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Effects of Dietary Furundu (Fermented *Hibiscus sabdariffa* Seed) Supplementation on the Performance, Some Blood and Serum Parameters and Histopathology of Wistar Rats

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Abstract: The present study was conducted to obtain information on the effects of various dietary doses of the fermented *Hibiscus sabdariffa* seed (Furundu) on Wistar rats. Emphasis was put on changes on growth, lesions, alteration in hematology and serobiochemical constituents and histopathology of treated rats. Fermented *Hibiscus sabdariffa* seed product (Furundu) was fed to Wistar rats at 10, 15 and 20%, respectively of the standard diet for 12 weeks. Incorporation in the diet of the three doses of both was toxic but not fatal. Depression in growth and hepatonephrotoxicity were observed in rats that had been given 10, 15 and 20% Furundu, respectively for 12 weeks. These findings were accompanied by leukocytosis, macrocytic normochromic anaemia in rats fed on 20% Furundu and macrocytic hypochromic, normocytic hypochromic anaemia in those fed on 10 and 15% Furundu respectively, in addition to alterations in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activity and albumin, globulin, bilirubin and cholesterol concentrations. At the end of the experimental period (12 weeks), the total protein of all the test groups and the globulin concentration of group 2 fed on 15% Furundu decreased significantly ($p<0.05$), albumin concentration of all the test groups and the urea concentration of group 3 fed on 15% Furundu remained unchanged. Histopathological examination showed Fatty cytoplasmic vacuolation of the centrilobular hepatocytes and lymphocytic infiltration, desquamation of the intestinal epithelium and intestinal congestion were noticed in rats fed on 20% Furundu. Fermented *Hibiscus sabdariffa* seed product Furundu is considered enterohepatonephrotoxic to Wistar rats at dietary levels of 10, 15 and 20%, respectively.

Key words: Body weight changes, fermented hibiscus sabdariffa, hepatotoxicity, furundu, hypoproteinaemia, macrocytic hypochromic anaemia, rats

INTRODUCTION

Fermented foods play an important socio-economic role in developing countries as well as making a major contribution to the protein requirements of natural populations. In general, traditional fermented foods are made under primitive conditions which result in low yields and poor quality products.

Generally, fermented oil seeds are not popular nutritious meat substitute protein foods but serve as condiments and flavors in soups. Traditionally, women carry out fermented food processing activities. In Africa food shortages, malnutrition and famines are the major and most persistent causes of human misery. Other sources such as diseases, civil wars and degenerate politics are intimately linked to the food problem (Dirar, 1993).

Although *Karkade* seeds are a promising source of dietary protein (Al-Wandawi *et al*., 1984) yet only meager information has been reported on their functional properties (El-Adawy and Khalil, 1994; Abu-Tarboush *et al*., 1997). Incidentally, the unpalatability of *Karkade* seeds renders them inconsumable as a food. Nevertheless, some Sudanese tribes in Kordofan region roast *Karkade* seeds, while other communities in western Sudan subject the seeds to a natural solid-state fermentation to produce the meat substitute, *Furundu* (Yagoub, 1998) similar to Dawadawa (Nigerian, Ghanaian fermented food).

The seeds of the plant contain toxic principals thus has no economic value. Villagers in production regions ferment the seeds into *Furundu* (fermented *Hibiscus Sabdariffa* seeds) and consume it. It is not yet fully understood whether or not such fermentation has a detoxication action on the seeds components. Epidemiological data might indicate the higher incidence of some morbid states in the consumption areas of *Furundu*. However, conducting some *in vivo* toxicological investigation might be useful in generating basic information on the wholesomeness of *Furundu* as a staple or famine food.

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Furundu is an interesting example of a high protein substitute produced by fermentation, a low-cost method of food preservation which markedly improves the digestibility, nutritive value and flavors of the raw seed.

Soups consumed with the staples are essential components of the diet and may contain a variety of seeds, nuts, pulses and leaves (Campbell-Platt, 1987). The bulk of the indigenous fermented condiments of Sudan are found in the western states. The north is very poor in food fermentations which are practically confined to the staple sorghum porridges and soured milks (Dirar, 1993). However, inter-state trade and population relocations have widened the spread of food fermentations throughout the country and beyond.

