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Research Article

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TOXICITY OF RAW DIETARY *Hibiscus sabdariffa* LINN *(MALVACEAE)* SEED MEAL TO WISTAR RATS

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Abstract

In the Sudan, fermented *Hibiscus sabdariffa* seeds are commonly known as Furundu, with high quality proteins consumed as substitute to meat mainly in the Western part of the country. The raw *Hibiscus sabdariffa* seed meal (HSSM), 10, 15 and 20 % of the basal diet was fed to Wister rats for 12 weeks. These doses were found to be non-toxic but not lethal to the treated rats. There was depression of growth and diverse enterohepatonephropathies which were correlated with highly significant ($P \leq 0.001$) elevation of serum AST, ALT and ALP activities and bilirubin ($P \leq 0.01$) concentration in addition to a significant decrease ($P \leq 0.01$) in cholesterol concentration. No changes were observed in the total protein, globulin and urea concentrations of the test groups and albumin concentration in Groups 1 and 2 fed on diets containing 10 and 15% HSSM. These findings were accompanied by leukocytosis and significantly lower red blood cells and haemoglobin concentration. Due to the elevated Mean corpuscular volume (MCV) and the normal values of the mean corpuscular Haemoglobin concentration (MCHC) in all the treatment groups, the toxicity of HSSM to Wistar rats resulted in macrocytic normochromic anaemia.

Key words: *Hibiscus sabdariffa* seed, Wistar rats, body weight, biochemical changes, pathological changes

Introduction

Sudan is the world largest exporter of Karkade (*Hibiscus sabdariffa*) and its production has been increasing during the last few years, where the area of production was about 1214 hectare. Average calyx yield is estimated to be about 50 Lb/acre. An approximately equal amount of seed is produced by the crop (MAF, 2010).

Hibiscus sabdariffa is a hardy herbaceous shrub belonging to the Family Malvaceae, known as Roselle or Red Sorrel (English) and Karkade (Arabic). It is thought of being native to Central and West Africa, South East Asia and elsewhere in parts of West Indies, Jamaica and Central America. The thick red and fleshy cup-shaped calyces of the flower are consumed worldwide as a cold beverage and as a hot (sour tea) drink (Morton, 1987).

The nutritional composition of Karkade seed as well as its functional properties (water absorption capacity, fat absorption capacity, bulk density, apparent viscosity etc.) is rarely studied compared to the calyces. The seed is among the highest protein- containing seeds when compared with others like passion fruit and black cumin seeds. Bakheit (1989) found that Karkade seed (El Rahad variety-Sudan) to contain 6.90, 16.65 and 7.1% moisture, crude fiber and ash, respectively. Al Wandawi *et al*. (1984) reported that Karkade seed contained 16.30% crude fiber, 96.64% total carbohydrates and 5.19% ash. Yagoub (1998) reported 26.79% protein 25.52% total carbohydrates, 22.39% crude fiber and 4.77% ash.The oil derived from *Hibiscus sabdariffa* seed was characterized by Salama *et al*. (1979) and concluded that the oil can be of great economic use and sreported the seed to contain 21.71% oil. Ahmed and Nour (1993) stated that the oil of *H. sabdariffa* seed resembled other vegetable oils in its saponification and iodine values and it rich in the essential fatty acid linoleic and considered edible after suitable methods of processing (refining, bleaching, deodorization, heating).

Beside the usual fatty acids, Karkade seed also contains unusual cyclopropenoid fatty acids which are normally found in the seed lipids of the order Malvales. It was reported to contain sterulic and malvalic acids, and 12, 13-epoxoleic in the percentages of 2.9, 1.3 and 4.5 % respectively (Ahmed *et al.,* 1979). It was also found that the seed contained anti-nutritional factors like gossypol which causes undesirable physiological disorders in non-ruminants, tannins (El-adawy and Khalil, 1994) and phytic acid (Yagoub, 1998).

