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IDENTIFICATION OF APHRODISIAC PROPERTIES OF *Ficus auriculata* LOUR. FRUIT EXTRACT

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ABSTRACT
The present study was aimed to evaluate the aphrodisiac potential and reproductive safety profile of methanolic extract of *Ficus auriculata* fruit in male Wistar albino rats. The dried fruit powder of *Ficus auriculata* was subjected to methanolic extraction adopting Soxhlet method. Male wistar albino rats weighing between 170 and 220 gm were used for this study. The aphrodisiac and reproductive toxicity potential were evaluated after grouping the rats into 3, namely control and methanolic fruit extract of *Ficus auriculata* treated groups at two dose levels, 250 and 500 mg/kg. The study was conducted for 28 days with daily once dosing of extract. Animals were sacrificed on day 29 by decapitation. Blood was collected. Sera were separated and used for various biochemical assays. The testes, epididymis, vas deferens, seminal vesicle and prostate were dissected out. Sperm count, fructose content in seminal vesicles, histopathological studies and hormonal assay were conducted. Sperm count and fructose level in treated animal were increased significantly. The increased sperm counts and increased weight of sexual organs are indicative of improved fertility. Fructose is the mail source energy for sperm motility. The result concluded that the Methaolic fruit extract of *Ficus auriculata* possessed aphrodisiac activity.

KEYWORDS: *Ficus auriculata*, Aphrodisiac activity, Wistar albino rats, Sperm count.

INTRODUCTION
Infertility has been a recurring problem among male and female individuals. Today, orthodox medicine has almost exceeded its limits in resolving problem of infertility. That's why the use of phytomedicine is becoming a main source in the treatment of infertility. Many factors are involved in the process of conception and ensuing pregnancy, including psychological, anatomical and immunological factors, which affect both men and women. 40-50% of infertility cases are the male infertility. Male infertility may occur the occlusion of the vas deferens, varicoceles, genital infections, immunological disorders and sperm abnormalities.

Fertility regulation with plant preparations in indigenous system of medicine has been reported in ancient literature. In recent times, extracts of *Nigella sativa*, *Lophira lanceolata*, *Cochlospermum planchonii* ,*Kaempferia parviflora*, *Ficuscarica*, *Dracaena arborea*, *Polycarpaea corymbosa* and *Tribulus terrestris* among others have been reported to enhance fertility.[1,2,3,4,5,6,7,8] A number of plant species have been tested for fertility regulation years ago and were subsequently fortified by national and international agencies. *Ficus auriculata* is a huge tropical, deciduous and evergreen tree with more than 800 species. Various parts of this plant (Bark, root, leaves, fruit seed and latex) are frequently used for the treatment of various illnesses. Therefore, in the present investigation, *Ficus auriculata* and its efficacy as aphrodisiac activity will open new avenues to scrutinize rich natural resources for further analysis in order to develop the potential of herbal medicine. Such screening and scientific validation may provide the basics for developing novel aphrodisiac agents without possible side effect. These can be expected to be used on a large scale as their cost and availability will pose no problem and there will be no limitation factor as in case of synthetic drugs.

MATERIALS AND METHODS
Preparation of Extraction
The dried fruit powder of *F. auriculata* was subjected to methanolic extraction adopting Soxhlet method. The extract was concentrated under few reduced pressure to yield semisolid mass which was dried in a desiccators and stored properly for further study.

Animals
Male wistar albino rats weighing between 170 and 220 gm were used for this study. Animals were housed at a temperature of 24±2˚C and relative humidity of 30-70%. All the experimental procedures and protocols used in

this study were reviewed by the Institutional animal ethics committee (688/02/C/CPCSEA) of NCP and were accordance with the guidelines of the IAEC. Approval was obtained from the IAEC, NCP, Erode.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-425 guideline (acute toxic class method), *Wister male albino rats* were used for acute toxicity study. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 28days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. The procedure was repeated for higher doses such as 50, 100 and 200mg/kg body weight.

Experimental Design

The *Wister albino male rats* were randomized into 3 groups comprising of 6 animals each. The animals were treated with respective extracts for 28 days and various test parameters were evaluated.

Group I: Rats received saline daily for 28 days (vehicle only).