MATERIALS AND METHODS

Furundu preparation: At the Food Research Centre, Khartoum North and in 2010, a typical Furundu was prepared under the supervision of an expert housewife from western Sudan. Karkade seeds were cleaned from stones, leaves and other debris, washed with water and then sun-dried. They were divided into two portions, one was kept raw and the other was used for the subsequent Furundu preparation. The seeds were roasted for 5-7 min (heated with a flame on a metal plate as dry heat) till they softened and crushed easily to give a characteristic smell. Then they were cooled and pounded using a pestle and mortar to give a soft powder which was then sieved to pass 40-mesh screen to remove the testa or seed coats.

Five hundred grams of the sieved powder were mixed well with 600 mL of tap water to form a dough which was packed into a Burma or Kalol (a small-necked earthenware pot). The vessels were tightly covered with metal trays which were wrapped with several layers of clothes packed together with a thick polyethylene bag to provide warmth and a humid atmosphere and then incubated at room temperature (35±2ºC). The cover was removed on the 4th day of fermentation when the dough became delicate and at this stage Combu (Weikab) (which was purchased from the local market in Omdurman) was added (2.5 g Combu powder in 5 mL of water).

The mash was mixed and the paste kneaded to a firm consistency and incubated for a further 24 h. The paste (called Kunafa) was then moulded by hand into small cubes flat or rounded shapes (around 5-7 g, 10 cm in diameter and 2 mm in thickness), sun dried for 3-5 days under aerobic conditions and stored till used (Fig. 1).

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 (2010), reared within the premises of the Institute under 12 h 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.

Fig.1: Furundu

Table 1: Percent inclusion rates (fresh basis) of ingredients of the basal diet fed to experimental rats

Ingredients	%
Meat meal	42.5
Grain starch	39.2
Granulated sugar	05.0
Cellulose powder	03.0
Corn oil	05.0
Super concentrate	05.0
Dl-methionine	00.3

Experimental design, dosing and analysis:

Experimental design and dosing: At the age of 60 days, the rats were allotted at random to four groups each of 8 rats. Furundu 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 (Table 1). Experimental feeding was continued for 12 weeks.

Four rats from each group were slaughtered at weeks 6 and 12 for post mortum 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 weights of rats were recorded at the first day of experimental feeding and thereafter weekly throughout the 12 weeks feeding period.

Serobiochemical parameters: Blood samples were collected at slaughter in dry test tubes, serum was separated and 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: Blood samples were collected in dry test tubes containing EDTA (Ethylenediaminetetraacetic acid) and examined 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).

Histopathological changes: Necropsy was conducted to identify gross lesions and specimens of the liver, kidneys, heart, spleen, 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 body weight, blood and serum data were compared using student's t-test after (Snedecor and Cochran, 1989).

RESULTS

Changes in body weight and weight gain: The effects of feeding diets containing 10, 15 and 20% Furundu, respectively on body weight and weight gain of the rats are presented in Table 2. After 6 weeks of feeding, the rats on diets containing 10 and 15% Furundu showed significant decrease ($p<0.05$) in body weight, while group 4 fed on diet containing 20% Furundu showed a significant increase ($p<0.05$) compared to the control (group 1). Body weight gain was higher ($p<0.05$) in groups 2 and 3 than the control, that of group 4 was the highest ($p<0.01$) among the test groups and the control rats.

At the end of the experiment (after 12 weeks), the body weight and weight gain of the test groups 2, 3 and 4 was lower ($p<0.01$-0.001) than the control animals.

Pathological findings: At 6 weeks after the beginning of the test feeding mild degenerative changes in the liver, heart, intestine and kidneys were detected in the vital organs of the rats of the three test groups fed on 10, 15 and 20%, respectively. The liver of the rats fed on 15 and 20% Furundu showed isolated cell necrosis, fatty change in the centrilobular hepatocytes, scattered renal tubules were degenerated and the medullary rays were mildly congested.

At the end of the experiment, infiltration of lymphocytes was seen in the liver, renal cortex and between cardiac muscle fibers. The liver revealed fatty cytoplasmic vacuolation of the centrilobular hepatocytes and lymphocytic infiltration (Fig. 2), the renal tubules appeared degenerated and the glomerular tufts became shrunken or infiltrated with lymphoid cells. There was intestinal desquamation in group 4 fed on 20% Furundu (Fig. 3).

