of their individual vesicular forms. The property of clusters responding to the minute perturbing changes in the individual constituting members, might be, in our view, an important tool in the study of cellular or vesicular assembly processes.

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The plant Banjauri (*Vicoa indica*) exhibits antifertility activity in adult female bonnet monkeys (*Macaca radiata*)

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The antifertility activity of the plant *Vicoa indica* was tested in proven fertile bonnet monkeys. The dry powder of the whole plant was fed to the cycling monkeys on day 1 to 14 of menstrual cycle or day 9 to 14 of cycle or on day 2 to 5 after delivery and the fertility was evaluated in the following cycle in cycle fed monkey or after weaning the young one in the post-partum fed monkeys. Results indicated that while feeding in the post-partum monkeys did not confer any protection against pregnancy feeding during day 1 to 14 of cycle, protected from pregnancy. The monkeys did not become pregnant even after exposure to the proven fertile male monkeys for 13 ovulatory cycles while all the vehicle fed monkeys became pregnant within 3 cycles.

BANJAURI is the colloquial name for the plant *Vicoa indica* which belongs to the family Compositae. It is a small plant of about 1–2 feet height with slender stem, long leaves and small yellow flowers. The plant grows wild in the months of July/August and fully mature plants with flowers are seen during September/October. It is reported[1] that the Adivasi tribes in Bihar use Banjauri as a contraceptive. According to the practice followed by Adivasis, the freshly collected plant is sun-dried and the whole plant equivalent is powdered along with seven pepper seeds and consumed each day as a suspension in water by woman on the second to fifth day after delivery of the child or from day 1 to 14 of three consecutive menstrual cycles. Such treatment reportedly induces permanent sterility. In view of the nonavailability of scientifically documented clinical study on the use of Banjauri, we have attempted to verify the activity profile of this plant product by determining the effect of feeding dried Banjauri powder both during post-partum period and early phase of the menstrual cycle on fertility of proven fertile adult female bonnet monkeys.

The procedures adopted for care and maintenance of bonnet monkeys have been reported in detail earlier[2]. The serum levels of estradiol-17β, progesterone and chorionic gonadotropin were determined according to methods described earlier[3].

Dried plant material was powdered and passed through a metal sieve (500 μ mesh) to remove coarse fibrous material. The finely powdered material was mixed with groundnut seeds and brown sugar in a ratio of (1 : 4 : 8) and thoroughly ground in a mortar and the resulting paste was fed to the monkeys. Care was taken

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to see that the entire material was consumed by the animal and those animals where uncertainty existed regarding intake were discarded from the study. In a separate study, a suspension of Banjauri plant powder in water was deposited in the stomach of female monkeys using a Riles tube.

The first set of experiments were carried out to determine whether the extract is toxic to the monkeys. To a set of monkeys, a total dose of 26 g/monkey (2 g of powder/day) was fed over a period of 13 days. A control set of 3 monkeys received only brown sugar and groundnut powder. The initiation of the feeding of the plant powder to each monkey was done on different days of menstrual cycle as the study was primarily aimed to examine whether the material has any toxic effects on the monkeys. Blood samples were collected before and after the feeding of the powder and analysed for haematological parameters. After feeding the animals were sacrificed and brain, liver, kidney, gonads, muscle and other tissues were collected for histological examination. The blood samples were subjected to haematological, biochemical and endocrinological studies.

Two regimens were employed to ascertain the antifertility effect of Banjauri. The first consisted of feeding Banjauri on the 2nd, 3rd, 4th and 5th day following delivery of the young (Group I). In the second, Banjauri was fed daily from day 1 to 14 of a menstrual cycle (Group II). The post-partum monkey study consisted of two subgroups. While group IA received a total dose of 6 g (1.5 g/day) for 4 days starting from 2nd to 5th day post-partum group IB monkeys were fed a total dose of 60 g (5 g/day given in two doses, one in the morning and one in the evening) over a period of 12 days from day 2 to 14 after delivery. The cycling monkey study was again made up of 2 subgroups. While monkeys in group IIA were given 1.0 g/day for 14 days from day one of the menstrual cycle, those in group IIB were given 2.0 g/day for 14 days from day one of menstrual cycle. A third group (IIC) was given a total dose of 50 g (5 g/dose) from day 1 to day 10 of menstrual cycle.

