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INHIBITION OF SPERMATOGENESIS WITH THE TREATMENT OF 50% METHANOLIC EXTRACT OF *MAYTENUS EMARGINATA* LEAVES IN ALBINO RATS

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ABSTRACT: The plant *Maytenus emarginata* belongs to the Family Celastraceae also known as Kankhera in Hindi have used effectively for the treatment of wide range of disorder as digestive ailments, nerve afflictions, Heart ailments, inflammation, nervous system, Rashes and as a tonic and anti stress. The present investigation is planned to evaluate safety and reversible contraceptive efficacy of aqueous methanolic extracts of *Maytenus emarginata* leaves to develop a orally effective and reversible male contraceptive. Animal were orally administrated with extract of *Maytenus emarginata* leaf at the dose of 50,100,200 mg/kg/ body wt/day per oral dose for 60 days. A marked dose dependent decrease in the count and motility of the sperms of treated rats was observed compared with control. Furthermore, pregnancy rate in females markedly reduced by 80% after the mating with male treated with 50,100,200 mg/kg *Maytenus emarginata* leaf extract. At the same time significant decrease were detected in seminal vesicles and testicular weight of rats that received.

INTRODUCTION: Now a day's world population has crossed 7 billion, while India population is around 1.27 billion. [1] In fact uncontrolled human population is one of the critical problems of the developing countries like India, because it creates many socio-economic problems leading to negative impact on environment and development. Therefore, the control of human fertility is the only means to sustainable development. Although, many contraceptive methods have been investigated to control human fertility includes Natural, chemical, hormonal and immunological approaches.

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Saponins are natural Surfectant widely occurring in many plants (*phytolacca dodecarda, calendula officinalis and acacia cassia*) are reported to have spermicidal actions. [2] However, no suitable method has emerged that is effective and free from side-effect. [3] Contraceptives containing estrogen and progesterone have been used effectively in women, but due to some risk factors associated to these, triggered the need to develop a new safe method.

A large number of plants species have been explored around the world including India and China in search to develop a male contraceptive agent because humans relied on plant products as sources of therapeutic agents without causing any side effects. [4-8] The plants have been explored to control fertility or abortion includes *carrica papaya, cassia occidentalis, citrullus colocynthis, daucus carota, gossypium herbaceum* etc. [9] In this search *Maytenus emarginata* have antifertility properties which are described in Ayurveda. The

ethanolic extract of *Maytenus emarginata* fruit was believed to suppress "Kam Vasna" (desire of sex). It was consumed by Sanyasees in shrines and the pupils studying in Gurukul for the same purpose. [10-12] The methanolic extract of *Piper betle* was also found having antifertility activity in female albino rats. [13] Therefore, the present preliminary study has been undertaken to investigate the reversible contraceptive efficacy and safety of the aqueous methanolic extract of *Maytenus emarginata* stem and leaves in male albino rats. [5, 3, 14] The development of new fertility regulating drug from medicinal plants is an attractive proposition, because from times immemorial humans have relied on plants and their products as sources of drugs and therapeutic agents [15].

MATERIALS AND METHODS:

Plant:

The plant *Maytenus emarginata* belongs to the Family Celastraceae also known as Kankhera in Hindi have used effectively for the treatment of wide range of disorder as digestive ailments, nerve afflictions, Heart ailments, inflammation, nervous system, Rashes and as a tonic and anti stress. The ethanolic extract of *Maytenus emerginata* has been also found having reversible contraceptive efficacy in male albino rats. [16]

Identification, Collection and preparation of the extract:

Plant of *Maytenus emarginata* was collected around the Jaipur district, India. Specimens voucher was deposit at Herbarium of Department of Botany, University of Rajasthan, Jaipur for identification. The leaves of *Maytenus emarginata* was grind in a mixture then, shocked in 50% methanol for overnight, boiling it at 40 °C for 24 hour and finally filtered with gauze. The filtrate were concentrated under the reduce pressure at 50 ± 5 ºC to obtained methanolic extract of this plant for experiment. [17]

Animal Model:

Colony-bred, healthy adult (4-5 months old) male albino rats (*Rattus norvegicus*) of the Sprague-Dawley strain, weighing between 150-200gm. were used. The animals were housed in polypropylene cages, measuring 430×270×150 mm, under controlled environmental conditions with provision of a 12 h light: 12 h dark regimen. The animals were fed a platted standard rat chow supplemented with soaked gram and wheat, water will be provided ad libitum. [18]

Treatment Protocol:

Rats of similar body weight, size age were grouped as under. Experiments were carried out during the course of study, to observe antifertility effect mode of action/effects nature of the extract and reversibility effects. The animals were divided into five treatment groups (A- E), each consisting of 8 animals in each.

