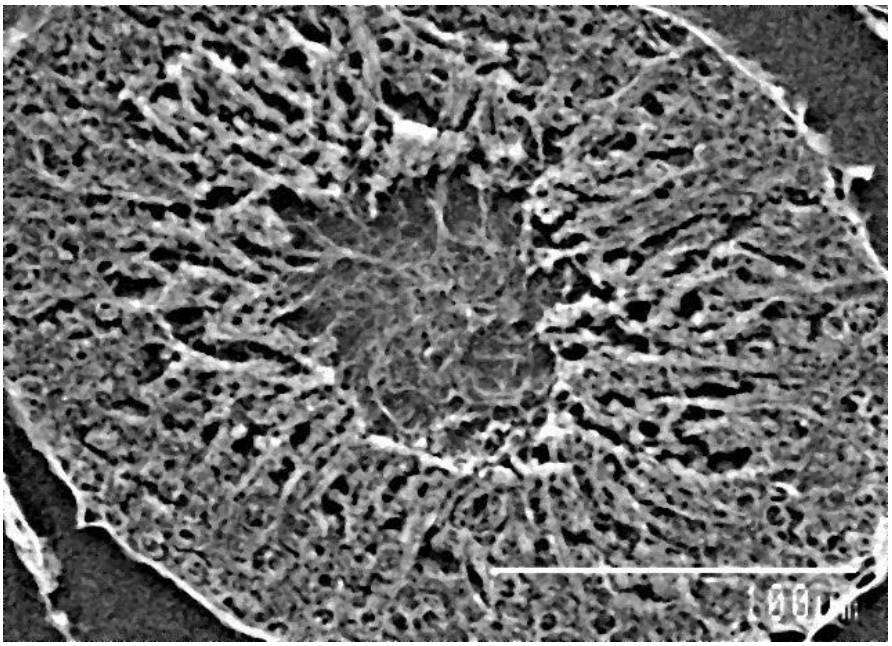

Fig. 10: SEM photograph of TS of a single seminiferous tubule in the testis of control/vehicle treated rat, showing the normal spermatogenic pattern with intact tunica albugenia and compactness of cell mass.

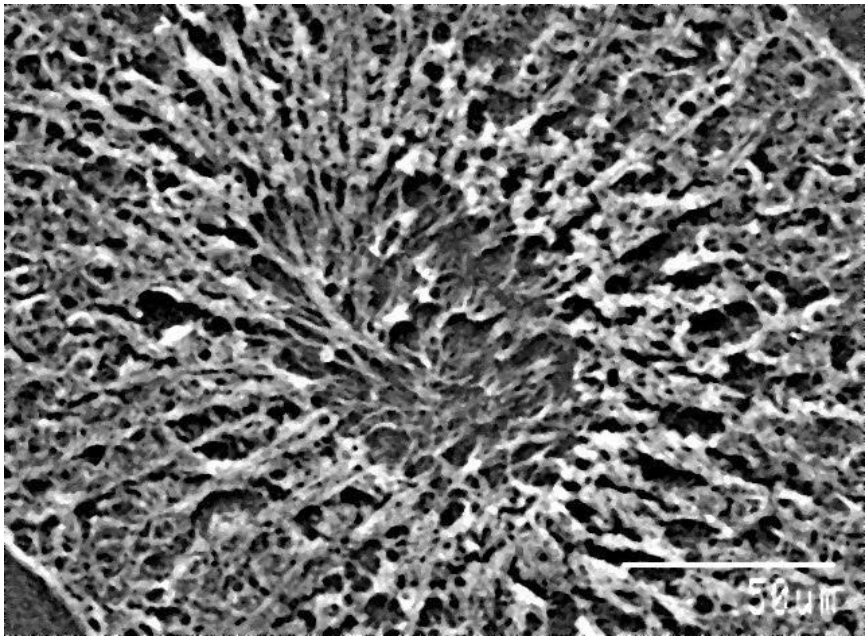

Fig. 11: SEM photograph of TS of a single seminiferous tubule in the testis of mangifera crude/mother treated rat, showing the reduced spermatogenic pattern with partial distorted tunica albugenia and loose cell mass.

