Safety evaluation of *Caryota urens* L. (Kithul) treacle in rats

P. Ranasinghe[1], G.A.S. Premakumara[1], C. D. Wijayarathna[2], W.D. Ratnasooriya[3]

[1]*Herbal Technology Section, Industrial Technology Institute, Colombo*

[2]*Department of Chemistry, Faculty of Science, University of Colombo*

[3]*Department of Zoology, Faculty of Science, University of Colombo*

Introduction

Traditionally, in Sri Lanka, treacle prepared from *Caryota urens* L. (Kithul) sap has been used as a sweetener for many centuries and it has been used to prepare a variety of sweet foods, drinks and some medicinal preparations such as Arista. In folklore it is claimed to posses health benefits such as anti-ageing and anti-diabetic. Recent scientific studies carried out by us showed that *C. urens* treacle posses different bioactive properties such as antioxidant, antihyperglycemic and glucosidase inhibitory properties. However, so far no scientific data is available to verify the safety of this traditional food. Therefore, in this study, toxicological evaluation of *C. urens* treacle was done using the normal rat model.

Materials and Methods

Sample

Authentic treacle sample (5 kg) prepared at Kithul treacle processing center at Rojasangama, Kotmale was used for this study.

Animals

Adult male Wistar rats obtained from Medical Research Institute, Colombo 08, Sri Lanka were used. The animals were maintained under standard laboratory conditions (12-h light/dark cycle, 25±2 ^{o}C and humidity 50 - 65%) and were fed with commercial diet (Master Feed Ltd., Colombo, Sri Lanka) for 2 weeks before start the experiment.

Diets

A standard diet was made having following composition (in weight %) protein, 10; oil, 2.5; fiber, 5.0; mineral, 2.5; methionine, 0.2; starch, 10; and sucrose 56. Treatment diet was prepared using 60 % (w/w) *C. urens* treacle and standard ingredients to meet same nutritional composition as in standard diet. Both standard and treacle added diets were prepared dried in oven at 50 ^{o}C until the moisture content reached to 14 - 13.5%. Then two diets were packed separately in polythene bags (40 g/bag) and stored in $^{-}$20 ^{o}C until use.

Experiment Design

After 14 days of acclimatization, rats were randomly divided into two groups (8 rats per group) and one group was given standard diet and other group was given treacle added diet *ad libitum* for 28 consecutive days. Both groups were given free access to tap water. Daily food intake and body weight gain (weekly) were recorded during the experiment. During the experiment, food intake was measured daily and body weight gained of the animals was recorded weekly. Overt sign of toxicity (salvation, rhinorrhoea, lachrymation, ptosis, writhing,

convulsions, tremors, erection of fur and exophthalamia) was monitored daily and reordered. On day 1 post treatment, rats were fasted for 14 h and blood samples were collected into EDTA treated and non-treated vials from tail vain under ether anesthesia with aseptic precautions. The EDTA non-treated samples were allowed to clot at 25±2 ^{o}C for 15 minutes and serum was separated by centrifuging at 6,000 rpm for 5 minutes at 4 ^{o}C. Randox test kits were used to determined different serum chemistry parameters such as glucose, serum proteins, serum albumin, creatinine, total cholesterol, triglyceride, urea, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT). The EDTA treated blood samples were used to count blood cells and other hematological parameters.

Data Analysis

Data were analyzed using GLM procedure in SAS 6.12 version and mean separation was done using Duncan's Multiple Range Test

Results and Discussion

Daily food intake and total body weight gain did not show significant (P>0.05) difference between treatment (25.0±1.6 vs 24.0±2.4 g respectively) and control (65.6±12.7 vs 72.8±10.6 g respectively) groups. An overt sign of toxicity (salvation, rhinorrhoea, lachrymation, ptosis, writhing, convulsions, tremors, erection of fur and exophthalamia) was not observed from rats in both treatment and control groups. The results of hematology parameters and serum chemistry tests of the two groups are given in Tables 1 and 2.

Table 1. Clinical hematology values of rats supplied with treacle and control diets for 28 consecutive days.

Hematology parameter	**Treacle**	**Control**
RBC (10^6/µl)	8.26 ± 0.15^a	8.19 ± 0.10
WBC (10^3/µl)	10.04 ± 0.70^a	11.81 ±1.25
Neutrophils (10^3/µl)	1.22 ± 0.10^a	1.37 ± 0.14
Lymphocytes (10^3/µl)	7.10 ± 0.76^a	9.16 ± 1.13
Monocytes (10^3/µl)	0.38 ± 0.08^a	0.34 ± 0.09
Eosinophils (10^3/µl)	0.99 ± 0.25^a	0.57 ± 0.04
Basophils (10^3/µl)	0.35 ± 0.06^a	0.37 ± 0.05
Hb (g/dl)	15.09 ± 0.29^a	15.04 ± 0.17
Hct (%)	67.93 ± 1.16^a	67.55 ± 0.58
MCHC (g/dl)	22.20 ± 0.16^a	22.25 ± 0.09
PLT (10^3/µl)	527.25 ± 105.03^a	640.38 ± 40.32

Values are presented as mean ± SEM of 8 independent replicates and values in a raw with same superscript letters are not significantly different (P>0.05). RBC, red blood cell count; WBC, white blood cell count; Hb, hemoglobin concentration; Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration, PLT, platelet count.

Kithul treacle did not show any toxicological effect on tested hematological parameters such as red blood cell count, white blood cell count, hemoglobin content and platelet count (Table 1) further according to hematological results kithul treacle did not a show pathological effect . According to the results of clinical chemistry parameters, Kithul

treacle did not show toxicological effect on metabolic functions (glucose, cholesyerol, triglyceride and protein levels), liver functions (SGOT and SGPT) and kidney functions (urea, albumin and creatinine) (Table 2).

Table 2. Clinical chemistry values of rats supplied with treacle and control diets for 28 consecutive days.

Parameter tested	Treacle	Control
Serum glucose (mg/dl) Total cholesterol (mg/dl) Triglycerides (mg/dl)	87.5 ± 1.8[a] 96.9 ± 3.1[a] 53.2 ± 3.5[a]	92.8 ± 1.5 88.0 ± 4.0 67.4 ± 5.4
Total proteins (g/dl)	7.4 ± 0.5[a]	7.1 ± 0.4
Serum albumin (g/dl)	4.31 ± 0.07[a]	4.32 ± 0.09
Creatinine (mg/dl)	0.6 ± 0.2[a]	0.5 ± 0.1
Serum urea (mg/dl)	13.2 ± 1.5[a]	13.8 ± 1.7
Serum Glutamic Pyruvic Transaminase (SGPT) (U/l)	15.1 ± 0.9[a]	14.5 ± 1.5
Serum Glutamic Oxaloacetic transaminase (SGOT) (U/l)	32.4 ± 3.3[a]	31.6 ± 2.4

Values are presented as mean ± SEM of 8 independent replicates and values in a raw with same superscript letters are not significantly different (P>0.05).

Conclusion

Caryota urens treacle did not show toxicological effect on blood physiology, carbohydrate metabolism, lipid metabolism, kidney and liver functions. Therefore, these findings may indicate the safety of Kithul (*C. urens*) treacle, a traditional sweetener of Sri Lanka.