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TOXICOLOGICAL STUDIES OF HERBAL BEVERAGE AND SEEDS EXTRACT OF *HIBISCUS SABDARIFFA* L. (ROSELLE)

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Toxicological effects of herbal drink prepared from exotic plant, *Hibiscus sabdariffa L.* (Roselle) were studied on albino miceand rats. For this purpose different doses of the drink were administered orally to different groups of test animals which were kept under observation for a period of three months. The roselle seeds extract was also administered by the same method and tested on the animals. After autopsy, the histopathological examination of liver and kidneys of the treated animals showed on cytotoxic effects.

Key words: *Hibiscus sabdariffa* L., Herbal beverage, Toxicology.

Introduction

Recent experiments on autecology and culture have shown that *Hibiscus sabdariffa* L. (Roselle), an exotic plant of economic importance, can be cultivated at Karachi successfully. The leaves are used as pot herb in many countries and have emollient effect [1]. The red fleshy calyces and involucles surrounding the young fruits are very acidic and source of a red beverage. They contain citric acid and salts serve as diuretic. The juice is also used for flavouring and in making jams, jellies, wines, sauces, chutneys, presserves and ices. The leaves are used in salads, as pot herb and for seasoning curry [2,3].

The drink made by placing the calyx in water, is said to be a folk remedy for cancer [1,4]. Flowers of Roselle contain gossypetin, anthocyanin and glucoside hibiscin which have diuretic and choleretic effects, decreasing the viscosity of the blood, reducing blood pressure and stimulating intestinal peristalsis. The seeds are used for debility, diuretic and tonic [5,6]. The fruits and seeds have been used as an aphrodisiac coffee substitute [7]. Dried fruits also contain vitamin C [8]. Roselle red from the calyces may be used as a food dye. The red pigment , high in anthocyanin is found in dried calyces of roselle [9].

Keeping the above facts in view, it was found worthwhile to utilize fleshy calyces for making herbal beverage. The beverage was prepared in the Karachi Laboratories Complex, Karachi. The present studies deal with the toxicity tests of Roselle beverage and seeds on animals model to asses its suitability for human consumption and for use in the preparation of food and feed.

Materials and Methods

Roselle beverage prepared from the calyces was tested for toxicological studies in albino mice and rats (Sprague Dawley strain) for chronic studies. The toxicity of beverage was determined by oral administration in normal and healthy albino mice and rats weighing between 25-30g and 250-300g respectively. They were reared at PCSIR Labs. Complex, Karachi, Animal House. The ani mals were kept under optimal experimental conditions and were observed for a period of seven days. They were housed in plastic cages measuring 35 x 45 x 35 cm. with sliding perforated stainless steel covers.

Dosage regimen. The test material was administered at a dose range of 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 ml/kg in mice and a set of control was kept on normal saline dose. Another batch was administered dose range of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5 ml/kg body weight in rats respectively, to ten groups of animals for three months and eleventh group was maintained as control and received normal saline. Each group comprised fo six animals of either sex (3 male and 3 female) and dept in separate cages which were observed for three months.

The toxicity of *Hibiscus* seeds was tested and determined by administration of dried powder via oral route in normal albino mice and rats, weighing between 20-25g, 110-120g by standard method [10]. The test material was administered at a dose of 100, 200, 300, 400, 500 and 600 mg/kg in albino mice and 1, 2, 3, 4, 5 and 69kg body weight in rats respectively to six groups of animals daily for three months and the seventh group was maintained as control and received the placebo. Each group comprised of six animals which was also observed for the same period. No mortality was recorded during the period of observation.

The physiochemical properties of the drink were also studied and compared with the standard drink available in the market. The colour, odour and smell of the herbal drink were kept natural. No synthetic and artificial chemical and flavour were added. The odour, pH, density and refractive index were

also studied and compared with the standard drink. The data was statistically analysed and the treatments were compared by Duncon's Multiple Range Test [11].

Histopathological studies. For histopathological studies, the treated animals were atuopsied, after three months. Liver and kidneys were taken out (Figs. 1,2). The small pieces of 2 to 3 mm were subjected to fixative Bouin's fluid for 18 to 24 hrs. After the fixation, the tissue was treated with different grades of alcohol in the following orders. 70% alcohol/hr, 80% alcohol/hr, 95% alcohol/hr, 100% alcohol/hr.