In the case of monkeys which were fed Banjauri during post-partum period, their fertility was tested following weaning of babies (done six months after delivery) and return to cyclicity (approximately 9–12 months after parturition). In the case of monkeys fed Banjauri during menstrual cycle, the animals were tested for fertility from second cycle onwards.

Female monkeys which had received the drug were placed with proven fertile breeder males (1 : 1) between days 9 and 14 of a menstrual cycle. Those monkeys which did not become pregnant during the first cycle

Table 1. Effect of feeding vehicle (for *Vicoa indica*) for 12 days to bonnet monkeys (*Macaca radiata*) on haematological parameters

	Pre-treatment ($n = 3$)	Post-treatment ($n = 3$)	Pre-treatment ($n = 4$)	Post-treatment ($n = 4$)
Haemoglobin (g%)	10.6 ± 0.3	11.4 ± 0.4	10.5 ± 0.5	11.25 ± 0.6
PCV (%)	36.0 ± 1.7	33.0 ± 1.5	35.0 ± 2.3	32.8 ± 2.5
TLC (10/cmm)	11.8 ± 1.7	9.4 ± 0.54	10.8 ± 2.3	9.5 ± 1.3
DLC:				
Polymorphs (%)	28.3 ± 3.2	37.0 ± 5.7	39.8 ± 6.0	40.5 ± 8.1
Lymphocytes (%)	64.3 ± 4.3	58.3 ± 4.4	54.5 ± 6.3	54.8 ± 8.4
Monocytes (%)	0	0	0	0
Eosinophils (%)	6.7 ± 2.4	4.7 ± 1.5	0.58 ± 0.1	0.5 ± 0.1
Basophils (%)	0	0	0	0
Platelets (10/cmm)	3.7 ± 0.4	4.2 ± 0.6	3.9 ± 0.3	4.2 ± 0.2
Reticulocytes (%)	0.97 ± 0.1	2.3 ± 0.2	0.7 ± 0.1	2.5 ± 0.3

Values are mean ± SE.

Table 2. Effect of feeding vehicle (brown sugar and groundnuts) for 12 days to bonnet monkeys (*Macaca radiata*) on biochemical parameters

		Pre-treatment ($n = 3$)	Post-treatment ($n = 3$)	Pre-treatment ($n = 4$)	Post-treatment ($n = 4$)
Total proteins	(g%)	7.5 ± 0.2	7.5 ± 0.26	7.85 ± 0.17	7.8 ± 0.3
Albumin	(g%)	3.8 ± 0.2	4.3 ± 0.2	3.75 ± 0.08	4.1 ± 0.15
Cholesterol	(mg%)	164.3 ± 12.8	160.3 ± 12.8	167.5 ± 5.4	171.8 ± 11.5
SGOT	(U/l)	19.3 ± 2.3	24.3 ± 3.7	28.8 ± 6.0	28.8 ± 6.3
Alk. phos.	(U/l)	47.7 ± 8.4	32.5 ± 5.7	43.3 ± 3.6	33.5 ± 5.7
Bilirubin	(mg%)	0.53 ± 0.09	0.64 ± 0.02	0.48 ± 0.04	0.4 ± 0.8
BUN	(mg%)	21.8 ± 2.1	22.2 ± 2.8	26.0 ± 4.8	28.5 ± 0.6
Glucose	(mg%)	91.7 ± 6.0	73.3 ± 4.7	84.3 ± 9.9	92.6 ± 10.9

Values are mean ± SE.

exposure were exposed to fertile males during subsequent cycles.

