subglobose, hyaline, cruciately septate metabasidium (5·2–7·8 μm diam.) which bears four long (up to 6·5 μm) and slender (1·3–2·0 μm wide) sterigmata. Basidiospores continuous, oval or short-cylindric, slightly curved, apiculate, hyaline, smooth and 4·6–5·9 × 3·3–3·9 μm. The basidiospores germinate by repetition and the secondary spores produced are similar to the basidiospores.

Material examined: On decaying wood, Tiger Shola, Kodaikanal, Tamil Nadu, Coll. K. V. Chandrashekara, 23–8–1977, Herb. MUBL No. 2366.

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ACID AND ALKALINE PHOSPHATASE ACTIVITY IN THE UTERUS OF RAT TREATED WITH *HIBISCUS ROSA-SINENSIS* LINN. EXTRACTS

THE flowers of *Hibiscus rosa-sinensis* Linn. (vern. Java), reported to possess contraceptive activity[1], have been confirmed for their antifertility efficacy in rats[2–4]. Its total 50% ethanolic and benzene extracts have also been reported to have significant antiestrogenic activity[5]. In view of the antiestrogenic action of these extracts, the present communication deals with their effect on acid and alkaline phosphatase activity of the uterus in adult rats as these enzymes are estrogen dependent.

Total 50% ethanolic and benzene extracts of *Hibiscus rosa-sinensis* (flowers) were obtained and each extract was macerated with gum-acacia supended in distilled water at dose of 75, 150 and 300 mg/kg body weight as described earlier[4] and was administered orally with the help of an intragastric catheter.

Colony-bred Swiss adult female albino rats, 3–4 months old, weighing 165 ± 15 g, were selected and two extracts were administered orally for 3 different durations as described earlier[6], to different batches of animals (Tables I, II). Control rats received vehicle only in a similar manner. The animals of each dose of the two extracts were sacrificed in diestrus stage and 48 h after the last dose, *i.e.*, on 8th, 14th and 20th day respectively. Rats showing stages other than diestrus were rejected. The uteri were carefully dissected out, trimmed, blotted on filter-paper and weighed to nearest 0·1 mg. The weighed tissue from different animals was kept separately in freezer for 48 hours, homogenized and processed for the estimation of the acid and alkaline phosphatases, using the method of Hawk *et al.*[7] with some modifications[8]. The results were statistically analysed using analysis of variance.

Table I summarizes the effect of these plant extracts on acid phosphatase activity of the uterus of rat. Both 50% ethanolic and benzene extracts evoke a significant increase in the activity and also reveal a clear-cut dose-response relation. 75 mg/kg dose of 50% ethanolic extract is significant and increases the activity when administered for 18 days only (*vs.* control $P < 0·01$). Similarly its 150 mg/kg dose when applied for 12 and 18 days schedule increases the activity of this enzyme significantly (*vs.* control $P < 0·01$ and $< 0·005$ respectively). Dose, 300 mg/kg is remarkably effective where the activity increases significantly at every schedule; however, it is maximum at 18 days level (*vs.* control $P < 0·001$). Benzene extract at doses 75 and 150 mg/kg when applied for 12 and 18 days have increased the activity significantly (*vs.* resp. control $P < 0·05$ and $< 0·001$ respectively). Its 300 mg/kg dose is highly significant at every duration to increase the activity (*vs.* control $P < 0·001$).

Table II shows the effect of these plant extracts on alkaline phosphatase activity of the uterus of rat. The effect of 50% ethanolic and benzene extract on uterine alkaline phosphatase activity is dose and duration dependent. 75 and 150 mg/kg doses of 50% ethanolic extract when administered for 18 days decrease the activity significantly (*vs.* control $P < 0·01$ and $< 0·001$ respectively). Its 300 mg/kg dose provokes more consistent results where every duration is effective to decrease this enzymatic activity; however, it is highly significant at 18 days schedule (*vs.* control $P < 0·001$). Similarly 75 mg/kg dose of benzene extract when administered for 18 days, diminishes the alkaline phosphatase activity (*vs.* control $P < 0·02$). Its 150 and 300 mg/kg doses when applied to adult rat at any of the schedules decrease the activity of this enzyme significantly (*vs.* respective control $P < 0·001$; $< 0·01$ and $< 0·02$). However, 300 mg/kg dose shows more encouraging results where every dose is statistically significant at highest level (*vs.* control $P < 0·001$).

Synthetic estrogen is well known to increase the acid phosphatase activity of the uterus of rat[9] but reports from other laboratories[10,11] suggest that progesterone is responsible for the increase in the concentration of acid phosphatase in the rat uterus. Similarly, inhibition in the alkaline phosphatase activity of the rat uterus is observed after progesterone treatment[10]. On the contrary, progesterone causes an increase in the alkaline phosphatase activity in the uterus of rat[12]. In the present investigation, both 50%

TABLE I

Effect of 50% ethanolic and benzene extracts of H. rosa sinensis *Linn. on uterine acid phosphatase activity in rats*

(Values and mean ± S. E., expressed in mg/100 g tissue/hr. Number of rats used in parentheses)

