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Stimulatory effect of the ethanol extract of *Melastoma malabathricum* L. (Melastomataceae) leaf on the reproductive system of male albino rats

K.Balamurugan, G. Sakthidevi and V.R.Mohan*
Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin-628008, Tamil Nadu, India.

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ABSTRACT

Fertility enhancement effect of ethanol extract of leaf of *Melastoma malabathricum* was observed in male albino rats. The relative weight of the testis and epididymis were increased. The epididymal sperm count, motility and sperm abnormality were increased significantly in treated rats. There was an increase in serum protein, albumin, globulin, urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels of treated rats. The activities of serum antioxidants (CAT, SOD. GPX, GST and GRD) in leaf extract treated rats were increased. The results of the hormonal assay showed that increased serum levels of LH and testosterone but decreased in the serum levels of FSH and estrogen compared to control. The results of fertility test indicated that the treated adult male rats increased the number of female's impregnation. In addition, the number of implantations and the number of viable fetuses were also increased. The results of the present study concluded that, ethanol extract of leaf of *Melastoma malabathricum* enhanced sperm concentration, motility and testosterone which might produce positive result in the male fertility.

INTRODUCTION

Infertility is a worldwide medical and social problem. It affects above 10-15% of married couples. WHO estimates that there are 60-80 million infertile couples worldwide. Infertility itself may not threaten physical health but it can certainly have a seriously impact on the mental and social well-being of infertile couple. In many countries the stigma of infertility often leads to marital disharmony, divorce or ostracism (Badami *et al.,* 2000; WHO, 1992). Research during the past two decades has an unfolded focus on impotence (erectile failure), premature ejaculation and male infertility. There are a number of prescription drugs which may act as sex stimulant and enhancing the sexual desire and activity in both men and women. Although the use of allopathic medicines have shown significant improvement in treating sexual disorders, but at the same time there are large number of side effects. These include irregularities of the rhythm of the heart, suicidal tendencies, mental disorders and tremors. The use of synthetic aphrodisiacs results in the dilation of blood vessels in other parts of the body causing headache and fainting.

Other side effects include facial flushing, stomach upset, burned vision and sensitivity to light which usually occur at higher doses (Kulkarni and Reddy, 1998). *Melastoma malabathricum* belongs to the Melastomataceae family. It is also called Singapore Rhododendron or Sendudok. It is a erect shrub or small tree 1.5-5m tall. It was traditionally used to treat diarrhoea, dysentery, leucorrhoea, hemorrhoids, wounds and infection during confinement, toothache, flatulence, sore legs and thrush and also it is used by the Jah hut people in Malaysia to cure diarrhoea (Sunilson *et al.,* 2009). In the light of the above findings, this work was conducted to monitor its effect on reproductive system and fertility in adult male rat.

* *Corresponding Author*
Mohan VR, Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin 628008, Tamil Nadu, India.
Tel +919787279902; Email: vrmohanvoc@gmail.com

MATERIALS AND METHODS

Plant material

The leaf of *Melastoma malabathricum* L. collected from Daudeli, Joide Taluk, Hubli District, North Karnataka. The collected plants were identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin for further reference.

Preparation of plant extract

The leaf of *Melastoma malabathricum* were dried separately under shade and then powdered with a mechanical grinder to obtain a coarse powder, which were then subjected to extraction in a Soxhlet apparatus using ethanol. The ethanol extract was concentrated in a rotatory evaporator. The concentrated ethanol extracts of leaf of *Melastoma malabathricum* were used for anti-fertility activity.

Animals

Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25 ± 2^0C) and light and Dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). 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 incubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight.

Experimental Design

The male rats were divided into 3groups consisting of 5 animals.

Group I: Rats received normal saline daily for 14 days, orally. (Normal control).

Group II: Rats received ethanol extract of leaf of *Melastoma malabathricum* at the dose of 250mg/kg body weight daily for 14 days.

Group III: Rats received ethanol extract of leaf of *Melastoma malabathricum*, at the dose of 500mg/kg body weight daily for 14 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 3000 rpm for 10 minutes and stored at 20^0C until used for various biochemical assays.

Then testis, epididymis, vas deferens, seminal vesicle and ventral prostrate were dissected out, trimmed off extraneous and weighed accurately on torsion balance. The organs weights were expressed in terms of mg/100g body weight.

Sperm count

Epididymal fluid (for sperm count) was collected from caput and cauda segments separately and diluted with Sorenson's buffer (pH7.2). The separated fluid was taken for sperm count. Sperm count was carried out by using Neubauer's haemocytometer as described by Zaneveld and Pelakoski (1997).

