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Effects of methanol extracts of *Cuscuta reflexa* Roxb. stem and *Corchorus olitorius* Linn. seed on male reproductive system of mice

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SUMMARY

The antifertility activity of methanol extract of *Cuscuta reflexa* Roxb. stem (MECR) and *Corchorus olitorius* Linn. seed (MECO) were studied on male Swiss albino mice. The extracts were found to decrease sperm count, percentage of motile sperm and testosterone level in treated mice when compared with vehicle control after 17 days of treatment. The weight of gonads, epididymis were decreased whereas no significant changes of the body weight of mice were observed after methanol extract treatments. The fertility test showed 100% negative result in MECR and MECO treated mice at medium and high dose level of treatment. MECR and MECO in low (25 mg/kg and 15 mg/kg, respectively), medium (50 mg/kg and 20 mg/kg, respectively) and high (75 mg/kg and 25 mg/kg, respectively) dose level caused a simultaneous fall in testicular Δ5-3β–hydroxy steroid dehydrogenase and glucose-6-phosphate dehydrogenase activities which are involved in testicular steroidogenesis. Total cholesterol and ascorbic acid content in testis were increased significantly in gonads. The activities of lactate dehydrogenase, malic dehydrogenase and ascorbic acid oxidase were reduced whereas that of carbonic anhydrase was increased significantly in the testis of MECR and MECO treated mice. All these observations indicate that the methanol extract of *C. reflexa* stem and *C. olitorius* seed produced antifertility activity in sexually matured male mice, which may be due to inhibition of gonadal steroidogenesis. This activity may be attributed due to the presence of flavonoids and steroids, respectively.

Key words: *Cuscuta reflexa*; *Corchorus olitorius*; male mice; reproductive system; MECR; MECO

INTRODUCTION

Cuscuta reflexa Roxb. (Swarnalata in Bengali, Amarvel in Hindi) family Convolvulaceae is a golden yellow dodder like parasite. The plant is common throughout India, found widely distributed in the plains of West Bengal, growing on thorny or other shrubs as parasite annuals. Various parts of this plant were used by tribes for the diseases like fits, melancholy and insanity. It is also useful externally against itch and internally in fevers, 'induration of the liver' (Chopra *et al*., 1992; Kirtikar and Basu, 2001). On preliminary chemical analysis, *Cuscuta reflexa* stem is found to contain large quantity of flavonoids (Rostogi and Mehrotra, 1993; Yadav *et al*., 2000; Mazumder *et al*., 2003) and its different extracts on preliminary investigation have been found to posses' antifertility effects (Rao *et al*., 1979).

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Corchorus olitorius Linn. (Jute) family Tiliaceae, is an annual herb with slender stems. It is cultivated in many parts of India. The seed are used as purgative and leaves as demulscent, diuretic, febrifuge (infusion) and in chronic cystitis and dysuria (Chatterjee and Pakrashi, 1992). *Corchorus olitorius* (jute) seed is a traditional tribal medicine for birth control (Chatterjee, 1996) and found to contain steroids and glycosides (Nakamura *et al*., 1998; Mazumder *et al*., 2003). Litterature survey reveals that no detailed study has yet been done regarding the antifertility activity of *C. reflexa* stem and *C. olitorius* seed. In our earlier investigation, we have found the antisteroidogenic activity of the methanol extract of *C. reflexa* Roxb. stem and *C. olitorius* Linn. seed in mouse ovary (Gupta *et al*., 2003a,b; Mazumder *et al*., 2003). As continuation of the previous work, the present communication deals with the effects of methanol extracts of *C. reflexa* stem (MECR) and *C. olitorius* seed (MECO) on male reproductive system of mice by monitoring the sperm count, sperm motility, weight of testis and epididymis, fertility test and biochemical parameters. The changes in cholesterol and ascorbic acid content in testis were measured, as they are the starting substances for androgen synthesis (Knorr *et al*., 1970; Verma *et al*., 1980). The effects of MECR and MECO were also studied on the activities of Δ5-3β–hydroxy steroid dehydrogenase (Δ5-3β–HSD), glucose-6-phosphate dehydrogenase (G-6-PDH), lactate dehydrogenase (LDH), malic dehydrogenase (MDH), ascorbic acid oxidase and carbonic anhydrase in the testis of treated mice.