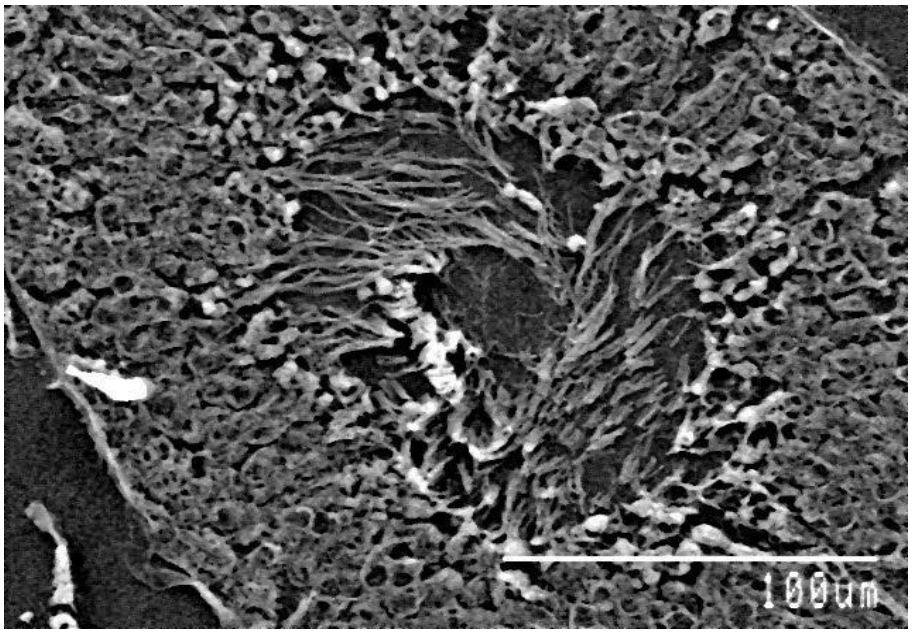

Fig. 12: SEM photograph of TS of a single seminiferous tubule in the testis of pure mangiferin treated rat, showing the spermatogenic pattern mostly normal with intact tunica albugenia and compactness of cell mass.

DISCUSSION

In the present study, the i.p administration of crude extract and pure mangiferin at the dose of 0.1ml in 1mg/100gm b.wt/day for 14 days consecutively to mature male albino rats caused no

significant change relative weights of testes showing the possibility of non toxicity of the drugs in a dose and duration dependent manner.[26,27]

The possibility of nontoxicity in extract and pure drug treated group was also supported by no change in serum cholesterol.

In other way, the significantly reduction in the wt. of ventral prostate and seminal vesicle indicates the antigonadal property of these treated compounds, because the activities of secondary sex organs and directly androgen dependent.[28] So these drugs may have antiandrogenic function. This antiandrogenic nature also can be stated by the reduction of fructose content in accessory sex glands i.e v. prostate and seminal vesicle.[29,30]

The estimation of ascorbic acid content in testes and liver showed significant reduction in both treated groups in compare to control, supporting the antigonadal nature of the drugs further.[31,33] But the rise of ascorbic acid level in treated epididymis in pure treated group is not clear.

The SEM study of testicular morphology showed qualitative degeneration of spermatogenic cells, rise of interstitial space and luminant diameter of seminiferous tubule in extract treated testes indirectly or via androgen depletion cause spermatogenic inhibition.[34,35] The spermatogenic inhibition was comparatively higher in crude extract treated group than in pure mangiferin treatment.

CONCLUSION

From the above study it can be concluded that crude extract of mangifera bark or pure mangiferin is nontoxic in a dose and dependent manner. Gonadal inhibitory nature is predominant in crude extract treated group than the pure mangiferin infusion. Pure mangiferin at the present dose and duration is somehow stimulatory or less inhibitory than crude extract. Possibly, due to the antioxidative property of pure mangiferin, the mild stimulatory action on male gonads and allied has been evolved. But, lastly it requires further vivid study in multivarious dose and duration manner.