Extract	Dose mg/kg/day	Treatment period (days) 6	12	18
Control (vehicle only)	—	96±1·58 (5)	97·75±1·70 (4)	101±1·02 (5)
50% ethanolic	75	94·6 ±1·94 (5)	112·8 ±5·80 (5)	121 ±5·35[e] (4)
	150	100·25±1·70 (4)	128±7·25[e] (4)	138·2±7·56[b] (5)
	300	117·4 ±5·59[d] (5)	150±7·90[a] (5)	161·8±7·69[a] (5)
Control (vehicle only)	—	96·25±1·70 (4)	98·75±0·95 (4)	99·8±1·78 (5)
Benzene	75	94·5 ±4·43 (4)	120·75±6·5[e] (4)	125·5±4·20[a] (4)
	150	98·6 ±2·40 (5)	119·75±8·26[e] (4)	146·8±9·31[a] (5)
	300	134·75±4·57[a] (4)	153·5 ±9·14[a] (4)	172±7·38[a] (5)

P values *versus* their respective controls : $a < 0{\cdot}001$; $b < 0.005$: $c < 0{\cdot}01$; $d < 0{\cdot}02$; $e < 0{\cdot}05$

TABLE II

Effect of 50% ethanolic and benzene extracts of H. rosa-sinensis *Linn. on uterine alkaline phosphatase activity in rats*

(Values are mean ± S. E., expressed in mg/100 g tissue/hr. Number of rats used in parentheses)

Extract	Dose mg/kg/day	Treatment period (days) 6	12	18
Control (vehicle only)		574·4 ±11·26 (5)	669·25 ± 6·5 (4)	583·4 ±9·76 (5)
50% ethanolic	75	573·6 ±11·61 (5)	561·4 ± 8·57 (5)	540·75±4·92[e] (4)
	150	550·5 ± 8·22 (4)	537·5 ± 9[e] (4)	503±14·08[a] (5)
	300	515·4 ± 8·73[c] (5)	480 8 ± 7·15[a] (5)	421·2 ±2·39[a] (5)
Control (vehicle only)	—	586·75±12·47 (4)	578±13·63 (4)	580·6 ±8·26 (5)
Benzene	75	560·5 ± 4·22 (4)	550·25 ±8·26[d] (4)	538·80±8·53[d] (4)
	150	540·6 ± 8·47[d] (5)	502·75±12·84[c] (4)	496·80±8·12[a] (5)
	300	488·25± 4·34[a] (4)	448± 8·90[a] (4)	398·40±4·72[a] (5)

P values *versus* their respective controls : $a < 0{\cdot}001$; $c < 0{\cdot}01$; $d < 0{\cdot}02$; $e < 0{\cdot}05$

ethanolic and benzene extracts of *H. rosa-sinensis* Linn. have increased the acid phosphatase activity but reduced significantly the alkaline phosphatase concentration in the uterus of rat. These alterations in acid and alkaline phosphatase activity of the uterus of rat under the influence of *H. rosa-sinensis* Linn,

extracts may be due to the strong antiestrogenic action of the extracts[6] which probably change the growth and secretory functions of the cell[13], cell permeability[14] and also the uterine milieu required for the implantation of an egg.

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COMPLEMENT FIXATION TEST IN EXPERIMENTAL SETARIASIS

Setaria cervi, a cosmopolitan filariid worm of buffaloes, has a high rate of incidence among cattle and buffalo population in India. The parasite does not usually cause any significant pathology in the natural hosts. However, evidence is available which indicates that the infective stages of the parasite under strange circumstances may enter the central nervous system of buffaloes, deers, sheep and goats and develop to partial maturity causing severe pathological condition known as 'cerebro-spinal nematodiasis'[1-3].

The present work deals with the serological diagnosis of experimental setariasis by means of Complement Fixation Test (CFT). Adult worms, collected from freshly slaughtered buffaloes, were implanted intraperitoneally into 36 laboratory bred white rats[4]. Each rat received 5 adult worms. CFT was performed according to Kolmer[5]. Before carrying out main CFT, hemolysin, complement and antigen were titrated. Two units of hemolysin contained in 0·5 ml were used in main CFT. Normal guinea pig serum was used as a source of complement. Two full units of complement in 1 ml were employed. 1% alcoholic extract of the powdered worms, prepared according to the methods of Minning and McFadzean[6], Pacheco[7], was employed as an antigen. Antigen was used in a dilution of 1 : 80 in 0·5 ml amount. Weekly blood, from 12 microfilaria-positive rats was collected intracardially, and the serum was separated. Pooled antisera diluted from 1 : 5 to 1 : 640 were used in 0·5 ml. A positive reaction was taken as the one which gave 50% hemolysis, and hemolysis above 50% was taken as negative[7]. In the test, immune serum control, hemolytic control, normal serum control and antigen control were also included. A weekly record of microfilaraemia in all these rats was also maintained. Another 24 infected rats were sacrificed at weekly intervals to note the condition and survival time of adult worms in the peritoneal cavity of white rats.

Microfilariae appeared in the peripheral blood circulation of white rats after a latent period of 8 ± 2 days with a maximum microfilarial density during 3rd and 4th weeks of initial infection. Adult worms survived in the peritoneal cavity for about 4-5 weeks, thereafter, they started disintegrating. Dead and exhausted worms were found to be embedded in the peritoneal wall and intestinal mesentries. Complement fixing antibody was detected in infected rat sera in 2nd week of initial infection with a titre of 1 : 5. The antibody titre rapidly increased and reached its maximum by 4th week with a titre of 1 : 80 which was found to be coinciding with the peak microfilarial density (Fig. 1). No antibody titre was detected during 8th week.

The above observations indicate that there is a definite correlation between the level of antibody titre, level of microfilaraemia and survival of adult worms. Higher levels of antibody titre during 4th and 5th weeks followed by a sharp fall during later phase of infection indicate that maximum antigenic stimulus is generated by live adult worms and micro-