Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2014; 6(1): 1-5

ISSN: 0975-5160

Research Article

Balanites aegyptiaca Carbohydrate Fractions and the Effect of Aqueous Extract of Mesocarp on Some Blood Parameters of CCl_4 Induced Rats

Salihu-Lasisi[1], M., *Akpabio C. J.[2], Ogunsola M.O.[3]

[1]*Biochemistry Unit, Biological sciences Department, Al-Hikmah University, Ilorin, Kwara State, Nigeria.*
[2]*Department of Biochemistry, Basic Medical Sciences, University of Ibadan, Ibadan, Oyo State Nigeria.*
[3]*Bowen University, Iwo, Osun State, Nigeria*

Available online: 1st March 2014

ABSTRACT

The mesocarp of fruits of *Balanites aegyptiaca* was analyzed for its biochemical constituents with estimation of different fractions of carbohydrates present in the fruit mesocarp. The fruits mesocarp was then administered orally to rats to assess blood parameters in CCl_4 liver damaged rats. White albino rats (*Rattus norvegicus*) assigned to four (4) groups containing fifteen (15) rats each were used to assess the effect of the aqueous extract of the mesocarp of the fruit *Balanites aegyptiaca* on the CCl_4 induced rats. Group 1 are the normal control (Nc) and group 2 are the diseased control (Dc), while groups 3 and 4 are the test groups of animals administered orally with 0.08mg of raw (Tr) extract and 0.19mg of concentrated (Tc) extracts per kg body weight respectively on daily basis. The result of the proximate composition revealed that the fruit mesocarp has 36.42% carbohydrates with 50.89% of it as reducing sugar. Compared to the controls, blood cells of the test rats revealed that there is significant increase ($p<0.05$) in the level of PCV, RBC, haemoglobin, MCH, MCHC and neutrophils count of the *Balanites aegyptiaca* treated rats. This indicates that in a diseased state, this fruits contains nutrients which can assist to restore and maintain biological functions of the body, if regularly consumed.

Keywords: *Balanites aegyptiaca*, Carbohydrate, Carbontetrachloride, Enzymes, Mesocarp.

INTRODUCTION

Balanites aegyptiaca (*Balanitaceae*) belongs to the family, *Zygophyloceae*, with the common names like desert date, soapberry, thorn tree (Gill, 1992). It is a savanna tree that grows up to12m high or less with a crown of tangled thorny twigs, drooping at the distal ends, easily recognized whether bearing flower or not with long straight green spines arranged spirally along the branches (Iwu, 1993). The fruits are produced between March and October with a thin hard skin and a pale brown sticky edible fleshy pulp (Hunchinson and Dalziel, 1958). The fruit fleshy outer part (the mesocarp) was effective as febrifuge (Egypt), and 80mg/kg were found to show hypoglycaemic activity on streptozotocin induced hyperglycaemia (Creach, 1943). Fruit and vegetables have many similarities with respect to their compositions, methods of cultivation and harvesting, storage properties and processing. Fruit in general is acidic and sugary and are important sources of both digestible and indigestible carbohydrates. The digestible carbohydrates are present largely in the form of sugars and starches while indigestible cellulose provides roughage, which is important to normal digestion (Dauthy, 1995).

Food can cause diseases when it contains too little or too much of a specific nutrient, which are supplied in a well-planned diet (Akanji, 2002). Thus diet is what a person or organism eats or drinks to maintain good health and it can be considered from two perspectives: the nutritionally adequate or balanced diet which maintains and promotes health and vigour and special diets prescribed for treatment or prevention and management of diseases (Shryock, 1992). Fruits are a class of food which made up 4 - 5% world food supply.

However, it is a known fact that treating disease is big business in any country. Because disease keeps people dependent on the system, while health sets people free. Disease turns individuals into victims, while health turns them into capable human beings with individual freedoms and free thinking. That's why this researcher is advocating for healthy living through right eating habit of locally available health promoting ingredient in our locality.

MATERIALS AND METHODS

Sample source and preparation: The fruits were purchased from Kurmi market at Kano State. The fruit coats (epicarp) were carefully removed using a cleaned knife and the fruit mesocarp were scraped into a clean air-tight container (this is the raw sample).