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 3. After 6 weeks of feeding, there were no significant differences in the concentrations of total protein, albumin, globulin, cholesterol and bilirubin between the test and the control animals. The activities of AST, ALT and ALP were higher ($p<0.05$-0.001) in the test groups compared to the control (group 1). Urea concentration was significantly higher ($p<0.05$) in groups 2 and 4 than the control, but did not change in group 3.

After 12 weeks of feeding, there was a significant increase ($p<0.001$) in the activities of AST, ALT and ALP, increase ($p<0.05$-0.01) in the concentration of bilirubin and a significant decrease ($p<0.05$) in serum cholesterol and globulin in group 2 than the control (group 1). Serum concentration of the total protein was

Table 2: Average (mean±S.E.) performance value (g) of rats fed on furundu for 12 weeks

Group	Initial weight	Body weight	Weight gain
6 weeks			
1 (control)	92.88±12.5	132.5±2.5	40±15.0
2 (10% furundu)	67.50±5.20	122.5±5.2*	55±5.4*
3 (15% furundu)	65.00±8.66	110±1.00*	45±8.7*
4 (20% furundu)	73.75±4.26	140.0±04.6*	66.3±8.0**
12 weeks			
1 (control)	82.50±2.50	210±5.0	127.5±7.5
2 (10% furundu)	66.25±6.57	166.3±8.8**	100±5.4**
3 (15% furundu)	70.00±10.6	137.5±4.8***	67.5±6.6***
4 (20% furundu)	70.00±2.04	178.8±5.2**	108.8±4.3**

NS: not significant; *: Denotes mean values significant at ($p<0.05$); **: Significant at ($p<0.01$);***: Significant at ($p<0.001$)

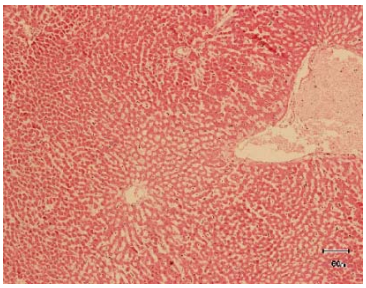

Fig. 2: Fatty cytoplasmic vacuolation of the centrilobular hepatocytes and lymphocytic infiltration in a rat fed on 20% Furundu for 12 weeks. H & E × 10

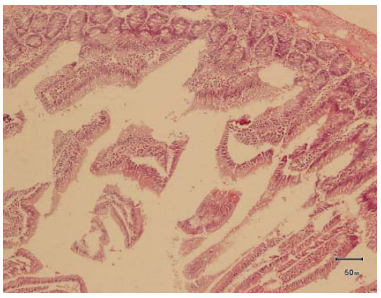

Fig. 3: Desquamation of the intestinal epithelium and intestinal congestion in a rat fed on 20% Furundu for 12 weeks H & E × 100

Table 3: Average (mean±SE) serobiochemical values in rats fed on various levels of furundu for 12 weeks

	Treatment groups			
Parameters	1 control	2 10% furundu	3 15% furundu	4 20% furundu
6 weeks				
AST (iu)	188±8.0	251.3±13.0*	244.6±12.5*	245±12.9*
ALT (iu)	34.5±1.5	49.8±3.42*	45.3±2.33*	60.5±1.04***
ALP (iu)	129±3.0	163.3±2.56***	161±2.33**	157±2.12*
Total protein (g/dL)	7.7±0.05	7.6±0.21^{NS}	7.5±0.12^{NS}	6.9±0.36^{NS}
Albumin (g/dL)	3.5±0.15	3.9±0.13^{NS}	3.7±0.16^{NS}	3.6±0.12^{NS}
Globulin (g/dL)	4.2±0.10	3.7±0.29^{NS}	3.8±0.17^{NS}	3.3±0.14 ^{NS}
Cholesterol (mg/dL)	53.5±6.5	55.6±1.08^{NS}	64±0.88^{NS}	64±2.16^{NS}
Bilirubin (mg/dL)	0.4±0.10	0.45±0.06^{NS}	0.73±0.12^{NS}	0.48±0.06^{NS}
Urea (mg/dL)	65.5±0.5	72.8±2.49*	66.3±0.33^{NS}	73.8±1.31*
12 weeks				
AST (iu)	62.3±0.80	170.2±1.50***	208.3±2.62***	228.1±9.17***
ALT (iu)	31.2±0.85	77.0±1.67***	73.3±1.52***	126.5±1.83***
ALP (iu)	125.5±2.5	166.0±0.70***	135.3±1.03***	160.8±0.47***
Total protein (g/dL)	7.7±0.25	6.9±0.12*	6.7±0.24*	6.6±0.23*
Albumin (g/dL)	4.2±0.05	3.9±0.10^{NS}	4.6±0.14^{NS}	3.6±0.29^{NS}
Globulin (g/dL)	3.5±0.30	3.0±0.29^{NS}	2.1±1.08*	3.0±0.06^{NS}
Cholestero (mg/dL)	69±1.00	62±1.63*	68±2.12^{NS}	69.5±1.84^{NS}
Bilirubin (mg/dL)	0.07±0.01	0.12±0.01*	0.16±0.01**	0.14±0.01**
Urea (mg/dL)	52.5±2.50	64.8±1.87**	51.3±1.10^{NS}	60.5±2.53*