MATERIALS AND METHODS

Preparation of extract

The stems of *C. reflexa* Roxb and the seeds of *C. olitorius* Linn were collected from Panua, Bankura district region of West Bengal in the month of August and were authenticated by the Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata. The voucher specimen has been preserved in our laboratory (Ref No: DMU 1 and 2). Shade-dried, powdered, sieved (40 mesh) plant materials were soxhlet extracted first with petroleum ether (40 - 60 °C) and then with methanol. The methanol extract (ME) was evaporated to dryness. The trace amount of methanol that might be present within the solid mass of methanol extract was removed by vaccum pressure. The yield of methanol extraction was 21.5 and 16.9% for *C. reflexa* stem and *C. olitorius* seed, respectively. For pharmacological testing, MECR and MECO were dissolved in propylene glycol (PG).

Chemical investigation of MECR and MECO

Preliminary analysis demonstrated the presence of large amount of flavonoids in MECR. Qualitative tests also indicated the presence of flavonoids and C-glycosides (Mazumder *et al*., 2003; Nakamura *et al*., 1998).

Two fractions from MECR were separated. The crude ME was extracted several times with benzene. The residue after benzene extraction was dissolved in ethanol and then divided into two portions. One portion was extracted with diethyl ether and it was concentrated when white flake like crystals were appeared. The crystals on preparative TLC using silica gel G as stationary phase and benzene: acetone (2:1) as mobile phase gave fraction A (Rf value 0.7, λmax 230 nm) having the IR (Jasco 530 FTIR) peaks at 1603 cm^{-1}, 3008 cm^{-1} (for aromatic ring), 1720 cm^{-1}, 1750 cm^{-1} (for ketone, ester group) suggesting the structural similarity with lactone compounds (Agarwal and Dutta, 1935).

The 2nd part of the alcoholic solution was treated with an excess of alcoholic lead acetate to remove tannins. The tannin free yellow alcoholic solution after removal of excess lead was evaporated to dryness under reduced pressure. The reddish yellow brittle amorphous mass was washed repeatedly with petroleum ether and collected as soft lemon yellow tufs (m.p. 2350) wherefrom on preparative TLC using silica gel G as stationary phase and

benzene: acetone (4:1) as mobile phase fraction B (Rf value 0.3, λmax 210 nm) is isolated. It is a colouring matter belonging to the hydroxy flavone group (Agarwal, 1936) which showed characteristic IR peaks at 1524 cm^{-1}, 1602 cm^{-1} (for aromatic); 923 cm^{-1} (methylene dioxy group); 3520 cm^{-1}, 3688 cm^{-1} (for –OH group); 1730 cm^{-1}, 1760 cm^{-1} (for ketone, ester group). It also showed fluorescence under UV.

MECO on preliminary analysis was found to contain large amount cardenolide glycosides. Qualitative tests (Liberman Burchard, Salkowski) for steroids and Cardenolide glycosides (Legal) were also found positive.

The dried ME of *C. olitorius* seed was partitioned between butanol and water and the organic layer was evaporated to dryness under reduced pressure to give a dark brown mass. The extract was chromatographed on silica gel G using graded elution techniques.

Fractions eluted with chloroform: methanol (17:3) yielded a solid, which on further purification by chromatography afforded a mixture of two compounds (TLC). This was subjected to further chromatography and erysimoside (Mahato *et al*., 1989) [mp 172-1730 C; [α]D+18.5 0 (C 0.51 in CH_3OH)] and olitoriside (Matsufuji *et al*, 2001) [mp 203-2050 C; [α]D-4.8 0 (C 0.51 in CH_3OH)] were identified in good agreement with those of authentic sample (mp, IR and comparative TLC).

Fraction eluted with chloroform: methanol (4:1) solvent system on further purification followed by chromatography afforded one compound which was identified with authentic strophanthidin (Nakamura *et al*., 1998) (mp, IR and comparative TLC).