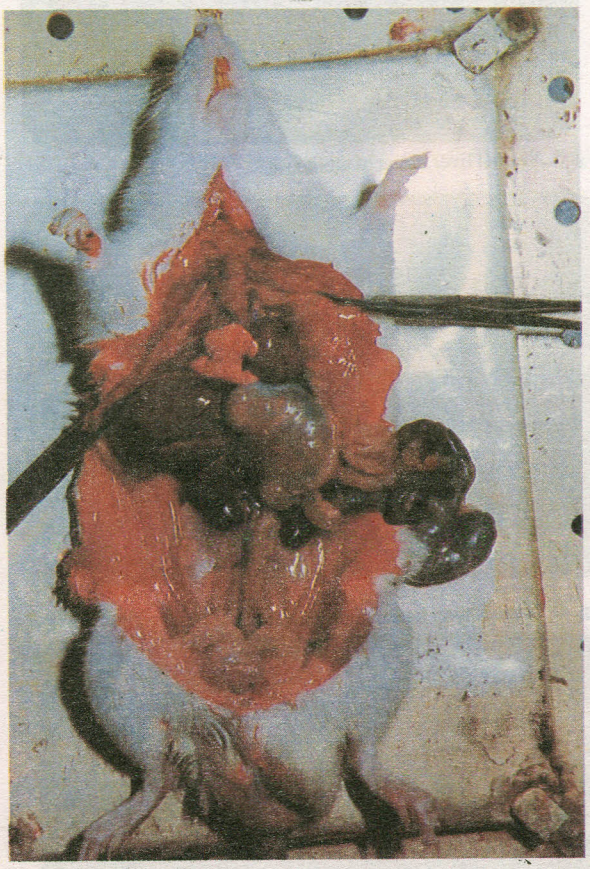

Fig. 1. Control albino rat.

Fig. 1A. Control and treated mice..

TABLE 1. ORAL TOXICITY TEST ON ALBINO MICE, (HERBAL DRINK).

Group No.	No. of animals	Mean initial weight in g	Mean final weight in g ml/kg	Oral dose of drink in ml/kg	Mortality	Toxic effect
1.	6	23 ± 2	24 ± 2	0.05	Nil	Nil
2.	6	24 ± 3	25 ± 3	0.1	Nil	Nil
3.	6	18 ± 2	29 ± 1	0.15	Nil	Nil
4.	6	27 ± 2	28 ± 2	0.2	Nil	Nil
5.	6	25 ± 2	26 ± 2	0.25	Nil	Nil
6.	6	29 ± 1	30 ± 1	0.3	Nil	Nil
7.	6	25 ± 2	26 ± 2	0.35	Nil	Nil
8.	6	24 ± 2	25 ± 2	0.40	Nil	Nil
9.	6	22 ± 4	23 ± 3	0.45	Nil	Nil
10.	6	23 ± 4	24 ± 4	0.5	Nil	Nil
11.	6	24 ± 4	25 ± 3	Normal saline	Nil	Nil

*Each group consisted of 6 animals (3 male and 3 female) and received syrup drink. Eleventh group was kept as control and received only normal saline. Means are followed by S.E.

Later the tissue was treated with Xylene for 1 hr in stage of 30 mins duration. Finally the tissue is ready for embedding. The transverse sections of the tissue were prepared of 6 mμ thickness. The section of the tissue were stained in the following manner using Harriss Haematoxylin and Eosine.

The section were treated with Xylene for 2 mins to deparraffinised, again treats with Xylene for 2 mins.

The sections were then subjected to dehydration with the following alcohol grades for 2 mins.

1. 100% alcohol to remove xylene.
2. 95% alcohol.
3. 80% alcohol 2 minutes each.
4. 75% alcohol.
5. Distilled water.

Then the sections which are dehydrated were washed to remove alcohol with distilled water. They were stained using haematoxylin stain for 1 1/2 or 2 mins. Later, they were washed with tap water for 10 mins and dehydrasted with 70%, 80% alcohol. Then section II were transferred to eosine 5% and again treated 95% alcohol to wash excess stain. This was subjected to 100% alcohol two changes and xylene two changes and finally fixed in Canada Balsam.