Group II: Rats received methanol fruit extract of *F. auriculata* at the dose of 250 mg/Kg body weight daily for 28 days.

Group III: Rats received methanol fruit extract of *F. auriculata* at the dose of 500 mg/Kg body weight daily for 28 days.After 24 hours of last treatment, the final weight was recorded and the animals were sacrificed by decapitation. Blood was collected. Sera were separated by centrifugation at 3000rpm for 10 minutes and stored at 20^0C until used for various biochemical assays.

Collection of reproductive organs

After the last dosing of fruit extract of *Ficus auriculata*, all the animals were sacrificed by employing euthenesia procedure and the testis, epididymides, vas deferens, seminal vesicles and prostates were identified, dissected out, blotted free of blood and cleared of connective tissue or fat. The organs were weighed immediately using an electronic balance.

Sperm count

In-vivo sperm count, epididymis of rats of each group were homogenized and taken into 5ml of 1% sodium citrate solution and squashed thoroughly with the help of needle and forceps until a milky suspension was obtained. The solution was filtered through 80 mm mesh and the volume was made up to 10 ml with the same solution; the made up volume was inclusive of washings of the filter. The suspension was shaken thoroughly and the spermatozoa were counted in five WBC counting chambers of the haemocytometer. The average numbers of sperms per chamber were recorded.

Fructose content in seminal vesicles

The seminal vesicles were macerated with 3ml of distilled water and centrifuged at 4000 rpm for 12 min. To the supernatant, 0.5 ml of resorcinol and 1.5 ml of HCL was added. The mixture was kept at 80^0C for 12 min. The reaction with resorcinol developed a rosy colour, which was measured at 500nm using spectrophotometer. A calibration curve was drawn using dilutions of fructose solution and measurement of the colour developed with resorcinol and HCL.

Tissue preparation for Histopathological studies

For histological work, testis of animals were cut into small pieces and fixed in Bovine's fixative. After dehydration with varying percentage of ethanol, sections were cut, stained with haemotoxylin and eosin, and then analyzed microscopically.

Hormone assay

FSH, LH and Testosterone hormones

Blood samples were spun at 2500 rpm for 10 minutes in a table top centrifuge. The serum samples were analysed to determine the concentration of testosterone, luteinizing hormones (LH), and follicle stimulating hormones (FSH) was measured following an immune enzymatic method with an enzyme-linked immunosorbent assay. The concentration of LH, FSH and testosterone hormones in blood sample was measured by special LH, FSH and testosterone hormones kits using ELISA (Enzyme-Linked Immunosorbant Assay) method.

Serum Biochemical analysis

All the groups of *Wister albino male rats* were sacrificed at the end of treatment on day 29 under ether anaesthesia in lethal chamber and the blood samples were collected by carotid bleeding. The samples were centrifuged and serum was separated and used for the estimation of total protein, urea, creatinine, SGOT, SGPT and ALP using respective standard biochemical kits.

Estimation of protein content[9]

Protein content of the tissue homogenate was assayed by the method of Lowry. The blue copper developed by the reduction of phosphomolybdic phosphotungstic components in the Folin-Ciocalteu reagent by the amino acid tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate were measured by the Lowry's method. About 50 mg of bovine serum albumin was weighed accurately and dissolved in distilled water and made up to 50 ml in a standard flask. About 10 ml of the stock solution was diluted to 50 ml with distilled water in a standard flask. One ml of this solution contained 200 µg proteins. About 0.2 to 1.0 ml of the working standard was pipetted out into series of test tube. About 0.1 ml of supernatant was pipetted out in other test tubes. The volume was made up to 1 ml in all the test tubes with distilled water. A tube with 1 ml of water served as the blank. Five ml of alkaline copper

solution was added to the test tubes and allowed to stand for 5 min. Then 0.5 ml of Folin reagent was added and incubated at room temperature in dark for 30 min. The absorbance was measured at 660 nm. Protein content was expressed as µg/mg of protein.

Estimation of urea and Creatinine content

All the groups of male rats were sacrificed at the end of treatment on day 29 under ether anaesthesia in lethal chamber and the blood samples were collected by carotid bleeding. The samples were centrifuged and serum was separated and used for the estimation of Urea and Creatinine using respective standard biochemical kits.