Animals and treatment

Adult albino male mice (22 ± 2 g; Swiss strain) were acclimatized to normal laboratory conditions for one week and given pellet diet (Hindustan Lever Ltd, India) and water ad libitum. The experiment was performed under the guidance of the Institutional Ethical Committee. The LD_{50} values of MECR and MECO are 435 mg/kg and 191 mg/kg, i.p. respectively (Pal *et al*., 2002). The mice were divided into eight groups and each group containing six mice. The animals of Group I and II received saline (0.9% NaCl w/v) and propylene glycol (5 ml/kg); Group III, IV, V received MECR (25 mg/kg, 50 mg/kg and 75 mg/kg, respectively); Group VI, VII and VIII received MECO (15 mg/kg, 20 mg/kg and 25 mg/kg, respectively). Normal saline, propylene glycol and ME were given intraperitoneally in alternate days for 17 days. The mating exposure tests on control and treated groups were performed using the method of WHO (WHO, 1983). The presence of sperms in the vaginal smear was the evidence of mating. On the 18th day, after 18 h fasting mice were sacrificed by cervical dislocation. Testis, cauda epididymis and adrenal glands were immediately dissected out, trimmed off adherent fatty materials, weighed and kept on ice for further processing.

Determination of sperm motility and count

Spermatozoa were extracted from mouse cauda epididymis at room temperature (32 °C ± 1) in a modified Ringer's solution. The conventional method (WHO, 1999) for visual assessment of flagellar motility was done by the following way. A portion of cell suspension was injected into the Neubauer counting chamber of haemocytometer. Immediately, total number of motile sperm and total cell number were counted under a phase contrast microscope at 900 X magnifications.

Biochemical methods

Testis was homogenized in chloroform-ethanol mixture (2:1) and non-polar part was extracted out and total cholesterol was quantified (Kingsley and Roscoe, 1949).

About 5 mg tissue of testis was homogenized in 45 µl ice cold 5% metaphosphoric acid and centrifuged for 20 min at 3500 X g. Then, 30 µl supernatant, 15 µl acetate buffer and 15 µl 2,6-dichlorophenolindophenol sodium (0.1 mg/ml)

were mixed and optical density was measured against water at 520 nm. Standard curve was drawn and ascorbic acid content was calculated (Omaye *et al*., 1979). Testis was homogenized in 0.1 M phosphate buffer (pH 7.4) and the activity of Δ5-3β–HSD was estimated (Rabin *et al*., 1961).

Testis was homogenized in 0.5 M Tris-HCl (pH 8.3) and centrifuged to estimate G-6-PDH (Lohr and Waller, 1974). Protein was estimated with folin phenol reagent and activity of enzymes was expressed in unit per mg of protein (Lowry *et al*., 1951).

Testis was homogenized in a 9-volume 0.25 M sucrose containing 1 mM EDTA and centrifuged to estimate LDH (Schatz and Segal, 1969). Enzyme velocity was expressed as change in absorbance per minute (Δ OD/min) per mg of protein.

Testis was homogenized in a pre chilled pestle mortar in a grinding medium containing 50 mM Tris HCl (pH 8.0), 50 mM $MgCl_2$, 5 mM 2 mercaptaethanol and 1 mM EDTA and then centrifuged to estimate malic dehydrogenase (Sottocasa, 1967). The reaction mixture was prepared by adding 2.4 mM oxaloacetate and 2.12 mM NADH. One enzyme unit is defined as the amount of enzyme causing an increase in optical density of 0.01 per min.

Testis was homogenized in Tris HCl buffer (pH 7.6) and centrifuged to estimate carbonic anhydrase (Waygood, 1957). Enzyme activity was expressed as change in absorbance per second per mg of protein.

Ascorbic acid oxidase enzyme was estimated as described by Charles and Richard (Charles *et al*., 1957). One unit of ascorbic acid oxidase activity is defined as the amount of enzyme that causes an initial rate of oxygen uptake of 10 ml per min.

Hormonal assay

Serum testosterone levels were assayed from frozen samples using radio immune assay method (readily available) (Belanger *et al*., 1980). The sensitivity of the assay was 10 pg/ml and intra assay error was 4.5%.

Statistical analysis

Results are expressed as mean ± S.E.M. ANOVA followed by Dunnett's '*t*' test was performed as a post hoc test of significance taking vehicle treated animals as control.

RESULTS

Results are presented in Tables 1 - 4. From Table 1, it is evident that MECR and MECO did not cause any significant change in the body weight of treated mice. However, the wet weight of testis, epididymis were reduced whereas that of adrenal glands were increased significantly in case of treated animals.