This study was conducted to investigate the organotoxicity of the dietary *Hibiscus sabdariffa* seed meal (HSSM) to Wistar rats.

Materials and Methods

Test Karkade (Hibiscus sabdariffa L.) seeds

Karkade (*Hibiscus sabdariffa L.*) seeds (Figure 1), originating from Northern Kordofan, El Rahad area, were purchased from a local market in Omdurman, cleaned off contaminants and contained in plastic bags before being crushed into meal prior to feeding.

Fig. 1: Karkade seeds

Experimental rats and feeding

Thirty two Wistar rats, of balanced sexes, were obtained from the Aromatic Herbs and Medicinal Plants Research Institute (A.H.M.P.R.I), Khartoum, reared within the premises of the Institute exposed to 12 hours photoperiod with feed and drinking water provided *ad libitum* before the commencement of experimental feeding. Room temperature was maintained at 25±2ºC at adequate house ventilation.

Rat basal diet

The Percent (%) inclusion rates (fresh basis) of ingredients of the basal diet fed to experimental rats are: meat meal= 42.5; Grain starch = 39.2; Granulated sugar = 05.0; Cellulose powder = 03.0; Corn oil= 05.0; Super concentrate = 05.0; and DI- methionine= 00.3.

Experimental design and dosing

At the age of 60 days, the rats were allotted randomly to four groups each of 8 rats. Karkade (HSSM) was thoroughly mixed with the basal diet in dilution and fed to rats at 10 (Group 2), 15 (Group 3) and 20% (Group 4) whereas Group 1 was fed the basal diet and served as control. Experimental feeding was continued for 12 weeks.

Four rats -selected randomly- from each group were slaughtered at weeks 6 and 12 for post mortem examination and vital organs sampling for histopathology. Blood samples were collected at slaughter for hematology and serum analysis.

Data collected

Body weight changes

Initial body weight was recorded at the first day of experimental feeding and thereafter weekly throughout the 12 weeks feeding period.

Serobiochemical parameters

Sera separated from blood samples collected at slaughter were stored at -20ºC until analyzed for the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and for the concentrations of total protein, albumin, globulin, bilirubin, cholesterol and urea.

Hematological parameters

EDTA-added blood samples were analyzed for haemoglobin concentration (Hb), red blood cells (RBC) counts, packed cell volume (PCV), mean corpuscular volume, mean corpuscular hemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) and total white blood cell (WBC).

Pathological changes

Necropsy was conducted to identify gross lesions and eventually specimens from the liver, kidneys, heart, spleen, and intestines were immediately fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 µm and stained routinely with haematoxylin and eosin (H & E).

Statistical analysis

Mean values in live weight, blood and serum were compared to the control using student's *t*-test **(Snedecor and Cochran, 1989)**.

Results

Changes in body weight and weight gain

The effects of feeding diets containing 10, 15 or 20% HSSM seed on body weight changes of rats are shown in Table 1. After 6 weeks of feeding, the rats fed the 20% HSSM had the highest ($p \leq 0.001$) gain in body weight. However, and during the same period, the rats on diets containing 10 and 15% HSSM showed significant decrease ($p \leq 0.05$-0.01) in both Final and body weight gains.

At the end of the experimental period (12 weeks), the rats fed on diet containing 10% HSSM had the lowest ($p \leq 0.001$) body weight gain and consumed less feed. None of the experimental rats died during the course of the experiment.

Pathological changes

After 6 weeks of treatment, mild macroscopic or microscopic changes were observed in the vital organs of the test groups 2, 3 and 4. Degeneration of the epithelial cell of scattered proximal convoluted tubules of the rats of groups 2 and 4 fed on 10 and 20% HSSM respectively. Individual cell necrosis and small fatty vacuoles in the centrilobular hepatocytes of rats in group 3 fed on 15% HSSM were observed.