The ovulatory nature of the cycle and establishment of pregnancy were determined by assaying serum for estradiol 17-β and progesterone and CG by methods standardized earlier in this laboratory[3].

Feeding trials revealed that the groundnut powder and brown sugar are very good additives as they completely masked the leafy odour. However, attempts to feed beyond 5 g/day were not successful as the monkeys wasted considerable quantities of the material fed.

Tables 1 and 2 show that there are no gross changes in the haematological, biochemical and histological parameters and as such the plant powder at the dose tried was considered to be non-toxic. Though a detailed study was not carried out even in the monkeys fed 5 g of Banjauri per day for 10 days no deleterious effects were observed.

Table 3 shows that feeding of 50 g of Banjauri had no significant effect on the serum estradiol on day 9 and 11 and progesterone on day 18, suggesting that Banjauri had no gross effect on the menstrual cyclicity. However, it should be noted that decrease in serum progesterone levels (less than 2 ng/ml Banjauri fed in monkeys compared to >2 ng/ml in control monkeys) did not result in any alteration in the length of the menstrual cycle.

Table 4 also shows that all the control monkeys fed vehicle become pregnant within 2.2 cycle exposures to proven fertile male monkeys, an observation which is in agreement with the earlier results[4].

Table 5 shows that the monkeys fed either 6 g or

Table 3. Serum estradiol from day 1 to 10 of menstrual cycle and progesterone levels in vehicle fed ($n=3$) and *Vicoa indica* (50 g/day $n=4$) fed monkeys

Group	Duration of cycle (days)	Serum estradiol (pg/ml) on		Serum progesterone (ng/ml)
		Day 9	Day 11	Day 18
Control	29 ± 3	136 ± 17	236 ± 21	1.52 ± 0.2
Vicoa indica	27 ± 5	242 ± 50	178 ± 18	1.60 ± 0.5

Mean ± SE.

Table 4. Effect of feeding dried Banjauri plant powder during post parturition period on fertility in the female bonnet monkey

Group	n	Total dose (g)	No. of ovulatory cycles/ total no. of cycles exposed	Mean no. of cycles needed to become pregnant	Parameter
Control	5	6 (vehicle)*	11/16	2.2	All delivered live young
IA	6	6 (Banjauri)	18/30	3.0	All delivered live young
IB	5	50 (Banjauri)	6/9	1.2	All delivered live young

*A mixture of groundnut powder and brown sugar was used as vehicle. The total dose was given orally in 4 equal portion on days 2, 3, 4 and 5 of lactation.
Each animal was exposed to a proven fertile male during day 9–14 of each cycle. The cycle was considered ovulatory if serum estrogen on day 8/9 was at least 200 pg/ml (range 200–960 pg/ml) and serum progesterone on day 18/19 was at least 2 ng/ml (range 2–9.6 ng/ml).

Table 5. Effect of feeding dried Banjauri plant powder during menstrual cycle in proven fertile female bonnet monkeys on their ability to conceive during subsequent exposure to fertile males

Group	n	Dose (g/day)*	No. of ovulatory cycles/ total no. of cycles exposed	Mean no. of cycles needed to become pregnant	Parameter
IIA	4	1.0	14/16	3.5	All delivered live young
IIB	6	2.0	82/126	–	None became pregnant even after 13.7 ovulatory cycle exposures
IIC	3	5.0	17/	–	One became pregnant after 6 cycles One became pregnant after 10 cycles One showed erratic cycles

Each animal was exposed to a proven fertile male during day 9–14 of each cycle. The cycle was considered ovulatory if serum estrogen on day 8/9 was at least 200 pg/ml (range 200–960 pg/ml) and serum progesterone on day 18/19 was at least 2 ng/ml (range 2–9.6 ng/ml).
†Mean cycle length of 28 g Banjauri fed monkeys computed from 54 cycles was 29.2 ± 4.4 days.
*Whereas groups IIA and IIB received treatment from days 1 to 14 of the first cycle, group IIC received treatment for days 1–10 of the first cycle.