Table1. Effects of MECR and MECO on body weight and organ weights (testis, epididymis and adrenal gland) in matured male mice

Treatment	Organ weights (mg/100 g body wt)			
	Body weight (g) (Final)	Testis (Both sides)	Epididymis (Both sides)	Adrenal gland (Both sides)
Saline (5 ml/kg, i.p.)	20.51 ± 0.21	1026.3 ± 4.70	77.0 ± 1.83	80.4 ± 1.21
Vehicle (PG) (5 ml/kg, i.p.)	20.83 ± 0.32	972.0 ± 3.85	74.9 ± 1.65	76.8 ± 1.19
MECR (25 mg/kg, i.p.)	20.73 ± 0.30	829.2 ± 2.71[a]	62.0 ± 1.51[b]	88.3 ± 1.13[b]
MECR (50 mg/kg, i.p.)	20.84 ± 0.26	795.1 ± 4.05[a]	55.2 ± 1.29[a]	94.0 ± 1.24[a]
MECR(75 mg/kg, i.p.)	21.14 ± 0.35	726.6 ± 3.15[a]	47.1 ± 1.40[a]	95.6 ± 1.25[a]
MECO(15 mg/kg, i.p.)	20.91 ± 0.36	797.7 ± 4.20[a]	58.3 ± 1.45[b]	86.6 ± 1.20[b]
MECO (20 mg/kg, i.p.)	21.10 ± 0.27	745.0 ± 3.55[a]	52.1 ± 1.43[a]	88.7 ± 1.23[b]
MECO (25 mg/kg, i.p.)	21.30 ± 0.34	684.0 ± 2.50[a]	49.3 ± 1.50[a]	90.9 ± 1.30[a]

Values are mean ± S.E.M. from 6 animals. Statistical analysis was done by ANOVA followed by post hoc test of significance, Dunnett's '*t*' test. [a]$P < 0.001$, [b]$P < 0.01$ as compared with vehicle control.

Table 2. Effects of MECR and MECO on sperm dynamics and testosterone level in matured male mice

Treatment	Sperm count (× 106/ml)	% of motile sperm	Fertility test	Testosterone (ng/ml)
Saline (5 ml/kg, i,p.)	43.8 ± 1.39	45.3 ± 1.3	100% (+) ve	4.31 ± 0.04
Vehicle (PG) (5 ml/kg, i.p.)	42.7 ± 0.87	43.5 ± 2.8	100% (+) ve	4.25 ± 0.03
MECR (25 mg/kg, i.p.)	24.4 ± 0.58[a]	22.5 ± 1.7[b]	75% (-) ve	3.05 ± 0.02[a]
MECR (50 mg/kg, i.p.)	21.7 ± 0.80[a]	21.0 ± 1.1[b]	100% (-) ve	2.65 ± 0.03[a]
MECR (75 mg/kg, i.p.)	18.9 ± 0.86[a]	19.9 ± 0.9[a]	100% (-) ve	2.28 ± 0.04[a]
MECO (15 mg/kg, i.p.)	22.6 ± 0.71[a]	21.2 ± 1.3[b]	83% (-) ve	2.90 ± 0.03[a]
MECO (20 mg/kg, i.p.)	16.8 ± 0.86[a]	20.1 ± 1.5[b]	100% (-) ve	2.72 ± 0.03[a]
MECO (25 mg/kg, i.p.)	15.8 ± 0.92[b]	19.0 ± 1.5[a]	100% (-) ve	2.31 ± 0.04[a]

Values are mean ± S.E.M. from 6 animals. Statistical analysis was done by ANOVA followed by post hoc test of significance, Dunnett's '*t*' test. [a]$P < 0.001$, [b]$P < 0.01$ as compared with vehicle control.

Table 3. Effects of MECR and MECO on content of ascorbic acid, cholesterol and the activities of G-6-PDH and Δ5-3β-HSD in mouse testis after 17 days of treatment

Treatment	Ascorbic acid (μg/mg of testis)	Cholesterol (μg/mg of testis)	G-6-PDH (U/mg of protein)	Δ5-3β-HSD (U/mg of protein)
Saline (5 ml/kg, i.p.)	76.4 ± 2.3	51.9 ± 3.7	2.8 ± 0.06	1.9 ± 0.08
Vehicle (PG) (5 ml/kg, i.p.)	78.2 ± 2.0	56.3 ± 2.9	2.9 ± 0.08	1.8 ± 0.07
MECR (25 mg/kg, i.p.)	96.9 ± 0.97[a]	123.8 ± 5.7[a]	1.7 ± 0.06[a]	1.3 ± 0.05[a]
MECR (50 mg/kg, i.p.)	102.5 ± 1.3[a]	139.5 ± 6.3[a]	1.6 ± 0.06[a]	1.1 ± 0.08[a]
MECR (75 mg/kg, i.p.)	107.7 ± 2.2[a]	153.8 ± 7.2[a]	1.4 ± 0.05[a]	0.9 ± 0.07[a]
MECO (15 mg/kg, i.p.)	92.7 ± 1.3[a]	157.5 ± 2.0[a]	1.1 ± 0.05[a]	1.2 ± 0.06[a]
MECO (20 mg/kg, i.p.)	104.8 ± 4.5[a]	181.9 ± 5.3[a]	0.9 ± 0.03[a]	0.9 ± 0.08[a]
MECO (25 mg/kg, i.p.)	113.2 ± 3.4[a]	205.3 ± 9.1[a]	0.8 ± 0.05[a]	0.7 ± 0.06[a]