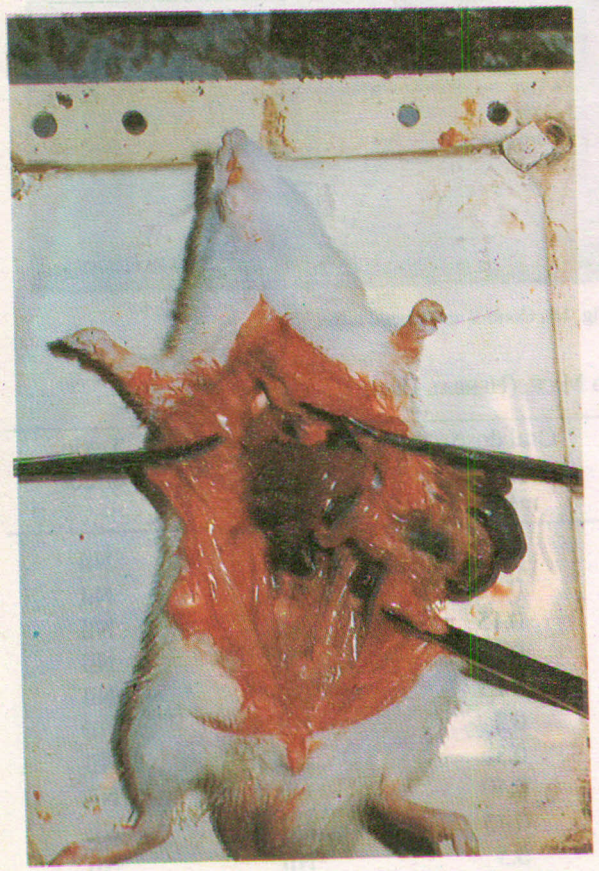

Fig. 2. Treated albino rat.

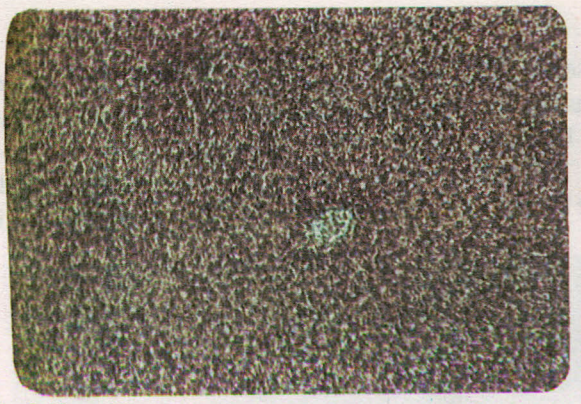

Fig. 3. Normal liver tissue of albino rat.

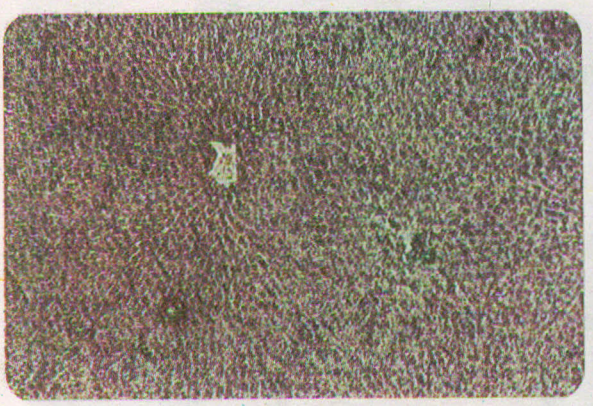

Fig. 4. Treatedl liver tissue of albino rat.

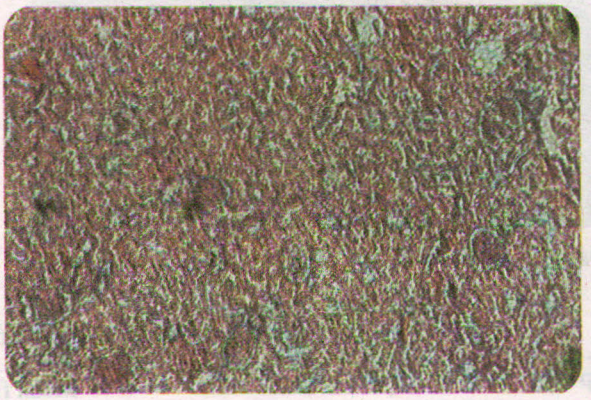

Fig. 5. Normal kidney tissue of albino rat.

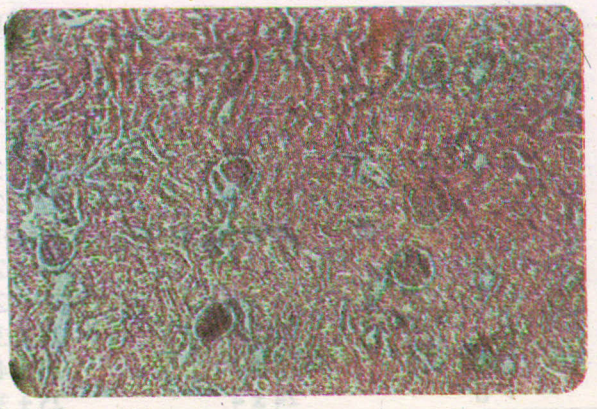

Fig. 6. Treated kidney tissue of albino rat.