Serum Antioxidant analyses

For studying serum antioxidative parameters analysis, tissue samples were minced with scissors and homogenized in 4 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4). The homogenate was centrifuged at 10,000 x g for 15 minutes at 40C to obtain the supernatant. Supernatant was diluted 5 times and used for estimating lipid hydroperoxides (LH), Malondialdehyde (MDA), SOD, CAT, GPx and GSH**.**

Estimation of malondialdehyde (MDA): (Niehius and Samuelssol, 1986)

Lipid per oxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) were measured by the method of Nieshus and Samuelsson, 1986. About 0.1 ml of lens homogenate (Tris HCl buffer, PH 7.4) was treated with 2 ml (1:1:1 ratio) of TBA –TCA-HCL reagent (Thiobarburic acid 0.37%, 0.25N HCl and 15% TCA) and placed in a water bath for 15 min, cooled and centrifuged at 1000 g at room temperature for 10 min. The absorbance of the clear supernatant was measured against a reference blank at 535 nm. The values are expressed as n moles of MDA/min/mg protein.

Estimation of lipid hydroperoxides (LH)[10]

About 0.1 ml of homogenate was treated with 0.9 ml of Fox reagent (188 mg Butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 mg ammonium ion sulphate were added to 90 ml of methanol and 10 ml 250mM sulphuric acid) and incubated for 30 min. the colour developed was read at 560 nm using a colorimeter. The values are expressed as n moles/mg protein.

Estimation of superoxide dismutase (SOD)[11]

SOD activity was determined by the inhibition of auto catalyzed adrenochrome formation in the presence of tissue homogenate at 480 nm. The reaction mixture contained 150 µl of homogenate, 1.8 ml of 30 mM carbonate buffer (pH 10.2), and 0.7 ml of distilled water and 400 µl of epinephrine (45 mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. The activity was expressed as units/mg tissue protein.

Estimation of catalase (CAT)[12]

The catalysis of H_2O_2 to H_2O in an incubation mixture adjusted to pH 7.0 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25 mM potassium phosphate buffer pH 7.0 and 0.1 ml of tissue homogenate and was incubated at 37ºC for 15 min and the reaction was started with the addition of 0.1 ml of 10 mM H_2O_2. The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for calculating of enzymatic activity. One unit of catalase activity was defined as the amount of enzymes causing the decomposition of µmol H_2O_2/mg protein/min at pH, 7.0 at 25^{o}C.

Estimation of glutathione peroxidase (GPx)[13]

Glutathione peroxidase activity was measured by the method described by Paglia and Valentine. The reaction mixture consisted of 0.2 ml of 0.4 M phosphate buffer pH 7.0, 0.1 ml of 10 mM sodium azide, 0.1 ml of 0.2 mM hydrogen peroxide, 0.2 ml of glutathione and 0.2 ml of supernatant. The contents were incubated at 37°C for 10 min. The reaction was arrested by the addition of 0.4 ml 10 % TCA and the absorbance was measured at 340 nm.

Estimation of reduced glutathione (GSH)[14]

The method was based on the reaction of reduced glutathione with dithionitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. Briefly after centrifugation, 0.5 ml of supernatant was taken and mixed with 2.0 ml of 0.3 mol/L di-sodium hydrogen phosphate (Na_2HPO_4) solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml, 1% sodium citrate) was added and the absorbance was measured immediately after mixing. Results were expressed in µ mol GSH/min/mg protein.

Statistical analysis

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.

RESULT AND DISCUSSION

Body and Reproductive organ weight

Slight increase in the body weight after administration of fruit extract of the *F. auriculata* with on significant increases ($P<0.05$) in the weight of testes, epididymis, seminal vesicle, prostrate and vas deference in treated male rats compared to control group and details were shown in **table (1)**. The drug may act on pituitary gland and increase the hormone of spermatogenesis. It is well established fact that weights and secretary functions of the epididymis, seminal vesicle and prostrate are closely regulated by the androgens, changes taking place in these organs after castration can be counteracted by administration of testicular hormones thus serving as "indicator test" for male hormones. This increase in the accessory glands weight might suggest an increase in the pattern of testosterone secretion.