NS: not significant; *: Significant at (p<0.05); **: Significant at (p<0.01); ***: Significant at (p<0.001)

Table 4: Average (mean ± SE) haematological values in rats fed on various levels of furundu for 12 weeks

	Treatment groups			
Parameters	1 control	2 10% furundu	3 15% furundu	4 20% furundu
6 weeks				
Hb (g/dL)	13.5±0.40	16.2±0.87^{NS}	15.6±1.38^{NS}	18.1±0.83**
RBC(x10^{6}mm)	8.90±0.75	10.4±0.56^{NS}	9.10±0.38^{NS}	10.0±0.52^{NS}
PCV (%)	54.9±3.75	62.7±4.35^{NS}	56.2±2.42^{NS}	60.5±2.48^{NS}
MCV (m^3)	61.5±0.50	60.5±1.04^{NS}	62.6±1.76^{NS}	60.5±1.50^{NS}
MCH (pg)	15.2±0.80	15.7±0.09^{NS}	17.5±1.26^{NS}	18.2±0.67*
MCHC (%)	24.7±1.00	25.9±0.40^{NS}	27.6±1.32^{NS}	30.0±1.02**
WBC(x10^{3}mm)	11.2±0.85	7.70±1.48**	7.20±1.16**	10.7±0.73^{NS}
12 weeks				
Hb (g/dL)	15.2±0.25	11.9±0.08***	11.5±0.23***	10.8±0.15***
RBC (x10^{6}mm)	8.90±0.25	6.60±0.11***	6.60±0.18**	06.3±0.33**
PCV (%)	34.5±0.80	36.1±0.17**	35.9±1.22^{NS}	34.0±2.14^{NS}
MCV (m^3)	51.1±1.20	55.0±0.57*	54.5±1.25^{NS}	53.8±0.47*
MCH (pg)	16.4±0.10	17.8±0.15**	17.9±0.58^{NS}	18.5±0.18***
MCHC (%)	34.6±0.55	32.7±0.14**	32.9±0.27**	34.4±0.40^{NS}
WBC (x10^{3}mm)	5.0±0.20	5.7±0.08^{NS}	7.9±0.33***	11.2±0.23***

NS: not significant; *: Significant at (p<0.05), **: Significant at (p<0.01); ***: Significant at (p<0.001)

lower (p<0.05), that of urea of groups 2 and 4 was higher (p<0.05-0.01) than the control, whereas, albumin and globulin concentrations of the test groups and urea concentration of group 3 remained unchanged.

Haematological findings: These data are shown in Table 4. At week six, there was no difference in the values of PCV, RBC and MCV between the test animals and the control group. The values of Hb, MCH and MCHC in group 4 was higher (p<0.05-0.01) than that at of the control animals. The value of WBC in groups 2 and 3 was lower (p<0.01) than that at of the control animals.

After twelve weeks, the values of Hb, RBC of the test animals, were significantly lower (p<0.01-0.001) and those of MCV and MCH were higher than the control rats. The value of PCV in group 2 and that of WBC of groups 3 and 4 were higher (p<0.01-0.001) than the controls. The value of MCHC was significantly lower (p<0.01) than the controls.