Sperm motility and abnormality

After anaesthetizing the rats, the caudal epididymis was then dissected. An incision (about 1mm) was made in the caudal epididymis and drops of sperm fluid were squeezed onto the microscope slide and 2 drops of normal saline were added to mobilize the sperm cells. Epididymal sperm motility was then assessed by calculating motile spermatozoa per unit area.

Morphology (abnormality) was evaluated on sperm from the caudal epididymis. The total morphological abnormalities were observed as described by Linde *et al* (1992).

Serum biochemical analysis

Serum proteins (Lowry *et al.*,1951) and serum albumins were determined by quantitative colorimetic method by using bromocresol green.

The total protein minus albumin gives the globulin, urea (Varley, 1976), creatinine (Owen *et al*.,1954), serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel (1957). Serum alkaline phosphatase (ALP) was measured by the method of king and Armstrong (1934).

Serum antioxidants

Serum antioxidant Catalase (CAT) (Sinha, 1972), Superoxidedismutase (SOD) (Das *et al.,* 2000), Glutathione peroxidase (GPX) (Rotruck *et al.,* 1984), Glutathione s-transferase (GST) (Habig *et al.,* 1974) and Glutathione reductase (GRD) (Goldberg and Spooner, 1983) were analyzed.

Hormonal Assay

Blood removed from the rats by intracardiac method. Blood was centrifuged at 3000 rpm to separate the serum for the measurement of testosterone, Luteinizing hormone (LH), estrogen and follicle stimulating hormone (FSH). The quantitative determination of hormones was done by using Enzyme Immunoassay Method (EIA). The EIA kit was obtained from Immunometrics (London, UK).

Fertility test

Fertility was estimated in adult male rats treated with ethanol extracts of leaf of *Melastoma malabathricum* and in the control male counterparts. Each male was placed in an individual cage with two virgin untreated females of the same strain. They were left together for 10 days during which two estron cycles had elapsed. One week after the removal of the exposed males, pregnant females were killed by cervical dislocation under light ether anesthesia and the number of implantation sites, the number of fetuses and the number of resorption sites were recorded (Rugh, 1968).

Statistical Analysis

Data were expressed as Mean ± SEM. Student's t test was used for statistical comparison.

RESULTS

Preliminary phytochemical screening and acute toxicity studies

Phytochemical screening of ethanol extract of leaf of *Melastoma malabathricum* revealed the presence of alkaloids, catechin, coumarin, tannin, phenols, saponins, steroid, flavonoid, glycoside and xanthoprotein. The acute toxicity study, ethanol extract of *M. malabathricum* leaf did not show any toxicity effect upto the dose of 2000mg/kg body weight, according 250 and 500 mg/kg body weight were taken as low and high dose of leaf of *M. malabathricum* for the experiment.

Body weight and reproductive organ weight

Table-1 shows slight increase in the body weight after administration of the leaf extract of the *Melastoma malabathricum* while the weight of the testis, epididymis, seminal vesicle, ventral prostrate and vas deferens were significantly increased (P<0.01) in treated male rats compared to control group.

Sperm density and motility

Table-2 shows that the motility of sperm in cauda epididimis was significantly increased (P<0.01) increased in treated animals that *M. malabathricum* leaf in comparison with control. Sperm density in treated animals, the seminiferous tubule diameter and leydig cell nuclear diameter of treated male rats was increased significantly. The sperm abnormality was decreased significantly in the treated animals.

Serum biochemical profile

Serum protein, albumin, globulin, urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels of control and treated rats were depicted in table-3. All the parameters were significantly increased.

Serum antioxidants

The activities of CAT, SOD, GPx, GST and GRD in the serum of control and leaf extract treated rats were presented in table-4. In the present study, plant extract treated rats had shown increased activities of all the studied antioxidants when compared to control rat.

Reproductive hormone level

Serum testosterone level

The ethanol extract of leaf of *M. malabathricum* (250 and 500 mg/kg body weight) repeated treatment for 14 days caused significant increase in serum level of testosterone in male rats. The level of testosterone increase was dosing related (Table-5).

Serum luteinizing hormone (LH) level

Repeated treatment of male rats with the ethanol extract of leaf of *M. malabathricum* for 14 days caused a dose related increase in the serum level of LH. Dose of 500mg/kg body weight daily for 14 days caused sharp rise in the serum level of LH. The level of increased was statistically significant (p<0.05).

Serum estrogen level

The ethanol extract of leaf of *M. malabathricum* caused decrease at dose related serum level of estrogen when compared with control. Dose of 500mg/kg body weight daily for 14 days caused sharp decrease in the serum level of Estrogen. The level of decreased was statistically significant (p<0.05).

Table. 1: Effect of *Melastoma malabathricum* leaf extract on the Body and Reproductive organ weight of adult male albino rats.