Table: 1 Effect of methanol Fruit Extract of *F. auriculata* Lour. on Body and Reproductive Organ Weight On *Wister Male Albino Rats.*

Groups	Body weight of animals on first day (gm)	Body weight of animals on 28th day (gm)	Body weight difference (gm)	Testis weight relative to body weight (gm/100gm)	Epididymis weight relative to body weight (mg/100 gm)	Vas deferens Weight relative to body weight (mg/100 gm)	Seminal vesicles weight relative to body weight (mg/100 gm)	Prostate weight relative to body weight (mg/100 gm)
Vehicle control	162.3±4.8	174.6±3.2	12.3	1.24±0.04	342.3±14.24	129.02±6.51	202.6±11.32	210.3±3.94
Extract 250mg/kg	161.8±6.1	176.3±5.6	14.5*	1.52±0.08*	363.9±11.3*	130.0±4.66^{ns}	212.3±9.24^{ns}	221.5±6.18^{ns}
Extract 500mg/kg	158.3±5.2	174.2±4.9	15.9**	1.75±0.09**	369.7±13.6*	130.4±3.1^{ns}	223.6±6.08*	237.2±2.32*

Values are mean ± SD; n=6 in each group; ^{ns}P>0.05, *P<0.05, **P<0.01 when compared to normal control; (one way ANOVA followed by Dunnett's test).

Sperm count and Fructose level

Sperm count and fructose level in treated animals was increased significantly (P<0.01) compared to control group **(Table 2).** The increased sperm counts and increased weight of sexual organs are indicative of improved fertility, and it is extract treatment. Fructose is the main source energy for sperm motility.

Table: 2 Effect of methanol fruit extract of *F. auriculata* on biochemical parameters on *Wister male albino Rats.*

Groups	Total protein (gm/dl)	Creatinine (mg/dl))	Serum urea (mg/dl)	ALP (U/L)	SGOT U/L	SGPT U/L
Vehicle control	6.3±0.46	0.58±0.09	16.6±0.97	153.2±10.09	17.42±0.86	23.62±2.02
Extract 250mg/kg	6.8±0.38*	0.54±0.04^{ns}	16.4±1.24^{ns}	149.6±9.0^{ns}	17.22±0.72^{ns}	22.56±1.62^{ns}
Extract 500mg/kg	7.2±0.41**	0.51 ±0.01*	15.8±1.02^{ns}	141.4±11.66*	16.86±0.98*	22.06±1.06*

Values are mean ± SD; n=6 in each group; ^{ns}P>0.05, *P<0.05, **P<0.01 when compared to normal control; (one way ANOVA followed by Dunnett's test).

Reproductive Hormone level

The development of normal mature sperm is the key to optimum male fertility. The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are released from the anterior pituitary. FSH stimulates spermatogenesis in the sertoli cells, while LH stimulates the production of testosterone in leydig cells of the testis. In order to understand these observation measurements of testosterone, FSH and LH levels after treatment was undertaken **(Table: 3).**

Serum testosterone level

The methanol fruit extract of *F. auriculata* (250 and 500 mg/kg body weight) treatment repeated for 28 days caused more significant increase (P<0.01) in serum level of testosterone in male rats **(Table: 3)**. Methanolic fruit extract of *F. auriculata* help in improving the testosterone availability to gonads. Increase in testosterone level has been associated with a moderate but significant increase in sexual desire. Clinical data on testosterone also suggest that a slightly increased level of testosterone in adult males results in an increased sexual desire and arousability. Therefore, an improved serum testosterone level after administration of extracts could be considered as one of the contributing factors responsible for an overall incremented sexual performance in treated groups. Androgen is essential for most of the stages of spermatogenesis, meiosis in particular. From a quantitative perspective, the most important androgen is testosterone. More than 95% of this hormone is produced by leydig cells, with the rest produced by the adrenal glands. The result of the present study suggests that methanolic fruit extract of *F. auriculata* may increase the function of sertoli and leydig cells.

Serum luteinizing hormone (LH) level

Repeated treatment of male rats with the methanol fruit extract of *Ficus auriculata* for 28 days caused a dose related more significant increase (P<0.01) in the serum level of LH **(Table: 3)**. In male, LH acts upon the leydig cells of the testes and is responsible for the production of testosterone, an androgen that exerts both endocrine activity and intra-testicular activity on spermatogenesis.