Group-A: The animals of this group were given vehicle (sterile distilled water) alone orally for 60 days to serves as vehicle controls.

Group-B: The animals of this group were treated with *Maytenus emarginata* extract at a dose of 50 mg/kg.b.wt./day for 60 days.

Group-C: The animals of this group were treated with *Maytenus emarginata* extract at a dose of 100 mg/kg.b.wt./day for 60 days.

Group-D The animals of this group were treated with *Maytenus emarginata* extract at a dose of 200 mg/kg.b.wt. /day for 60 days.

Group-E: The animals of this group were administrated at a dose of 100 mg/kg.b.wt./day for 60 days will be kept for a recovery period of 30 days.

Schedule of Sacrifice:

After 24 h from the last dosing/recovery for each group, the animals were weighed and sacrifice under mild ether anesthesia.

Body and Organ Weights:

On 61 day initial and final body weights of the animals were recorded. Testes, cauda epididymis, seminal vesicles and ventral prostrate were dissected out, freed from adherent tissues, and weighed to the nearest milligram on an electronic balance.

Fertility Test: Successful mating was carried out with all the animals 5 days prior to sacrifice (male

female ratio 1:2). The mated females were allowed to complete the gestation. The numbers of pups were recorded and litter size and percent fertility was calculated. [17]

Sperm Motility and Density:
For sperm motility and density was measured by routine procedure and express as millions/mm3 suspension. [18]

Serum Biochemistry:
Serum Alanine amino transaminase [19], Aspartate amino transaminase [19], Acid Phosphatases [20] and Alkaline Phosphatases. [21]

Tissue Biochemistry:
The testis, cauda epididymis, seminal vesicles and ventral prostrate were dissected out, freed from adherent tissues and weighted at nearest milligram balance. Protein [22], Glycogen. [23] Cholesterol [24], Sialic acid [25], Ascorbic acid [26] and Fructose. [27] were estimated in right side of testis and other accessory reproductive organs.

Hormone Assay:
Blood samples were also collected and serum was separated for estimation of FSH, LH and testosterone by radioimmunoassay. Serum samples were separated by standard procedures and stored at -20° C for subsequent analysis. [28]

Statistics:
Data are expressed as mean ± S.E. and analyze for statistical significance by using student's "t" test. The data were considered as significant and highly significant at $p \leq 0.001$, respectively. [29] Data exposed as Mean ± S.E., ns = non-Significant ($P< 0.05$), **Significant ($P< 0.01$), *** highly significant ($P<0.001$), analyze for statistical significance by using student's t- test for 8 animals. In which Groups 2, 3, 4 and 5 was compared with Group 1.

RESULTS:
Body and organ weight: The weight of reproductive tissues were decreased significantly with the treatment, however in recovery group non-significant changes were observed while heart, liver, kidney, adrenal and other vital organs were non-significantly affected with the dose.

Serum biochemistry:
Alkaline phosphatase, acid phosphatase, SGOT and SGPT levels in serum of all groups were non significantly changed after treatment of 50, 100 and 200mg./kg. body weight of *Maytenus emarginata* leaf extract.

Tissue biochemistry:
Protein, glycogen, cholesterol, Sialic acid and fructose levels were significantly decrease in testes, epididymides, seminal vesicle and ventral prostate while non-significant changes were observe in vital organs

Sperm motility and density:
Cauda epididymal sperm motility was significantly decline in the dose dependent manner. Sperm density was also significantly decline in dose dependent manner, but 30 days withdrawal of treatment no significantly changes were observed in recovery group.

Fertility:
Fertility was decreased in dose dependent manner in treated groups. However after 30 days withdrawal of the treatment reversible antifertility activity was observed in recovery group. All the deliver pups were normal and healthy.

Histology of testes:
Histological studies showed all events of spermatogenesis, where the lumen was filled with sperm. Sertoli and Leydig cells situated at their correct position and normal structure in control as well as recovery rat testes while the treated rat testes arrest of spermatogenesis. The seminiferous tubule appeared reduce in size. The lumen contained sloughed debris and few germ cells.

