

thus preventing their intimate contact with gastric mucosa for a long time. In addition, the slow release of small quantities of the drug over a long period of time, will lead to improvement of dissolution and absorption properties of ketoprofen. Consequently, the contact time of the drug with gastric mucosa decreases, so the extent of ulceration due to drug entrapped in the microparticles is reduced compared to the free drug (Table 1).

The calculated percent protection was 89% and the extent of ulceration of mucosa by free drug was 2.1 times that calculated for microparticles containing equivalent amount of drug. Analysis of variance for the calculated parameters (Table 1) indicated the significant high extent of protection of mucosa ($p \leq 0.05$) after administration of ketoprofen floating microparticles.

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Induction of Carbonic Anhydrase by *Cuscuta reflexa* Stem and *Corchorus olitorius* Seed in Mice

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The effects of multiple intraperitoneal doses of methanolic extract of *Cuscuta reflexa* Roxb. stem and *Corchorus olitorius* Linn. seed on the activity of carbonic anhydrase in the uterus of mice were studied. These methanolic extracts caused a significant increase in the carbonic anhydrase activity in the uterus of mice. The increased rate of enzymatic activity might be associated with elevated level of progesterone induced by these methanolic extracts. This further establishes the antifertility nature of methanol extract of *C. reflexa* stem and *C. olitorius* seed.

Cuscuta reflexa Roxb., family Convolvulaceae known as *Swarnalata* (Bengali) and *Amarvel* (Hindi) is a golden yellow dodder like parasite. The plant is common throughout India, found widely in the plains of West Bengal, growing on thorny or other shrubs as parasite annuals. Various parts of this plant were used in tribal medicine for the diseases like epilepsy, melancholy and insanity¹. It is also use-

ful externally against itch and internally in fevers, flatulence and induration of the liver²⁻⁵. The *C. reflexa* stem and its different extracts on preliminary investigation have been found to possess antifertility effect⁶.

Corchorus olitorius Linn. (jute) family Tiliaceae is an annual herb with slender stems. It is cultivated in many parts of India. The seeds are used as purgative and leaves as demulcent, diuretic, febrifuge (infusion) and in chronic cystitis and dysuria⁵. *C. olitorius* seed is a traditional tribal medi-

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cine for birth control.

Literature survey reveals that no detailed study has yet been done regarding the antifertility activity of methanolic extract (ME) of *C. reflexa* stem and *C. oltorius* seed. In our earlier investigation, we have found that ME of *C. reflexa* stem and *C. oltorius* seed exhibited antifertility (antisteroidogenic) activity in mice (unpublished work). As continuation of the previous study, an attempt has now been made to assess the carbonic anhydrase activity in the uterus of mice to correlate it with the antifertility activity of ME of *C. reflexa* stem and *C. oltorius* seed.

The stems of *C. reflexa* Roxb. and the seeds of *C. oltorius* Linn. were collected locally in West Bengal and were authenticated by the division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata. A voucher specimen has been preserved in our laboratory. Shade-dried, powdered plant material (#40 mesh) was Soxhlet extracted first with petroleum ether (40-60°) and then with MeOH. The methanolic extract was evaporated to dryness. The trace amount of methanol, which might be present within the solid mass of methanolic extract, was removed by washing with ethyl alcohol. For pharmacological testing, methanolic extract of *C. reflexa* stem and *C. oltorius* seed were dissolved in propylene glycol (PG).

Adult female Swiss-Webster mice (22±2 g) were acclimatized to normal laboratory conditions (25-30°, 75-85 % relative humidity, 12 h light/dark cycle) for 1w and given pelleted diet (Hindustan Lever) and drinking water *ad libitum*. The experiments were performed after getting the experimental protocols approved by the Institutional Animal Ethics Committee, Jadavpur University, Kolkata. The LD₅₀⁷ values of ME of *C. reflexa* stem and *C. oltorius* seed were 435 and 191 mg/kg, respectively. The mice showing a normal oestrus cycle for a period of 2 w were then divided into 8 groups with each group containing 6 mice. Group I and II served as the normal saline control (5 ml/kg, i.p.) and vehicle (propylene glycol) control (5 ml/kg, i.p.), respectively. Groups III, IV and V were treated with ME of *C. reflexa* at the doses of 25, 50 and 75 mg/kg, i.p., respectively. Groups VI, VII and VIII were treated with ME of *C. oltorius* at the doses of 15, 20, and 25 mg/kg, i.p., respectively. The extracts were administered on alternate days for 17 d. On the 18th d after 18 h of fasting the mice were sacrificed by cervical dislocation. Uterus was dissected out, freed from fatty materials, weighed and kept on ice for further processing.