50 g of Banjauri during post-partum period were not protected against pregnancy and all the animals became pregnant within 2 to 3 cycle exposures to proven fertile males.

To test the efficacy of Banjauri to block fertility of regularly cycling monkeys totally three different regimens were employed. Monkeys which were fed 1 g/day over 14 days ($n=4$) became pregnant within 3 exposures to males. However, monkeys which were fed 2 g/day for 14 days remained non-pregnant even after 22 (mean) exposures to proven fertile male monkeys (Table 5). The number of ovulatory cycle exposures varied from a minimum of 14 to a maximum of 25. Out of these cycles 65% were ovulatory as judged by serum estradiol levels on day 9/10 and progesterone levels on day 18 of cycle. In the group which received 5 g/day over a period of 10 days one became pregnant after six cycles, one conceived after 10 cycles while one exhibited very erratic cycles and as such breeding studies with this were discontinued. It was also noticed that the animals which were fed 2 g Banjauri/day did not cycle properly during later part of the study (i.e. 18 months after termination of feeding) as judged by serum steroid (E_2 and P_2) hormone levels. A comparison of the number of exposures needed for pregnancy in our colony revealed that while in the control monkeys an average of 2.2 cycle exposure is needed to become pregnant, in the monkeys fed Banjauri during the menstrual cycle the animals remained non-pregnant even after 20–22 cycle exposures.

Indian folklore has several claims of medicinal plants having contraceptive efficacy. The majority of the claims, however, have not been subjected to strict scientific verification. The present study reveals that the dried powder of *Vicoa indica* administered as suggested by the folklore, i.e. in post-partum monkeys is not effective in conferring protection against pregnancy during subsequent exposures to proven fertile males. The dose administered was based on that claimed to be effective in the human. However, the animals which were fed a total dose of 28 g during days 1–14 of menstrual cycle, remained non-pregnant even after 150 ovulatory cycle exposures. It should be emphasized that the colony data suggest that an average of 3 ovulatory cycle exposure is adequate for pregnancy establishment and the fertility index of the female colony is fairly high. Also of the total number of cycles exposed, only ovulatory cycle was considered and even this is 5 times more than the number required for the control and in spite of so many exposures the animals have remained non-pregnant. A similar effect was observed in animals fed Banjauri by intragastric deposition. In view of the fact that the administration of plant powder is effective only when fed during the menstrual cycle, it is possible that the active principle in the plant induces some irreversible changes in the reproductive tract (fallopian tube or uterus) resulting in infertility. Although this is only a preliminary study providing evidence only for the efficacy of the plant and not identifying the active principle, we feel that this observation needs attention in view of the possible potential as safe oral contraceptive. It is pertinent to note here that in a recent clinical trial conducted with Banjauri on 362 lady volunteers who were given 50 g of powder in a single dose on 2nd day of abortion along with water and eleven black pepper seeds only 14 cases of drug failure were reported[5]. It is currently unclear if the active principle of this plant product is affecting implantation or early embryonic development in the fallopian tube itself. Three compounds (sesquiterpene lactones) designated as Vicolide A, Vicolide B and Vicolide C have been isolated and characterized from the plant *Vicoa indica*. Of these, vicolide B was found to have antifertility activity in Wistar rats when administered at a dose of 100 mg/kg body weight[6] and this was ascribed to the compound having antiestrogenic activity[7]. The possibility of these compounds bringing about occlusion of the fallopian tube has also to be considered. In this connection, the ability of quincarine to bring about occlusion of the fallopian tube may be of significance[7]. Considering that this plant extract provides extended protection against contraception following ingestion for one cycle only, it is felt that further controlled studies with the crude plant extract and few of the vicolide derivatives in non-human as well as human primates are warranted.

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