DISCUSSION: Several plants have been evaluated for their antifertility potential in the hope of developing a contraceptive for use in man.[30-36] The result of this investigation show interferes with the structure and function of major elements of male fertility by a marked decrease in the rate of fertility. *Maytenus emarginata* extract reduce male fertility as a dose dependent manner after administration of (50,100,200 mg/kg./body weight) and also decrease in counts and motility of cauda epididymal sperms. Decreased level of testosterone and LH hormones

leads to degenerative changes in testis and accessory reproductive organs resulted inhibition of sperm production and motility. The testes of treated animals were arrest of spermetogenesis. Appearance of such inclusions in the epithelial cells of the corpus epididymides is ascribed to the absence of spermatozoa in the epididymal duct. [37] Sertoli cells, spermatogonia and spermatocytes structure was disform in dose dependent manner. The significant decrease in sperm motility, density and body weight of testis, epididymis, seminal vesicle, ventral prostate and vas-deferens due to significant reduction of tissue biochemical level.

The reduction of sperm density is confirmed by histological and hormonal investigation of testis and serum along with fertility status of the animals. [38] The treatment also caused marked reduction in the level of Sialic acid in the epididymis. Since Sialic acid is a true secretory product of the epididymis. [39] In male contraception, it is not necessary to stop spermetogenesis but rather to eliminate the fertilizing ability of the spermatozoa by causing changes in morphology or in the function of sperm. [40] Vacuolization was observed in the Sertoli cells, spermatogonia and spermatocytes. [41] After treatment rather of *maytenus emarginata* leaves extract there is no significant changes was observed in serum biochemistry of treated and recovery group of animal which showed *maytenus emarginata* extract are free from side effect.

TABLE 1: FERTILITY OF MALE RATS IN CONTROL AND 50,100,200MG/KG./BODY WEIGHT TREATED WITH MAYTENUS EMERGINATA LEAF EXTRACT TREATMENT FOR 60 DAYS WHEN TREATED WITH FEMALE (MALE: FEMALE RATIO 1:2)

Treatment	No. of mated Male	No. of mated Female	Pregnant female	Fertility (%)
Group-1 (Control)	8	16	15	93.75%
Group-2 (50mg/kg.b.wt./ Day for 60 days)	8	16	12	75%
Group-3 (100mg/kg.b.wt./ Day for 60 days)	8	16	08	50%
Group-4 (200mg/kg.bt.wt./ Day for 60 days)	8	16	03	18.7%
Group-5 Recovery (withdrawal of 90 days)	8	16	15	93.75%