Treatment Groups	Body wt(gm)		Testis (gm)	Epididymis (mg)		VD (mg)	SV (mg)	Prostrate (mg)
	Before	After		Caput	Cauda			
Group-I	259.56±9.43	278.50±8.92*	1.914±0.38	126.33±3.04	284.56±2.44	116.39±2.84	294.11±6.85	163.22±2.94
Group-II	284.25±6.54	299.54±7.56	2.153±0.84*	191.39±2.54*	354.63±4.93*	134.59±1.98	298.69±5.43	196.16±2.82*
Group-III	264.68±7.81	287.34±8.13**	2.654±0.62*	224.58±3.65**	398.59±5.22**	149.63±2.56*	316.37±6.54*	209.52±2.94**

Each Value is SEM of 5 animals * P < 0.05; ** P < 0.01. Control Vs Treated

Table. 2: Effect of *Melastoma malabathricum* leaf extract on the sperm concentration and motility in the epididymis of adult male albino rats.

Treatment Groups	Sperm Concentration (Counts x 10^6 mil)		Sperm Motility (FMI) @ (cauda)	Sperm Abnormality #	
	caput	cauda		Head (%)	Tail (%)
Group-I	356.24±10.84	419.54±15.36	153.26±10.36	5.94±0.18	8.93±0.34
Group-II	379.36±11.56*	431.66±13.39ns	168.51±9.88ns	4.13±0.21	7.05±0.39
Group-III	394.15±12.65**	486.30±14.83**	189.36±4.88**	2.54±0.13*	3.05±0.74*

Each Value is SEM of 5 animals * P < 0.05, ** P<0.01 Control Vs Treated

@ : Motility is movement recorded after 5 min in the suspension of caudal epididymal spermatozoa in phosphate buffered solution.

: Expressed in percentage

Table. 3: Effect of *Melastoma malabathricum* leaf extract on few serum biochemical profile of adult male albino rats.

Parameter	Group I	Group II	Group III
Protein (gm/dl)	7.11±0.24	8.34±0.84	8.94±0.55*
Albumin (gm/dl)	4.65±0.65	4.68±1.43	4.94±0.23
Globulin(gm/dl)	2.46+0.12	3.66+0.36	3.60+0.21
A/G Ratio:	1.89:1	1.27:1	1.37:1
Urea(mg/dl)	15.33±0.52	16.54±1.05	19.32±0.93
Creatinine(mg/dl)	0.63±0.03	0.73±0.07	0.88±0.03
SGOT (U/L)	11.84±1.08	13.48±0.93	18.39±1.34
SGPT(U/L)	15.36±2.94	16.35±0.84	22.63±1.85
ALP(U/L)	167.55±4.86	168.54±1.24	183.56±2.16*

Values are given as means □ S.D from six rats in each group * P < 0.05 Control Vs Treated.

Table. 4: Effect of *Melastoma malabathricum* leaf extract on the activity of serum Catalase, Glutathione peroxidase, Glutathione-S transferase, Superoxide Dismutase and Glutathione reductase in rats.

Treatment	Catalase (µmoles of H2O2/ decomposed/min/ mg protein)	Glutathione peroxidase (µmoles of NADPHoxidized/min/ mg protein)	Glutathione-S transferase (µmoles ofconjugate formed/ min/mg protein)	Superoxide dismutase (Units)	Glutathione reductase (µmoles of NADPH oxidized/min/ mg protein)
Group I	7.93±0.24	0.274±0.03	10.14±0.93	22.66±1.85	28.04±1.31
Group II	8.24±0.54	0.309±0.02ns	11.68±0.71ns	29.17±0.93*	39..68±0.73*
Group III	10.65±1.24**	0.354±0.13*	13.99±0.34*	34.86±1.85**	41.16±0.28**

Values are given as means □ S.D from six rats in each group * P < 0.05, ** P<0.01 Control Vs Treated.

Table. 5: Effect of *Melastoma malabathricum* leaf extract on Sex hormones levels and pituitary gonadotrophins in male albino rats.

Treatment Groups	Parameters			
	Testosterone (mg/ml)	LH/ICSH (µIu/ml)	Estrogen(pg/ml)	FSH (µIu/ml)
Group I	3.03±0.85	1.98±0.05	18.31±0.24	0.98±0.05
Group II	3.54±0.73	2.08±0.06	16.22±1.24	1.24±0.07
Group III	4.88±0.93*	2.59±0.09*	12.54±1.91*	1.88±0.09*

Values are given as means □ S.D from six rats in each group * P < 0.05 Control Vs Treated.
Group I. : Rats given normal saline daily for 14 days consequently orally (by using an intragastric catheter tube (IGC).
Group II: - Rats given MML extract at the dose of 250 mg/ Kg b.wt, daily, orally for 14 days consequently by IGC).
Group III: Rats given MML extract at the dose of 500 mg/ Kg b.wt, daily, orally for 14 days consequently (IGC).