Values are mean ± S.E.M. from 6 animals. Statistical analysis was done by ANOVA followed by post hoc test of significance, Dunnett's '*t*' test. [a]$P < 0.05$ as compared with vehicle control.

Table 4. Effects of MECR and MECO on the activities of ascorbic acid oxidase, LDH, MDH and carbonic anhydrase in mouse testis after 17 days of treatment.

Treatment	Ascorbic acid oxidase (U/mg of protein)	Lactate dehydrogenase (U/mg of protein)	Malic dehydrogenase (U/mg of protein)	Carbonic anhydrase (U/mg of protein×10^{-5})
Saline(5 ml/kg, i.p.)	0.075 ± .008	0.088 ± .007	0.085 ± .009	0.17±.005
Vehicle(PG)(5 ml/kg, i.p.)	0.072 ± .005	0.086 ± .006	0.082 ± .005	0.19±.004
MECR(25 mg/kg, i.p.)	0.041 ± .005[a]	0.050 ± .004[a]	0.052 ± .006[a]	0.25± .005[a]
MECR(50 mg/kg, i.p.)	0.035 ± .004[a]	0.043 ± .005[a]	0.045 ± .004[a]	0.34 ± .007[a]
MECR(75 mg/kg, i.p.)	0.028 ± .003[a]	0.035 ± .004[a]	0.037 ± .004[a]	0.46 ± .009[a]
MECO(15 mg/kg, i.p.)	0.050 ± .004[a]	0.049 ± .005[a]	0.051 ± .003[a]	0.27 ± .005[a]
MECO(20 mg/kg, i.p.)	0.042 ± .005[a]	0.040 ± .005[a]	0.037 ±0.004[a]	0.37 ± .007[a]
MECO(25 mg/kg, i.p.)	0.031 ± .004[a]	0.032 ± .003[a]	0.033 ± .003[a]	0.49 ± .008[a]

Values are mean ± SEM from 6 animals. Statistical analysis was done by ANOVA followed by post hoc test of significance, Dunnett's 't' test. [a]P<0.05 as compared with vehicle control.

The sperm count and percentage of motile sperm were decreased markedly in case of MECR and MECO treated mice, at all dose levels of treatment (low dose by 43% and 48%, 47% and 51%, respectively)

with respect to controls ($P < 0.05$). The fertility test showed 100% negative fertility in MECR and MECO treated mice at medium and high dose level. Serum testosterone level of MECR and MECO treated mice were decreased significantly in comparison to control group (Table 2). As depicted in Table 3, MECR and MECO significantly elevated the level of cholesterol (low by 120% and 180%) and ascorbic acid contents (medium by 31% and 34% and maximum by 37% and 45%, respectively) in mouse testis as compared to vehicle control. The activities of G-6-PDH and Δ5-3β–HSD were inhibited significantly ($P < 0.05$) by MECR (medium by 69% and 50% whereas the level maximum by 72% and 61%, respectively).

From the Table 4, it is observed that the activities of LDH, MDH and ascorbic acid oxidase were decreased significantly ($P < 0.05$) by MECR (low by 42%, 37 %, 43% and high by 59%, 55%, 61%) and MECO (low by 43%, 38%, 31% and high by 63%, 60%, 57%, respectively) whereas treatment with MECR and MECO resulted in significant elevation of carbonic anhydrase activity (low by 32% and 42%, respectively) in mouse testis compared to vehicle control.