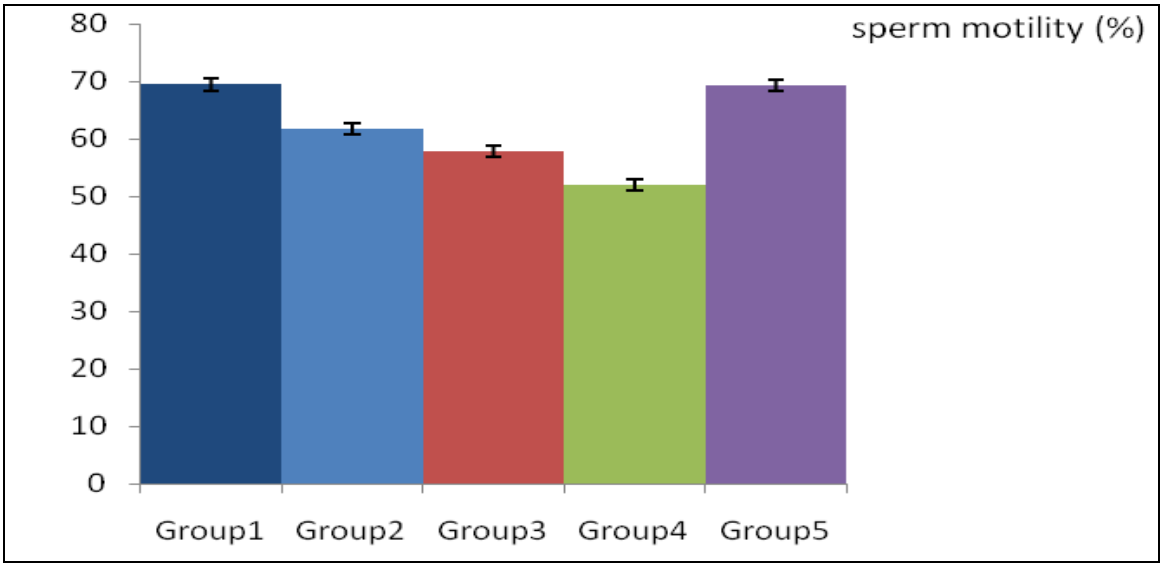


FIG.1: CHANGES IN SPERM MOTILITY OF CAUDA EPIDIDYMIS AFTER 60 DAYS TREATMENT OF *M.EMARGINATA* LEAF EXTRACT (P>0.01).

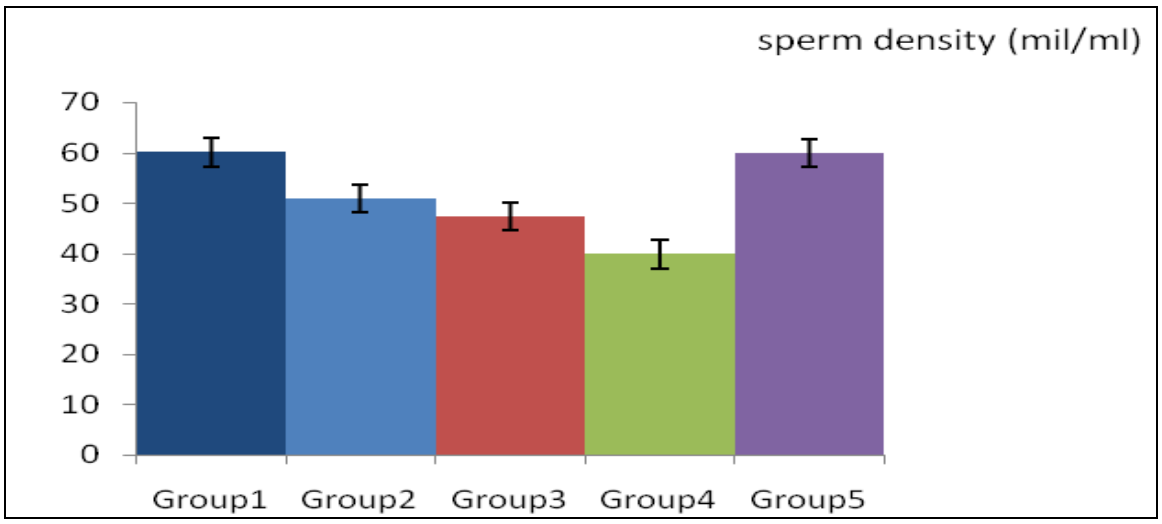


FIG. 2: CHANGES IN SPERM DENSITY OF CAUDA EPIDIDYMIDES AFTER 60 DAYS TREATMENT OF *M. EMERGINATA* LEAF EXTRACT (P>0.01)