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REFERENCES

1. Ghosh A, Pakhira BP, Tripathy A, Ghosh D. Male contraceptive efficacy of poly herbal formulation contracept- TM, composed of aqueous extracts of Terminalia chebula fruit and Musa balbinosa seed in rat. Pharmaceutical biology, 2017; 55(1): 2035-2042.
2. Tharanathan RN, Yashoda HM, Prabha TN. Mango (*Mangifera indica* L.) "The king of fruits"— an overview. Food Rev. Int, 2006; 22: 95–123.
3. Rodeiro I, Donato MT, Martínez I, Hernández I, Garrido G, González-Lavaut JA, Menéndez R, Laguna A, Castell JV, Gómez-Lechón MJ. Potential hepatoprotective effects of new Cuban natural products in rat hepatocytes culture. Toxicol in Vitro, 2008; 22: 1242-1249.
4. Garrido G, Blanco-Molina M, Sancho R, Macho A, Delgado R, Muñoz E. An aqueous stem bark extract of *Mangifera indica* (Vimang®) inhibits T cell proliferation and TNF-induced activation of nuclear transcription factor NF-*κ*B. Phytotherapy Research, 2005; 19(3): 211–215.
5. Garrido G, Gonzalez D, Lemus Y, Garcia D, Lodeiro L, Quintero G, Del- porte C, Nuñez-Selles AJ, Delgado R. In vivo and in vitro anti-inflammatory activity of Mangifera indica L. Extract. Pharmacol Res, 2004; 50: 143-149.
6. Jain C, Singh A, Kumar P, Gautam K. Anti diabetic potential of flavonoids and other crude extract of stem bark of *Mangifera indica* L. a compare study. Journal of scientific and innovative research, 2014; 3(1): 21-27.
7. Bhowmik A, khan LA, Akhter M, Rokeya B. Studies on antidiabetic effect of *Mangifera indica* stem barks and leaves on non diabetic, type 1 & type 2 diabetic model rats. Bangladesh journal of pharmacology, 2009; 4(2): 110-114.
8. Ojewole J. Anti-inflammatory, analgesic and hypoglycemic effects of *Mangifera indica* Linn. (Anacardiaceae) stem-bark aqueous extract. Methods Find Exp Clin Pharmacol, 2005; 27: 547-54.
9. Thomas S, jose Ck, Mathew L, Akbar R, Varghese JS, Fathma J. screening of aqueous extract of mangifera indica leaves for invitro antioxidant activity. inventi journal. 2013; 806(13).
10. Garrido G, Gonzalez D, Romay C, Nuñez-Selles AJ, Delgado R. Scavenger effect of a mango (*Mangifera indica* L.) food supplement's active ingredient on free radicals produced by human polymorphonuclear cells and hypoxanthine–xanthine oxidase chemiluminescence systems. Food Chem, 2008; 107(3): 1008–1014.
11. Ribeiro SMR, Queiroz JH, Antioxidant in Mango (*Mangifera indica*L.) Pulp. Plant

Foods for Human Nutrition. 2007; 62(1): 13-17.