Table 1: Average (mean ± S.E.) body weight (g) changes of rats fed on raw HSSM for 12 weeks.

Group	Initial body weight	Final body weight	Body weight gain
6 weeks			
1 (Control)	92.88±12.50	132.50±2.50	40.00±15.00
2 (10% HSSM)	78.75±2.39	111.30±5.90*	320.5±8.30**
3 (15% HSSM)	73.75±4.73	107.50±5.20**	33.80±4.30**
4 (20% HSSM)	71.66±6.00	173.03±4.40***	101.70±8.80***
12 weeks			
1 (Control)	82.50±2.50	210.00±5.00	127.50±7.50
2 (10% HSSM)	67.50±3.22	143.80±6.60***	76.30±5.90***
3 (15% HSSM)	62.50±6.29	147.50±2.50***	85.00±7.10***
4 (20% HSSM)	70.00±2.04	155.00±7.40***	85.00±5.40***

NS=Not significant; * Denotes mean value significant at (P≤0.05), ** Denotes mean value significant at (P≤0.01), *** Denotes mean value significant at (P≤0.001).

At the end of week 12, congestion of the cardiac vessels and hepatic portal and central veins and necrosis of the centrilobular hepatocytes (Figure 1) were seen in rats of group 2 fed on 10% HSSM. In the rats fed 15 and 20% HSSM respective to groups 3 and 4, there was congestion of the blood vessels of the kidney and heart, severe necrosis of the cardiac muscle fibers, fatty cytoplasmic vacuoles and focal necrosis of the centrilobular hepatocytes and the renal proximal convoluted tubules and shrinkage of the Glomeruli (Fig. 2).

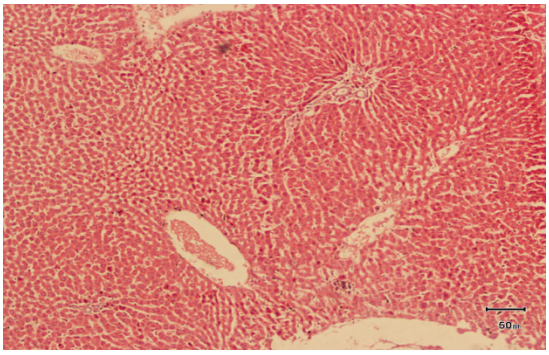

Fig.2. Fatty cytoplasmic vacuolation and necrosis of the centrilobular hepatocytes of a rat fed on 10% HSSM for 12 weeks. H & E x100.

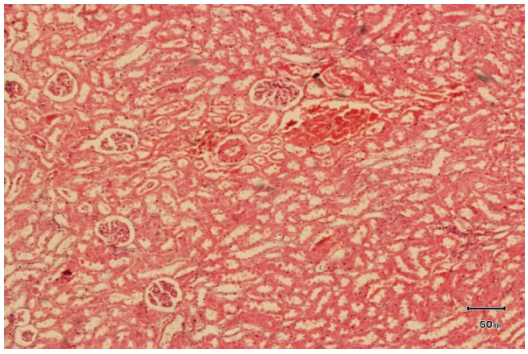

Fig. 3: Necrosis of the renal proximal convoluted tubules and packing of the glomeruli and hemorrhage in a rat fed on 20% HSSM for 12 weeks. H & E x 100.

Changes in serum constituents

Changes in the activities of AST, ALT and ALP and the concentrations of total protein, albumin, globulin, cholesterol, bilirubin and urea in the serum of the test and control rats are presented in Table 2. After 6 weeks of feeding, there were no significant differences in the concentrations of total protein, albumin, globulin and bilirubin between the test and the control animals. The activities of AST and ALP were higher (p≤0.05-0.001) in the test groups when compared to the control (group 1).