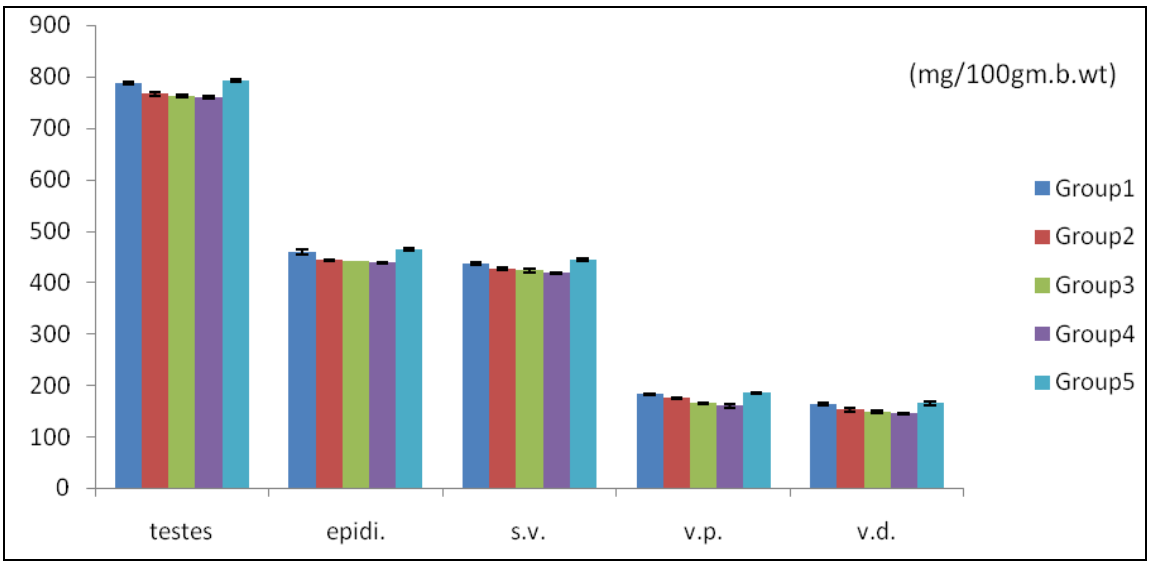


FIG. 3: CHANGES IN WEIGHT OF TESTES, EPIDIDYMIDES AND SEMINAL VESICLE,VENTRAL PROSTATE, VAS DEFERENS AFTER 60 DAYS TREATMENT OF *M.EMARGINATA* LEAF EXTRACT (P<0.01)

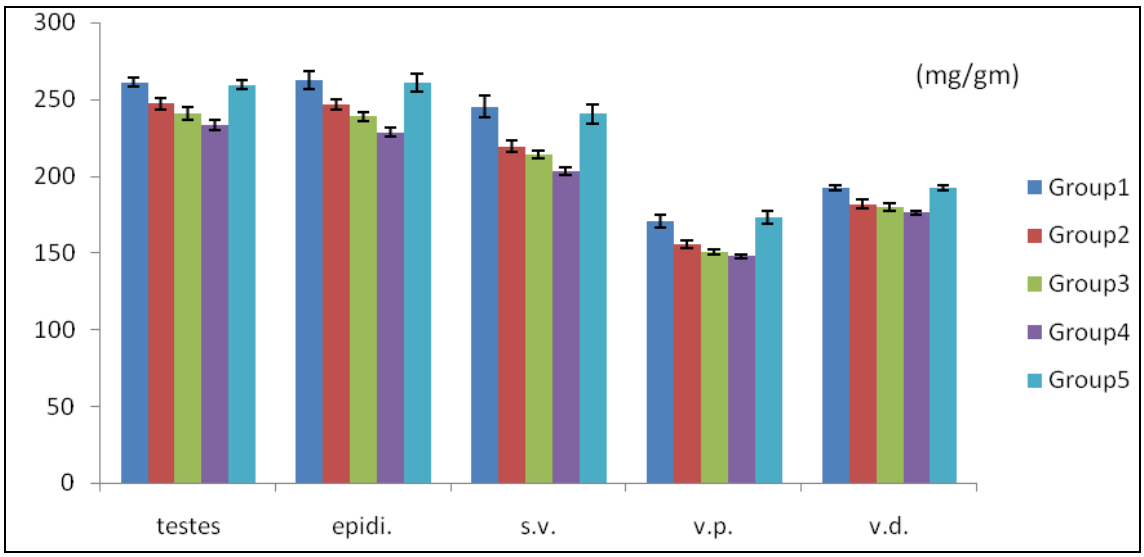


FIG.4. CHANGES PROTEIN LEVEL IN TESTES, EPIDIDYMIDES, SEMINAL VESICLE, VENTRAL PROSTATE, VAS DEFERENS AFTER 60 DAY TREATMENT OF *M.EMARGINATA* LEAF EXTRACT.