Serum follicle stimulating hormone (FSH)

Methanol fruit extract of *F. auriculata* for 28 days caused a significant increased (P<0.01) in the level of FSH in male rats **(Table: 3)**. Follicle stimulating hormone regulates the growth of seminiferous tubules and maintenance of spermatogenesis. FSH is also critical for sperm production, it supports the function of sertoli cells, which in turn support many aspects of sperm cell maturation. The increase level of FSH reveals a possible role of *F. auriculata* fruit extract in influencing the release of gonadotrophic hormones from pituitary. The rise of FSH by itself is of critical importance in the initiation and expansion of spermatogenesis in mammals.[15] The levels of FSH and LH were also increased after treatment with the extract. Both testosterone and FSH have an important function in spermatogenesis. FSH has key roles in the development of a normal complement of functional sertoli cells (and thus adult spermatogenic potential), in the maturation of sertoli cells at puberty and the maintenance of their cytoskeleton and cell junctions, and in the maintenance of spermatogonial development.

Table: 3 Effect of methanol fruit extract of *F. auriculata* on sperm count and fructose content in seminal vesicles on *Wister male albino Rat.*

Groups	Sperm count (millions/ml)	Fructose content (mg/gm)
Vehicle control	111.9±7.2	1.41±0.64
Extract 250mg/kg	119.2±4.6**	1.72±0.36*
Extract 500mg/kg	121.4±3.7**	2.16±0.22**

Values are mean ± SD; n=6 in each group; *P<0.05, **P<0.01 when compared to normal control; (one way ANOVA followed by Dunnett's test).

Serum biochemical profile

Serum protein and creatinine level in *F. auriculata* fruit extract treated rats shows a significant increase (P<0.01) compared to control group and shows no difference in serum urea level (P>0.05). In fruit extract treated animals, the activity of liver marker enzymes (SGOT, SGPT and ALP) levels were significantly increased (P<0.05) than untreated rats **(Table 4)**.

Table: 4 Effect of methanol fruit extract of *F.* auriculata on hormone levels on *Wister male albino rats.*

Groups	FSH (µIU/ ml)	LH (µIU/ ml)	Testosterone (ng/ml)
Vehicle control	1.25±0.02	2.21±0.12	3.04±0.07
Extract 250mg/kg	1.39±0.06ns	3.26±0.08**	4.56±0.12**
Extract 500mg/kg	1.56±0.03*	3.85±0.09**	5.16±0.26**

Values are mean ± SD; n=6 in each group; nsP>0.05, *P<0.05, **P<0.01 when compared to normal control; (one way ANOVA followed by Dunnett's test).

Serum antioxidants

MDA and LSH in fruit extract treated rats had no difference between (P>0.05) the control rats, SOD and CAT levels were significantly increased (P<0.01) in fruit extract treated rats compared to control group, GPx and GSH levels were more significantly increased (P<0.01) in fruit extract treated rats compared to control group **(Table 5)**. Superoxide dismutase (SOD) scavenges both extracellular and intracellular superoxide anion and prevents lipid peroxidation (LPO) of the plasma membrane. [16] The increased level of CAT, GPx and GSH might be due to the insufficient production of anions in response to the methanolic fruit extract of *F. auriculata*. It is possible that, these antioxidant may cause decreased oxidative stress. This will result in decreased LPO, increased sperm motility, viability and function.