12. Percival S, Talcott S T., Chin S T., Mallak A C., Lounds-Singleton A, and Pettit- Moore J. Neoplastic Transformation of BALB/3T3 Cells and Cell Cycle of HL-60 Cells are Inhibited by Mango (*Mangifera indica* L.) Juice and Mango Juice Extracts. J. Nutr. 2006; 136(5): 1300-1304.
13. Makare N, Bodhankar S, Rangari V. Immunomodulatory activity of alcoholic extract of *Mangifera indica* L. in mice. J. Ethnopharmacol, 2001; 78(2–3): 133–137.
14. Akinpelu DA, Onakoya TM. Antimicrobial activities of medicinal plants used in folklore remedies in south-western. African Journal of Biotechnology, 2006; 5(11): 1078-1083.
15. Shree Devi M.S. Acute toxicity and diuretic activity of *Mangifera indica* L. bark extracts. International Journal of Pharma and Bio Sciences, 2011; 2(3): 141-146.
16. Ansari MA, Reddy KK, Sastry KNS & Nayudamma Y. Dicotyledonae anacardiaceae. Polyphenols of Mangifera indica. Phytochemistry, 1971; 10: 2239.
17. Dar A, Faizi S, Naqvi S, Roome T, Zikr-ur-Rehman S, Ali M, Firdous S & Moin S T. Analgesic and antioxidant activity of mangiferin and its derivatives: The structure activity relationship. Biol Pharm Bull, 2005; 28: 596.
18. Nunez Selles A J, Velez Castro HT, Aguero-Ageuro J, Gonzalez-Gonzalez J, Naddeo F, De Simone F & Rastrelli L. Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. J Agri Food Chem, 2002; 50: 762.
19. Ghosal S, Rao G, Saravanan V, Misra N & Rana D. A plausible chemical mechanism of the bioactivities of mangiferin, Indian J Chem, 1996; 35 B: 561.
20. Ichiki H, Miura T, Kubo M, Ishihara E, Komatsu Y, Tanigawa K & Okada M. New antidiabetic compounds, mangiferin and its glucoside. Biol Pharm Bull, 1998; 12: 1389.
21. Andreu GP, Delgado R, Velho JA, Curti C & Vercesi AE. Iron complexing activity of mangiferin, a naturally occurring glucosylxanthone, inhibits mitochondrial lipid peroxidation induced by Fe^{2+} citrate. Eur J Pharmacol, 2005; 47: 513.
22. Das A, Bose C, Ghosal S, Chakraborty I, Pradhan N. Effect of pure mangiferin vis a vis crude ethanolic extract from mangifera indica bark on haematological parameters in male rats. World journal of pharmaceutical research, 2014; 3(9): 1390-1403.
23. Abell LL, Levy BB, Brodie BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J. Biol. Chem, 1952; 195: 357-366.
24. Francy, Amador. Determination Of Total Cholesterol From Testes Modified Method Of

ZAK, 1968.

25. Roe JH, Kuether CA. Estimation of total ascorbic acid from tissue. J. Biol. Chem, 1943; 147: 399-407.
26. Pradhan NK, Ghosal S, Chakraborty I. *Jussiae repens* is a nontoxic antigonadal herb— A dose dependent study on male rats. Int J Pharm Bio Sci, 2013; 4(2): 131-143.
27. Thanabhorn S, Jaijoy K, Thamaree S, Ingkaninan K, Panthong A. Acute and subacute toxicity study of the ethanol extract from Lonicera japonica thumb. Ethnopharmacol, 2006; 107: 370-373.
28. Gutman AB, Gutman EB. Estimation of "acid phosphatase" activity of blood serum. J Biol Chem, 1940; 136: 201–209.
29. Ostrowki W. In "Male Accessory Sex Gland, Biology and pathway", Eds. Mills and E.S.F. Hafez. Elesevier, North Holland Biochemical Press, N.Y, 1980; 4: 1197.
30. Chakraborty I, Ghosal S, Pradhan N. *Jussiaearepens* (L) Acts As Antifertility Agent-A Search for Herbal Male Contraceptive. Int.J Pharm Sci Rev Res, 2014; 24(2): 288-296.
31. Frunder H, Blume E, Kluge H. Biochem, Biophys. Acta, 1962; 65: 146.
32. Deane H W. Histochemical observations on the ovary and oviduct of the albino rat during the estrous cycle. Am J Anat, 1952; 91: 363-393.
33. Pradhan NK, Deb C. Thimet induced biochemical changes on testicular activities in rats. Indian Biologists, 1995; 27(2): 30–34.
34. Steinberger E. Hormonal regulation of the seminiferous tubuli function. Curr. Top. Mol. Endocrinol, 1975; 2: 337-52.
35. Chowdhury AK. Dependence of testicular germ cells on hormones: a quantitative study in hypophysectomized testosterone-treated rats. J. Endocrinol, 1979; 82: 331– 340.