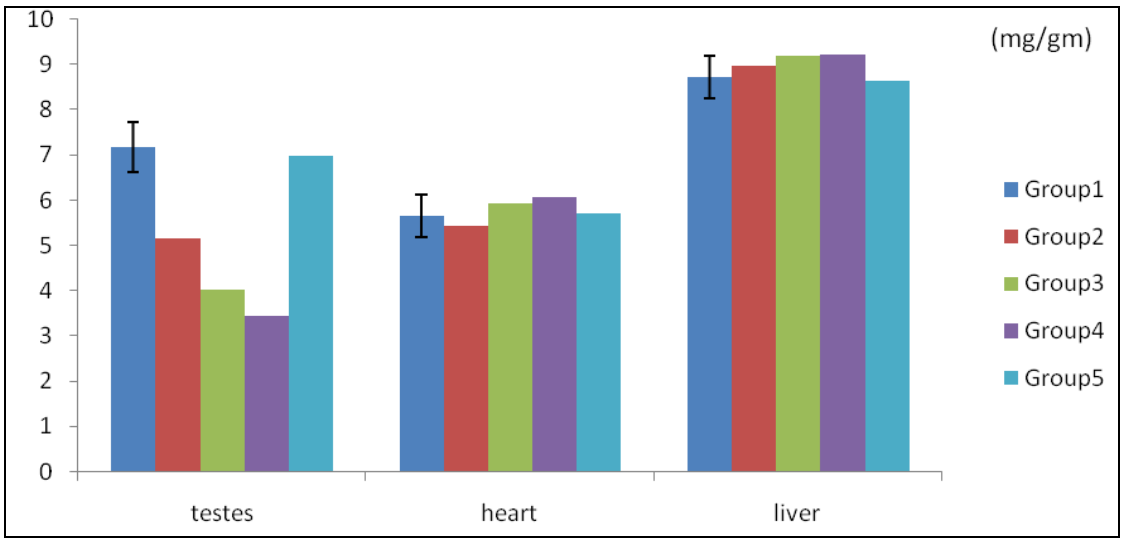


FIG. 5: CHANGES GLYCOGEN LEVEL IN TESTES, HEART, LIVER AFTER 60 DAY TREATMENT OF *M.EMARGINATA* LEAF EXTRACT.

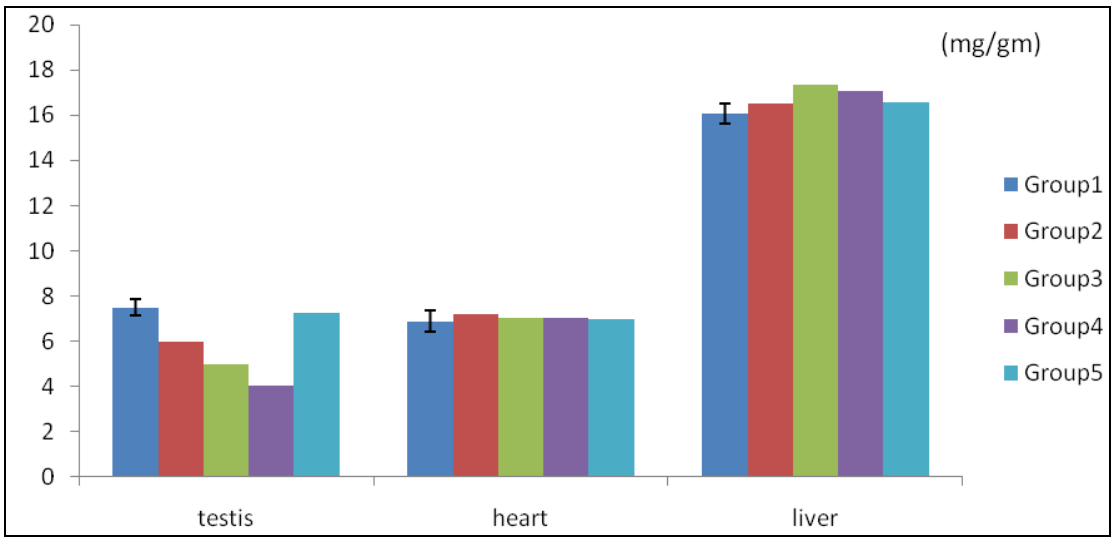


FIG.6. CHANGES CHOLESTROL LEVEL IN TESTES, HEART, LIVER AFTER 60 DAY TREATMENT OF *M.EMARGINATA* LEAF EXTRACT.