TABLE 2. ORAL TOXICITY TEST OF HERBAL DRINK ON ALBINO RATS.

Group No.	No. of animals	Mean initial weight in g	Mean final weight in g ml/kg	Oral dose of drink in ml/kg	Mortality	Toxic effect
1.	6	250 ± 10	252 ± 10	0.5	Nil	Nil
2.	6	260 ± 5	262 ± 5	0.1	Nil	Nil
3.	6	260 ± 10	261 ± 10	1.5	Nil	Nil
4.	6	245 ± 10	247 ± 10	0.2	Nil	Nil
5.	6	250 ± 5	252 ± 5	2.5	Nil	Nil
6.	6	255 ± 10	256 ± 10	0.3	Nil	Nil
7.	6	260 ± 5	261 ± 5	3.5	Nil	Nil
8.	6	260 ± 10	261 ± 10	4.0	Nil	Nil
9.	6	260 ± 50	259 ± 5	4.5	Nil	Nil
10.	6	260 ± 5	258 ± 6	5.0	Nil	Nil
11.	6	250 ± 10	252 ± 10	Normal saline	Nil	Nil

*Each group consisted of 6 animals (3 male and 3 female) and received syrup/drink. Eleventh group was kept as control and received only normal saline. Means are followed by S.E.

TABLE 3. TOXICITY TEST OF DRY MATTER ON ALBINO MICE (SEED).

Group No.	Mean weight g	Oral dose in ml/kg	Toxic effect
1.	20 ± 3	100	Nil
2.	20 ± 2	200	Nil
3.	20 ± 4	300	Nil
4.	18 ± 3	400	Nil
5.	18 ± 3	500	Nil
6.	21 ± 3	600	Nil
7.	22 ± 2	Basal diet	Nil

*Each group consisted of 6 animals and received *Hibiscus seeds* (dry matter). Means are followed by S.E.

TABLE 4. TOXICITY TEST OF DRY MATTER ON ALBINO RATS (SEED).

Group No.	Mean weight g	Oral dose in ml/kg	Toxic effect
1.	100 ± 10	1	Nil
2.	100 ± 20	2	Nil
3.	95 ± 15	3	Nil
4.	100 ± 10	4	Nil
5.	100 ± 20	5	Nil
6.	100 ± 20	6	Nil
7.	100 ± 15	Basal diet	Nil

*Each group consisted of 6 animals and received *Hibiscus seeds* (dry matter). Seventh group was kept as control and received only basal diet. Means are followed by S.E.

Results and Discussion

The results recorded that oral administration of syrup in doses of 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 ml/kg body weight to albino mice (Table 1) and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5 ml/kg body weight to albino rats (Table 2) did not show any toxic effect. During three months observation period it was found that there was no change in physical condition of the animals and no mortality was observed. After autopsy, no gross changes were found in heart, lungs, liver, spleen, g.i. tract, kidneys, ovaries and testes. Since the results showed that the herbal drink to be no-toxic (5 to 15g/kg), (10, 12, 13), it is concluded that it has the potential of being used as source of energy in food and feed.

No histopathological changes have been observed in both organs, size of organs also remained normal within limited sources and in view of technique employed, no change in morphology was note. There was no change in histology of liver and kidney. (Fig. 3-6).

The results recorded that oral administration of *Hibiscus* seeds in doses of 100, 200, 300, 400, 500 and 600 mg/kg body

TABLE 5. PHYSIO-CHEMICAL PROPERTIES OF THE TEST & STANDARD SYRUPS.

Sr. no.	Syrup (drink)	Colour	Smell	Odour	pH	Weight/ml (density at 20°C)	Refrective index at 14°C
1.	Test drink	Bright red	Pleasant	Odour-less	4	1.1747g	1.39640
2.	Standard	Red	Pleasant	Odour-less	3	1.3793g	1.47977

weight to albino rats (Table 4) did not show any toxic effect. It was also observed that there was no change in physical condition of animals during three months observation period.

The physico-chemical properties revealed that the colour, small, odour, pH, density and refractive index of the drink as compared to standard which has been incorporated in the (Table 5).

Precisely, the herbal drink prepared from Roselle and tested clinically is safe ad refreshing for human consumption.

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