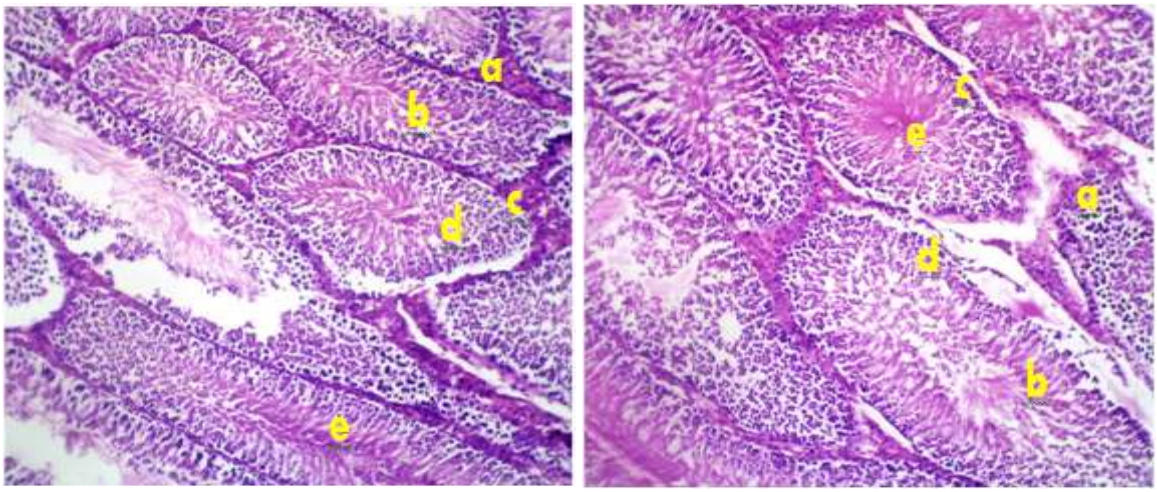
Table: 5 Effect of methanol fruit extract of *F*. auriculata on antioxidant levels on *Wister male albino rats.*

Groups	MDA (nmol/mg of protein)	LH (nmol/mg of protein)	SOD (nmol/mg of protein)	CAT (µmol/mg of protein)	GPx (µmol/mg of protein)	GSH (µmol/mg of protein)
Vehicle control	2.9±0.26	0.58±0 .04	1.06±0.06	7.83±0.92	0.24±0.008	0.46±0.02
Extract 250mg/kg	2.4±0.06^{ns}	0.56±0.02^{ns}	1.26±0.04^{ns}	8.62±0.26^{ns}	0.35±0.01*	0.58±0.05*
Extract 500mg/kg	2.2±0.08^{ns}	0.52±0.06^{ns}	1.62±0.09*	9.84±0.12*	0.43±0.03**	0.64±0.04**

Values are mean ± SD; n=6 in each group; ^{ns}P>0.05, *P<0.05, **P<0.01 when compared to normal control; (one way ANOVA followed by Dunnett's test).

3.6. Histopathological observation

Transverse sections of testis of control group animals showed testicular parenchyma with normal seminiferous tubules. Spermatogenesis appears normal with step wise maturation. Basement membrane shows tubules and interstitial cell appear focal with mild destruction. There is no evidence of maturation arrest. In the transverse sections of extract-treated group animals, testicular parenchyma with normal seminiferous tubules, basement membrane of the tubules and interstitial cells appear unremarkable. These results revealed that the methanolic fruit extract of *F. auriculata* showed aphrodisiac activity and it is dose dependent.



Methanolic extract 250 mg/kg **Methanolic extract at 500mg/kg**

a – Leydigs, **b** – sertolic cell, **c** – spermatid, **d** – spermtogonia, **e** – flagellae

CONCLUSION

In aphrodisiac activity of Methanolic fruit extract of *F. auriculata* on *male wistar albino rats*, *F. auriculata* did not show any toxicity effect up to the dose of 1000mg/kg body weight, accordingly 250 and 500 mg/kg weight was taken as low and high dose. The animals were treated with respective extracts for 28 days and various test parameters were evaluated. After 28 days, reproductive organ weight, change on animal body weight, histology of tests, sperm count, fructose level, biochemical parameters, hormone levels, serum antioxidants were recorded.

The administration of fruit extract significantly increased the body weight while the weight of the testis, epididymis, seminal vesicle, prostrate and vas deference's in treated male rats compared to control group, sperm count and fructose level in treated animals was increased significantly in extract treated rats. The methanol fruit extract of *F. auriculata* caused significant increase in serum level of testosterone, serum luteinizing hormone (LH) and serum follicle stimulating hormone (FSH) level increase. Protein, urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels were significantly increased in treated rats. Fruit extract treated rats had shown increase in activity of

all the studied antioxidants (MDA, LH, SOD, CAT, GPx, and GSH) when compared to control rat. Transverse sections of testis of control group animals of testicular parenchyma with normal seminiferous tubules, spermatogenesis appears normal with step wise maturation. Basement membrane shows tubules and interstitial appear focal mild destruction. There is no evidence of maturation arrest. In the transverse sections of extract treated group animals, testicular parenchyma with normal seminiferous tubules. Basement membrane of the tubules and interstition appear unremarkable.

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