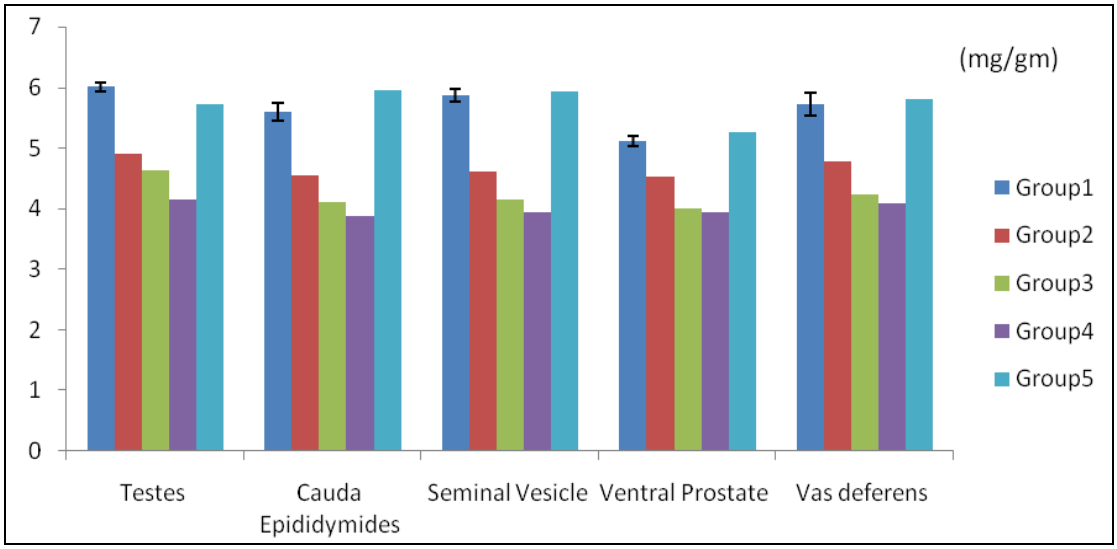


FIG.7. CHANGES SIALIC ACID LEVEL IN TESTES, EPIDIDYMIDES, SEMINAL VESICLE, VENTRAL PROSTATE, VAS DEFERENS AFTER 60 DAY TREATMENT OF *M.EMARGINATA* LEAF EXTRACT.

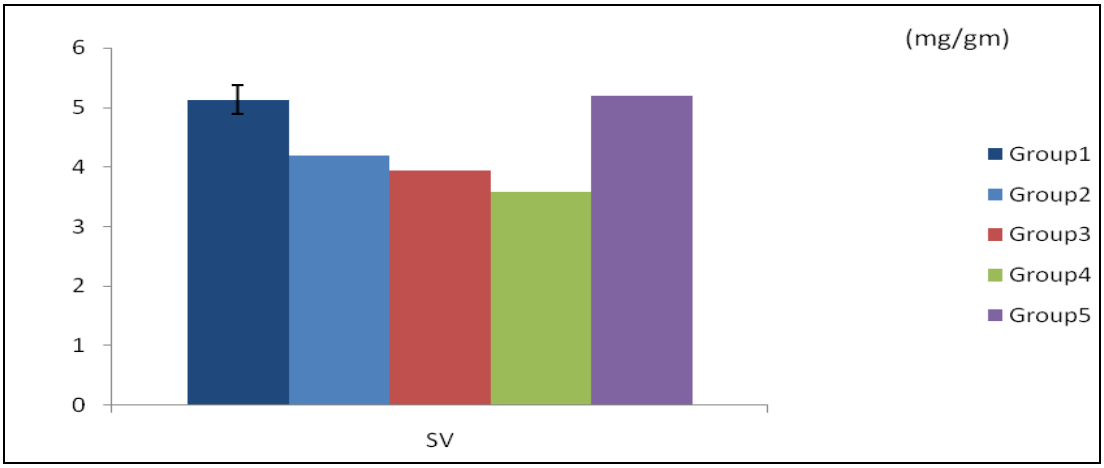


FIG.8: CHANGES FRUCTOSE LEVEL IN SEMINAL VESICLE AFTER 60 DAY TREATMENT OF *M.EMARGINATA* LEAF EXTRACT.