Table. 6: Effect of *Melastoma malabathricum* leaf extract on the Fertility of male albino rats.

Groups	No. of male	No.of females	No.of pregnant females	No.of implantation	No. of viable fetuses	Total No. of resorption sites
Group-I	2	6	5/6	9.31±0.84	4.18±1.26	4
Group-II	2	6	5/6	6.8±0.14	4.05±1.13	5
Group-III	2	6	6/6	8.54±0.24	6.84±1.05*	5

Each Value is SEM of 5 animals * P < 0.05, Control Vs Treated
Group I. : Rats given normal saline daily for 14 days consequently orally (by using an intragastric catheter tube (IGC).
Group II : - Rats given MML extract at the dose of 250 mg/ Kg b.wt, daily, orally for 14 days consequently by IGC).

Serum follicle stimulating hormone (FSH) level

Pre-treatment with the ethanol extract of leaf of *M. malabathricum* caused decrease in the serum level of FSH male rats compared to control. The decrease in the serum level of FSH in male rats statistically significant(p<0.05) when treated with *M. malabathricum* leaf extract (500mg/kg body weight).

Fertility test

The results presented in table-6 show that intra-gastric administration of the ethanol extract of leaf of *M. malabathricum* at doses 250 and 500 mg/kg body weight for 14 days to male rats caused a significant increase in the number of females impregnated by treated male rats. The number of implantations and the number of viable fetuses calculated after cesarean suctions were significantly increased in female rats impregnated by treated males when compared with female rats impregnated with untreated male rats. On the other hand, the number of resorption sites were found to be increased to significant values in female impregnates by treated male rats when compared to controls.

DISCUSSION

In the present study, the weight of reproductive organs markedly increased. The weight and secretary functions of testis, epididymis, seminal vesicles, ventral prostrate and vas deferense are closely regulated by androgens. The drug may act on pituitary gland and increased main hormone of spermatogenesis. It is well established fact that weights and secretary functions of the epididymis, seminal vesicle and ventral 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 the male hormones (Choudhary and Steinberger, 1975; Agarwal *et al.*, 1986). The results presented in this work also show that the seminal vesicles

weights were increased in adult male rats ingested *M. malabathricum*. This increase in the accessory glands weights might suggest an increase in the pattern of testosterone secretion. Significant increase in the sperm motility of cauda epididymis was observed in treated group. This may be due to activity effects of *M. malabathricum* on the enzymes of oxidative phosphorylation. Sexual cells can occur during the reproductive phase, mitotic division of the spermatogenesis or during the maturation of the spermatozoa, thereby increasing the number and quality of the sperm cells produced in the testis.

Among the ethanol extract of *M. malabathricum* leaf (Group-II and III) (250 and 500 mg/kg body weight) produced a significant increase in total sperm count and viable sperms. This may be as a result of the ability of the extract at the given doses, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadodropins which may result in alteration of spermatogenesis.

The presence of mature sperm concentration was increased in the experimental rats treated with 500 mg/kg body weight *M. malabathricum* leaf extract. This suggests that the 500 mg/kg dose could influence the maturation of the spermatozoa in the male rats, which might also be a contributory factor to the increase in the mean total sperm count. In the present investigation the observed increase in the cauda epididymal sperm motility might be due to an alteration in the microenvironment in the cauda epididymis, which also had a synergistic action on the of the spermatozoa of the treated rats as a result of the androgen-stimulatory effect of the extract of *M. malabathricum* leaf.

The increase in the cauda epididymis sperm counts in the treated animals substantial the spermatogenic nature of the extract. The extract had a direct effect on the testis resulting in an increase in the number of spermatozoa and the increased level of testosterone production. Also, the extract had no spermatotoxic effct of previously indicated by Shah *et al* (1991).

The increased level of superoxide dismutase, catalase, glutathione peroxidase, glutathione s-tranferase and glutathione reductase were reported in the present study. Similarly, total protein, SGOT, SGPT and ALP levels were increased in the serum of extract treated rats.

The extract did not show an antigonadotrophic nature, demonstrated by the increased levels of FSH and LH in the treated rats. The increased level of FSH reveals a possible role of *M. malabathricum* leaf extract in influencing the release of gonadotrophic hormones from the pituitary. The rise of FSH by itself is of critical importance in the initiation and expansion of spermatogenesis in mammals, as is generally agreed (Lohiya *et al*., 2002).

The results presented in this paper also show that the ingestion *Melastoma malabathricum* by adult male rats increased the number of impregnated females. The number of implantations and the number of viable fetuses were increased. This effect may be due to increase in sperm motility and sperm density. In conclusion, these results confirmed that the long term *M. malabathricum* ingestion produces increased effects on fertility on reproductive system in adult male rat. However, the exact mode of action requires further studies.

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