ALT activity increased (p≤0.05-0.001) in groups 3 and 4 but did not change in group 2 fed on 10% H. sabdariffa seed. Serum cholesterol and urea concentrations were significantly higher (p≤0.01-0.001) in group 2 than the control rats. Cholesterol concentration in groups 3 and 4 did not change but serum urea of the two groups was lower (p≤ 0.01) than the control rats.

At the end of the experimental period (12 weeks), there was a significant increases (P≤0.001) in the activities of AST, ALT and ALP, increase (p≤0.05) in the concentration of bilirubin and a significant decrease (p≤0.05-0.001) in serum cholesterol in the test groups than in the control (group 1). Albumin concentration decreased (p≤0.01) in group 4 and that of globulin was reduced (p>0.05) in groups 2 and 3 compared with the controls. Serum concentrations of the total protein and urea did not change in any of the test groups when compared with the controls of group 1.

Haematological findings

These data are shown in Table 3. At week six, there was no difference in the values of PCV, RBC, MCV and WBC between the test animals and the control group. The values of Hb, MCH and MCHC in group 4 were higher (p≤0.05) than that at of the control animals.

After twelve weeks, the values of Hb, RBC of the test animals, were significantly lower (p≤0.01) and those of MCV and MCH were higher than the control rats. The value of PCV in group 4 and that of WBC of groups 4 and group 2 were higher (p≤0.01-0.001) than the controls. The value of MCHC was not different among the test groups and the control.

Table 2: Average (mean ± SE) serobiochemical values in rats fed on various levels of HSSM for 12 weeks

Parameters	Treatment groups			
	1 Control	2 10% HSSM	3 15% HSSM	4 20% HSSM
6 weeks				
AST (iu)	188.00±8.00	238.70±10.70*	263.00±15.40*	257.70±13.10*
ALT (iu)	34.50±1.50	36.50±1.77^{NS}	65.00±2.12***	56.30±6.40*
ALP (iu)	129.00±3.00	159.70±1.08***	161.80±3.06**	162.30±6.35*
Total protein(g/dl)	7.70±0.05	7.70±0.14^{NS}	8.30±0.18^{NS}	7.60±0.19^{NS}
Albumin (g/dl)	3.50±0.15	3.60±0.14^{NS}	3.90±0.20^{NS}	3.90±0.19^{NS}
Globulin (g/dl)	4.20±0.10	4.10±0.04^{NS}	4.40±0.10^{NS}	3.70±0.23^{NS}
Cholesterol(mg/dl)	53.50±6.50	79.00±1.77**	57.00±1.47^{NS}	59.30±1.45^{NS}
Bilirubin (mg/dl)	0.400±0.10	0.33±0.06^{NS}	0.38±0.06^{NS}	0.37±0.08^{NS}
Urea (mg/dl)	65.50±0.50	83.80±1.08***	53.70±0.85**	54.70±1.45**
12 weeks				
AST (iu)	62.30±0.80	176.6±1.50***	148.6±1.50***	160.30±0.42***
ALT (iu)	31.20±0.85	66.8±3.22***	52.30±3.41***	68.80±2.74***
ALP (iu)	125.50±2.50	157.3±3.15***	148.50±1.04***	203.3±4.51***
Total protein(g/dl)	7.70±0.25	7.60±0.27^{NS}	7.50±0.31^{NS}	7.20±0.27^{NS}
Albumin (g/dl)	4.20±0.05	4.60±0.17^{NS}	4.20±0.13^{NS}	3.60±0.06**
Globulin (g/dl)	3.50±0.30	3.00±0.05^{NS}	3.30±0.20^{NS}	3.60±0.11^{NS}
Cholesterol(mg/dl)	69.00±1.00	57.00±1.73**	64.00±1.51*	51.30±2.28**
Bilirubin (mg/dl)	0.07±0.01	0.15±0.10**	0.19±0.17**	0.14±0.01**
Urea (mg/dl)	52.50±2.50	51.00±1.22^{NS}	52.50±0.50^{NS}	048.30±0.85^{NS}

NS=Not significant; * Denotes mean value significant at (P≤0.05), ** Denotes mean value significant at (P<0.01),*** Denotes mean value significant at (P<0.001)

Discussion

In spite of the use of *H. sabdariffa* seed in Western Sudan in the production of popular fermented product, "Furundu" toxicological information on the seed is unavailable. The results of the present study indicated that feeding rats with 10, 15 and 20% HSSM in of the basal conventional diet proved toxic, as evidenced by depressed growth, serobiochemical, haematological and histopathieson.