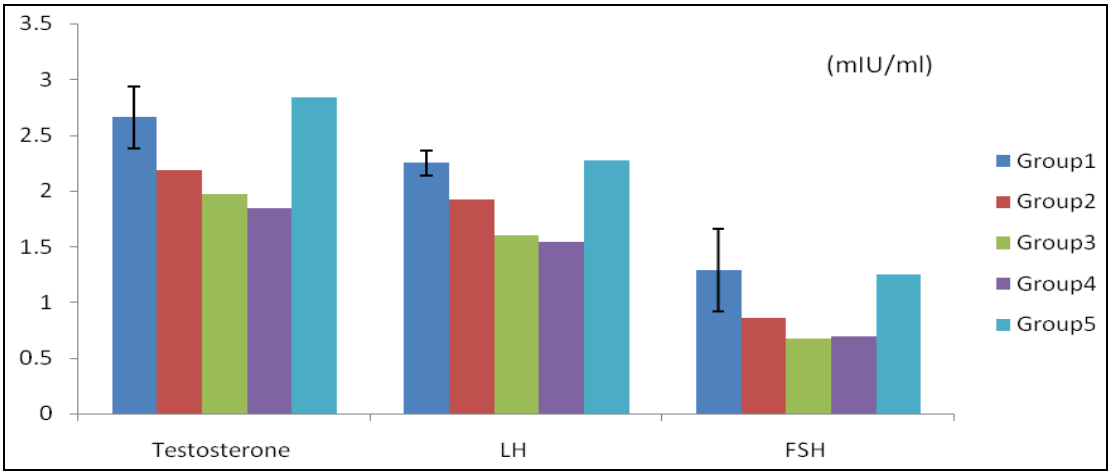
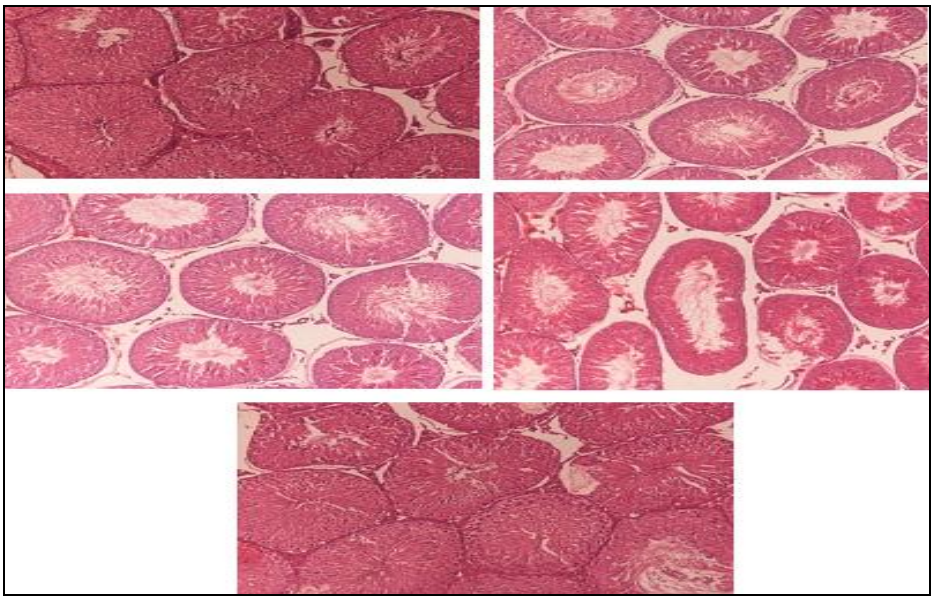


FIG.9: CHANGES HORMONES (TESTOSTERONE, LH, FSH) LEVEL IN SERUM AFTER 60 DAY TREATMENT OF *M.EMARGINATA* LEAF EXTRACT



Photomicrograph of testes of a rat of group 1 (1: control) and group 2 (**Fig.2** treated, with 50 mg /kg /.wt) after 60 day of treatment showing normal features in group 1. while group 2 showing cellular damage of testes.

Photomicrograph of testes of rat of group 3 (**Fig.3:** treated with 100 mg./kg./b.wt. and fig.4:treated with 200 mg./kg./b.wt.) after 60 days of treatment showing reduced somniferous tubular diameter and cellular damage of testes.

Photomicrograph of testes of rat of group 5 (**Fig.5:** 100 mg./kg./wt. *M.emarginata* leaf extract) after 60 days of treatment further 30 days recovery period showing normal cellular structure as compare to group 1.

CONCLUSIONS: It can be concluded that oral administration of 50% methanolic extract of *Maytenus emarginata* leaves decreased fertility of male rats might be due to the decreased level of proteins, fructose and sialic acid contents .Reduction of sperm density is confirmed by histological and hormonal investigation of serum and testis.[42] Further study is needed in higher animal models to observe effects and to develop a male contraceptive from *Maytenus emarginata*.

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