*Author for correspondence: E-mail: comfortsamonline@yahoo.com

Table 1: Proximate Composition of Mesocarp of Balanites aegyptiaca Fruit

Parameters	Percentage Composition
Apparent Moisture content	17.67± 0.012
Dry matter	82.33± 0.409
Organic matter	69.02± 0.117
Ash	30.98± 0.021
Crude fiber	0.84 ± 0.021
Crude protein	12.17 ± 0.174
Carbohydrate	36.42± 0.285
Crude fats	2.42 ± 0.183

Each value is a mean ± SEM (n = 5)

Table 2: Carbohydrate Fractions in the Mesocarp of the *Balanites aegyptiaca* Fruit

Carbohydrates fraction	Percentage composition
Ethanol soluble sugar (glucose)	22.4 ± 0.12
Total soluble extract (glucose)	31.26 ± 0.37
Starch	1.25 ± 0.01
Total soluble carbohydrate (WSP – Starch)	20.96
Water soluble polysaccharides	22.20 ± 0.25
Hemicellulose	1.52 ± 0.61
Pentose	0.96 ± 0.13
Oligosaccharide	Not detected
Fructose	19.63 ± 0.47
Sucrose	1.72 ± 0.82
Cellulose	0.39 ± 0.43
Lignin	32.8 ± 0.36
Cell wall constituents	4.12 ± 0.01
Total reducing sugar (Fructose + Glucose)	50.89

Each value is a mean ± SEM (n = 5)

Carbohydrate fractions determination: 1g of the sample was extracted with 80% ethanol (v/v) using Nelson - Somogyi (1945) reagent to determine soluble sugars as glucose. Filtrate from starch was hydrolyses with 52% perchloric acid was treated with 10ml H_2SO_4 and 50% NaOH (w/v) for water-soluble carbohydrates determination and its residue treated with 0.5ml of 72% (v/v) for cellulose determination. The residue from starch hydrolysis was boiled with 72% H_2SO_4 for hemicellulose determination. Also the residue from cellulose hydrolysis was ashed at 600^0C for 1hour and weighed. The difference in the two weightings represents the value of the lignin fraction. The estimation of fructose content was however determined by the method of Johnson *et al.* (1964), while the amount of pentose in the hemicellulose extracts was determined by the method of Majbaum (1947) as modified by Albaum and Umbreit with slight modification of reagent (Soughgate, 1969). The sucrose from the hemicellulose extract was determined by the modified by Anthrone method of Firley *et al.* (1973) at 610nm and total soluble sugar at 625nm using spectrophotometer.

Animals grouping

Group 1 - The normal control rats (Nc)

Group 2 - The test control rats (Dc)

Group 3 - The treated with raw extract of 0.08mg/kg body weight of the rats (Tr)

Group 4 - The treated with concentrated extract of 0.19mg/kg body weight rats (Tc)

Blood parameters determination

The packed cell volume was measured after centrifuging the blood sample that was collected in a heparinized capillary tube as described by Cartwright (1968). Mean Cell Haemoglobin Concentration (M.C.H.C.) was calculated using the haemoglobin concentration value divided by the packed cell volume. The total leucocyte count was determined by visual method as described by Dacie and Lewis (1991).

Liver function indices determination

In determining total protein, the Biuret method was used (Plummer, 1978). The method described by Doumas *et al.*, (1971) was used for the determination of serum albumin level using Bromocresol Green.

RESULTS AND DISCUSSION

Hulme (1987) reported that the main sugars in fruits are glucose, fructose and sucrose. The present study confirms the earlier report by Anon (1977) that the mesocarp of *Balanites aegyptiaca* contains 50 – 60% free sugar. 81.3 – 91.1% of which is present as reducing sugars, particularly the fructose. The high content of fructose (Table 2) in the mesocarp as observed in the in this study may be advantageous for diabetes patients who may utilize fructose as source of energy since no insulin is required for its transportation (Oloyede, 2005). Furthermore, the presence of water soluble polysaccharides and cell wall constituents, as obtained in this study may help in reducing blood cholesterol level and consequently reduce the risk of cardiovascular diseases (Barker, 2002; British Nutrition Foundation,

Table 3: Haematological Parameters of Rats Administered Aqueous Extracts of Mesocarp of *Balanites aegyptiaca* Fruit Following CCl_4 Induced Liver Damage