It is well known that response of animals to feeding plant material is dependent to the type of the active constituents and concentrations of the amount added to the diet as well as the rate of their metabolic conversion to metabolites and consequent excretion.

Previous phytochemical investigations of HSSM have demonstrated the presence of antinutritional factors like gossypol (Al-Wandawi *et al*., 1984 and Bakheit, 1989), tannins and phytic acid (El-Adawy and Khalil, 1994 and Yagoub, 1998) and protease inhibitors (Abu-Tarboush and Ahmed, 1996) that have been associated with reduction in food digestibility the decrease in nutrient bio-availability and flatulence production.

On the other hand, many different species of the genus Hibiscus were reported to contain particularly large

percentage of cyclopropenoid fatty acids in their oils, much larger level than those found in crude cotton seed (Ralaimanarivo *et al.,* 1982 and Sundar and Laksh, 1984). Cyclopropenoid fatty acids, however, can inhibit various enzymes involved in fatty acid biosynthesis, particularly in fatty acid desaturation (Fogerty *et al.,* 1972 and Gurr, 1974). There are reports showing that these fatty acids may be toxic to higher animals and perhaps (co-) carcinogenic (Rukmini *et al.,* 1982 and Gunstone *et al.,* 1994).

In rats fed 10, 15, and 20% HSSM damage to the vital organs probably contributed to the increase in serum enzymes activity and metabolites concentrations. Although, serum urea concentration in rats fed different concentration of raw HSSM pathological examination did not provide evidence of antinephrotoxic effect of the seed. Laskar *et al*. (1998) described antinephrotoxic activity of the plant, *Dolichos biflorus* against paracetamol induced hepatotoxicity in rats and suggested that biotransformation paracetamol into a nephrotoxic compound, p-aminophenol, is responsible for the elevation of blood urea nitrogen.

Feeding of rats to concentrations of 10, 15, and 20% of raw HSSM resulted in increased serum bilirubin. It has been found that the rise in serum bilirubin is due to periportal liver injury or periportal proliferation of the bile ducts previously described in sheep (Gopinath and Ford, 1972), in goats (Ali and Adam, 1978) and in rats (Adam,1999).

The anaemia is macrocytic normochromic as indicated by high MCV and normal MCHC. These findings suggested that the plant constituent (s) may be involved in the derangement of the haemopoietic process. This type of anaemia has been observed in sheep fed on *Rhayza stricta* (Adam, 1998) and chicks fed on *Cassia italica* (Bakheit and Adam, 1996).

Although, leukocytosis was observed in groups 2 and 4 fed on 10 and 20% raw HSSM, normal WBC count was seen in rats receiving intermediate (15%) dose of raw HSSM. Leukocytosis resulted from neutrophillia was noticed in sub-chronic toxicity of *Cassia obtusifolia* on rats (Voss and Brenncke, 1991)

From the results of the present investigation it was noticed that the liver is the most sensitive organ than the kidneys and intestines to the toxic action of the active constituents of the HSSM utilized.

Conclusion

Although *H. Sabdariffa* seed is highly nutritious and contained an oil of high value, its incorporation in the diet at 10, 15 and 20%, respectively is undoubtedly hazardous to Wistar rats and capable of biochemical and performance changes, anaemia, and leukocytosis.

Histopathological changes were not evident at 6 weeks of feeding but sufficient to impose significant haematological and serobiochemical changes.

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