DISCUSSION

In the present study, administration of MECR and MECO caused decrease in weight of testis, epididymis which may be due to low plasma level of testosterone or alteration of androgenic synthesis (Bartlett *et al*., 1990; kasturi *et al*., 1995; Udoh and Kehinde, 1999; Handelsman, 2000). The reduction in the number of spermatozoa (sperm count) and their motility in MECR and MECO treated mice are of importance with regard to fertilization (Bedford, 1983). In many of the plant based contraceptives, inhibition of male fertility after administration of natural substance has been ascribed to describe sperm motility and sperm count (Qian *et al*., 1995; Sharma and Jacob, 2001). Decrease in sperm motility and sperm count in the MECR and MECO treated mice suggests alteration of sperm maturation in the epididymis (Sarkar *et al*., 2000). A decrease in sperm reserve may be a reasonable cause for reduction in the weight of epididymis. The significant decrease in testosterone level in the treated animals supports this view (Sandhyakumary *et al*., 2002; Gupta and Sharma, 2003).

This was associated with an elevation in the level of cholesterol, which serves as a precursor for the synthesis of androgens in biogenic pathway in the testis (Knorr *et al*., 1970; Verma *et al*., 1980; Bedwal *et al*., 1994; Pal *et al*., 2003) and may suggest the non-utilization of lipid towards testosterone biosynthesis (Krum *et al*., 1964). Ascorbic acid, an easily diffusible water-soluble reductant is found abundantly in testis (Gupta *et al*., 2004) where it plays an important role in testicular hormonogenesis and the elevation of ascorbic acid content is responsible for inhibition of steroidogenesis. To substantiate these facts, the estimation of G-6-PDH and Δ5-3β–HSD, the two key enzymes involved in steroidogenesis was performed. It is well documented that Δ5-3β–HSD is a key enzyme involved in androgen and steroid biogenesis (Knorr *et al*., 1970). Therefore, in the present investigation a fall of G-6-PDH and Δ5-3β–HSD after treatment with MECR and MECO suggests a diminution of testicular steroidogenesis (Gupta *et al*., 2003). It is also reported that gonadotrophins through the activation of G-6-PDH metabolism in pentose phosphate pathway increased the rate of formation of NADPH essential for hydroxylation reaction in the formation of steroid hormones from cholesterol (McKerns, 1965).

The other three enzyme activities of the testis of the MECR and MECO treated mice exhibited significant changes. LDH and MDH being metabolic enzyme, decreased activity of these may be due to decreased metabolic activity going on in the treated mice (Dhanju *et al*., 2001). Lower level of MDH decreases the synthesis of NADH, which is responsible for synthesis of steroid hormones (Satyanarayana, 1999). The anti-steroidogenic activity of MECR and MECO in males may be mediated through its inhibitory

effect on dehydrogenase enzymes, particularly LDH-X and MDH as well (Giridharon *et al*., 1982). The importance of LDH-X in male reproduction is demonstrated by the fact that antibodies against this enzyme have a contraceptive effect in males (Goldberg and Wheat, 1976).

Ascorbic acid is oxidized to dehydroascorbic acid by ascorbic acid oxidase enzyme. So, the decreased activity of ascorbic acid oxidase increases deposition of ascorbic acid in the testis (Das, 2000). The increase in carbonic anhydrase activity in MECR and MECO treated mice may be due to the enhanced level of progesterone (Crossland, 1980) induced by these extracts. However, the mechanism by which the carbonic anhydrase activity is increased in the testis of treated mice is not clear. It might be due to feedback regulation of CNS, as carbonic anhydrase is also present in a number of extrarenal tissues including CNS (Jackson, 1996). More extensive studies are required to establish it.

MECR and MECO showed a dose dependent sedative activity (Mazumder *et al*., in press). Sedatives are known to inhibit libido and several plant products with sedative potential have inhibited libido in rats (Ratnasooriya and Dharmasiri, 1997; Ratnasooriya *et al*., 1999; Ratnasooriya and Dharmasiri, 2000). In conclusion, this study shows that MECR and MECO have short lasting depressive effects on male mice sexual function (Ratnasooriya and Jayakody, 2002).

Phytochemical tests indicate the presence of flavonoids in MECR (Rostogi and Mehrotra, 1993; Yadav *et al*., 2000) and steroids in MECO (Nakamura, 1998; Mazumder *et al*., 2003). Since various flavonoids (Hiremath *et al*., 1994) and sterols (Hiremath *et al*., 1999) have been reported to possess antifertility activities, the anti-steroidogenic property of the MECR and MECO in male mice might be due to the presence of such compounds (Gupta *et al*., 2003a,b).

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