DISCUSSION

The incorporation of fermented *Hibiscus sabdariffa* seed (Furundu) in diets at 10 and 20% was chosen for several reasons. In chickens and rats, dietary levels of 10% represent non-toxic concentrations of some plants exemplified by *Nigella sativa* (Al-Homidan *et al*., 2003), *Thymus vulgaris* (Haroun *et al.*, 2002) and *Rhanterium*

epapposum (Adam, 2007). On the other hand, the inclusion of 20% in the diet of *commiphora myrrha* oleogum resin and *Trichdesma africanum* is fatal to rats (Omer, 1997; Adam, 2007) and of *Rhazya stricta* in the diet at 50% is lethal to rats (Adam, 1999). The susceptibility of animals fed plant materials seems at least dependent on the type of active constituents in the plant, the concentration added to the diet and the rate of their metabolic conversion in the liver to metabolites and their consequent excretion.

Meager research has been done to investigate the safety of fermented *H. sabdariffa* seed product (Furundu). The present study suggested that the seriousness of feeding Furundu at 10, 15 and 20%, respectively may be related to the concentration and characteristics of the active compounds in the plant. This toxicity showed that fermentation alone cannot be used to ensure food safety. The probability is that fermentation is only one technique amongst several in the processing operation. Other unit operations such as the final cooking of the product may contribute significantly to its overall safety. An example of this is in *gari* (a West African cassava fermented product) processing where the final roasting step ensures the volatilization of a high proportion of the remaining cyanogens (Westby *et al*., 1997).

The incorporation of Furundu in the diet at 10, 15 and 20%, respectively proved non-fatal but toxic to rats. Damage to liver, kidneys and intestine could explain the depressed growth. The mechanism whereby the seed constituents injured body tissues cannot be defined from the present study. In rats fed 10, 15 20% Furundu, respectively, damage to these organs probably contributed to the increase in serum AST, ALT and ALP activity, decreased total protein, globulin and cholesterol concentrations.

Laskar *et al*. (1998) described antinephrotoxic activity of the plant, *Dolichos biflorus* against paracetamol induced hepatotoxicity in rats and suggested that the biotransformation of paracetamol into a nephrotoxic compound, p-aminophenol, is responsible for elevation of blood urea nitrogen. The degeneration or necrosis of the renal tubular epithelium, packing of the glomerular tufts, lymphocytic infiltration seen in the rats fed on 10 and 20% Furundu, respectively, might have accounted for the rise in urea concentration.

The hypoproteinaemia in rats fed on different concentrations of Furundu might have resulted from hepatocellular dysfunction. This as well as the occurrence of hypocholesterolemia is further evidence of liver damage characterized by the development of cytoplasmic fatty vacuolation and necrosis of the centrilobular hepatocytes with lymphocytic infiltration.

Feeding of rats to concentrations of 10, 15 20%, respectively of Furundu 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).

As there were decreases in Hb and MCHC and an increase in MCV in rats fed on 10 and 15% of Furundu, the anaemia was of a macrocytic hypochromic type. The same type of anaemia was noticed in rats that fed on *Cassia senna* and *Citrullus colocynthis* (Adam *et al*., 2001) and *Chrozophora oblique* (Adam *et al*., 1999).

The decreases in Hb, RBC and increase in MCV without significant effects on MCHC indicate macrocytic normochromic anaemia. These findings suggest that the plant constituent [s] may be involved in a derangement of the haematopoietic process. Previous investigations showed macrocytic anaemia in rats which had been fed a diet consisting of 10 or 50% *Rhazya stricta* leaves (Adam, 1999) or in chickens which had received a diet consisting of 10% *Cassia italica* seeds (Bakhiet and Adam, 1996).

Although leukocytosis was observed in groups 3 and 4 fed on the fermented product, Furundu, normal WBC count was seen in rats receiving intermediate (15%) dose of the product. Leukocytosis resulted from neutrophillia was noticed in sub-chronic toxicity of *Cassia obtusifolia* on rats (Voss and Brennecke, 1990).

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 Furundu (fermented *H. sabdariffa* seed) utilized.

CONCLUSION

- Although fermented *H. Sabdariffa* seed is highly nutritious (high quality protein), its incorporation in the diet at 10, 15 and 20%, respectively is undoubtedly hazardous to Wistar rats and capable of enterohepatonephrotoxicity, anaemia, leukocytosis and depression in growth.
- Fermentation only does not completely detoxify the Hibiscus seed and further processing (cooking) step is needed.
- Histopathological changes were not evident at 6 weeks of feeding but sufficient to impose significant haematological and serobiochemical changes.

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