The uterus was homogenized with 3.5 ml normal sa-

line. Then 8 ml of 40 % ethanol and 4 ml of chloroform were quickly added. The mixture was stirred in a centrifuge tube for 3 min to a thin sludge and allowed to stand for 20 min. The mixture was centrifuged for 10 min at 3500x g and the supernatant was taken for the estimation of carbonic anhydrase.

Three millilitres of veronal buffer (3 ml of 0.22 M veronal, pH 7.95), 3 drops of bromothymol blue and 0.3 ml of enzyme and 2 ml of water were mixed in a 15 ml stoppered weighing bottle and placed in ice water for 15 min. Five millilitres of ice cold water saturated with CO₂ (0.071 M) was added anaerobically from a long nozzleed all-glass syringe. The time was observed for the pH to drop to 6.3, determined with the aid of a bromothymol standard at this pH. The solutions were mixed in less than 1 s without bubbling or loss of CO₂. Similarly blank sample was prepared without the addition of enzyme. The enzyme activity was calculated by using the following formula: the rate of enzymatic hydration of CO₂ = (t₀-t)/(t-1)·6.91x10⁻⁵ mol/l/s. Where t and t₀ are time of reaction in the presence and the absence of enzyme, respectively.

Results are expressed as mean ± S.D. Statistical analysis was done by ANOVA followed by Tukey's test⁹ and the difference was considered statistically significant at P<0.05. Results are shown in Table 1. The carbonic anhydrase activity of different groups were compared to that of control. It was found that ME of *C. reflexa* stem and *C. oltorius* seed at the medium and high dose level (50 and 75 mg/kg and 20 and 25 mg/kg, respectively) showed significant elevation of enzyme carbonic anhydrase with respect to control. The increase in carbonic anhydrase activity may be due to the presence of materials (under investigation) is possibly due to enhanced level of progesterone¹⁰. The higher level of progesterone inhibits the release of LH and thus prevents ovulation and it also makes the cervical mucus less suitable for the passage of sperm. It also alters the endometrium in such a way as to discourage implantation^{10,11} and thus prevents fertilization.

In conclusion, the mechanism by which progesterone mediates its effect on carbonic anhydrase is yet to be elucidated. Progesterone may have depressant and hypnotic action in the CNS (including the region of the pulse generator of hypothalamus). Carbonic anhydrase is also present in a number of extrarenal tissues including CNS¹². Thus, the elevation of carbonic anhydrase might be due to feedback regulation of CNS. However, further investigation is required to establish it. The result of the present study is definitely

TABLE 1: EFFECT OF METHANOLIC EXTRACT OF *C. REFLEXA* STEM AND *C. OLITORIUS* SEED ON CARBONIC ANHYDRASE ACTIVITY

Group	Treatment (dose)	Enzyme Activity (mol/l/s)
I	Normal saline as control (5 ml/kg)	0.30±0.08
II	Propylene glycol as control (5 ml/kg)	0.31±0.06
III	ME of <i>C. reflexa</i> in PG (25 mg/kg)	0.38±0.10
IV	ME of <i>C. reflexa</i> in PG (50 mg/kg)	0.47±0.06*
V	ME of <i>C. reflexa</i> in PG (70mg/kg)	0.60±0.05*
VI	ME of <i>C. olitorius</i> in PG (15 mg/kg)	0.43±0.18
VII	ME of <i>C. olitorius</i> in PG (20 mg/kg)	0.51±0.09*
VIII	ME of <i>C. olitorius</i> dissolved in PG(25 mg/kg)	0.56±0.07*

Enzyme activity is expressed as rate of enzymatic hydration of $\text{CO}_2/6.9 \times 10^{-5}$. All values are mean±S.D. of n=6. *indicates $P < 0.05$ vs Control, by ANOVA followed by Turkey's test. PG stands for propylene glycol.

encouraging, as this may be exploited during future study where carbonic anhydrase level would serve as an index for determining the antifertility efficacy of a compound.

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