Parameter	Animals Grouping	2nd day	4th day	8th day	14th day	21st day
Haemoglobin (g/dl)	Group 1	14.0 ± 0.94^{ba}	14.1 ± 1.21^{ba}	14.3 ± 0.86^{b}	15.1 ± 2.22^{c}	14.7 ± 0.89^{b}
	Group 2	11.5 ± 0.82^{a}	11.9 ± 1.13^{a}	12.3 ± 1.22^{a}	13.2 ± 0.41^{a}	12.8 ± 0.31^{a}
	Group 3	15.1 ± 0.41^{ca}	14.6 ± 0.56^{ba}	15.1 ± 0.96^{ca}	15.6 ± 1.83^{cb}	15.9 ± 1.41^{cb}
	Group 4	13.8 ± 1.08^{ba}	14.3 ± 1.24^{ba}	14.5 ± 1.26^{cb}	14.9 ± 0.72^{b}	14.9 ± 0.84^{b}
RBC (x $10^{12/l}$)	Group 1	6.5 ± 0.01^{b}	6.7 ± 0.15^{a}	7.0 ± 0.22^{b}	6.1 ± 0.34^{b}	6.8 ± 0.32^{b}
	Group 2	6.2 ± 0.21^{a}	6.6 ± 0.12^{a}	$5.7 \pm 0.16^{a\,b}$	5.7 ± 0.22^{ab}	4.9 ± 0.36^{a}
	Group 3	9.5 ± 0.18^{c}	7.3 ± 0.14^{b}	7.6 ± 0.14^{c}	7.2 ± 0.19^{c}	7.7 ± 0.42^{c}
	Group 4	6.8 ± 0.11^{b}	7.8 ± 0.41^{c}	6.5 ± 0.20^{b}	7.3 ± 0.29^{c}	7.9 ± 0.13^{c}
PCV (%)	Group 1	52.3 ± 0.06^{c}	52.3 ± 0.03^{b}	$48.3 \pm 0.03^{c\,b}$	46.8 ± 0.42^{ba}	49.3 ± 0.02^{c}
	Group 2	35.0 ± 0.12^{a}	$31.0 \pm 0.04^{a\,b}$	30.8 ± 0.01^{a}	$31.7 \pm 0.17^{a\,b}$	32.1 ± 0.16^{ac}
	Group 3	45.6 ± 0.05^{ba}	53.3 ± 0.12^{c}	$48.3 \pm 0.03^{c\,b}$	$51.3 \pm 0.02^{d\,b}$	$50.\ 9 \pm 0.08^{c}$
	Group 4	43.4 ± 0.11^{ba}	52.7 ± 0.06^{bc}	44.3 ± 0.21^{b}	49.3 ± 0.57^{cd}	47.5 ± 0.13^{bc}
MCV	Group 1	73.0 ± 1.21^{c}	74.0 ± 1.32^{c}	63.3 ± 0.02^{b}	59.7 ± 1.25^{a}	64.6 ± 0.73^{b}
	Group 2	63.0 ± 0.99^{b}	67.3 ± 0.24^{a}	62.0 ± 0.87^{a}	60.0 ± 0.52^{b}	52.1 ± 0.86^{a}
	Group 3	59.7 ± 0.34^{a}	70.3 ± 0.11^{b}	66.3 ± 1.31^{c}	65.0 ± 2.16^{c}	64.9 ± 1.29^{b}
	Group 4	$61,7 \pm 0.90^{b}$	70.3 ± 1.67^{b}	67.7 ± 0.80^{c}	69.7 ± 1.25^{d}	73.3 ± 2.27^{c}
MCH (pg)	Group 1	20.0 ± 0.22^{b}	22.0 ± 0.37^{b}	19.0 ± 0.04^{c}	21.0 ± 0.13^{c}	19.8 ± 0.02^{b}
	Group 2	18.3 ± 0.04^{a}	17.7 ± 0.03^{a}	21.3 ± 0.10^{b}	18.7 ± 0.81^{b}	18.9 ± 0.12^{a}
	Group 3	32.3 ± 0.61^{c}	18.3 ± 0.21^{a}	17.7 ± 0.81^{a}	22.0 ± 1.26^{c}	24.6 ± 0.05^{c}
	Group 4	18.3 ± 0.03^{a}	18.0 ± 0.03^{a}	18.7 ± 0.02^{a}	18.1 ± 0.04^{a}	18.6 ± 1.02^{a}
MHCH (g/dl)	Group 1	25.3 ± 0.01^{ab}	24.3 ± 0.04^{a}	26.7 ± 0.01^{ac}	27.7 ± 0.02^{ad}	27.6 ± 0.32^{ad}
	Group 2	30.7 ± 0.03^{b}	25.7 ± 0.01^{ba}	32.7 ± 0.03^{bc}	39.2 ± 0.02^{cd}	37.4 ± 0.04^{c}
	Group 3	30.7 ± 0.14^{b}	24.7 ± 0.14^{a}	27.3 ± 0.51^{a}	29.5 ± 0.11^{b}	26.8 ± 0.01^{a}
	Group 4	30.7 ± 0.01^{b}	26.0 ± 0.02^{ba}	33.7 ± 0.02^{bc}	32.2 ± 0.06^{bc}	33.1 ± 0.07^{bc}

Each value is a mean ± SEM (n = 5 in a group) Values along the same column and row with different superscripts are significantly different (p< 0.05)

2004), while the low crude fibre makes the fruit suitable to be included in low rough diet therapy (Tandon and Prasad, 1989) and this also improve the digestibility of protein and thus increasing the utilization of the associated amino acids (Fetuga *et al.,* 1973). The total crude protein content (Table 1) of the mesocarp of *Balanites aegyptiaca* obtained in this study, when compared to that of Abdel-Azim *et al.* (1985) and Anon(1977) revealed that the sample will be a better substitute which may be incorporated in the diet of low protein requiring diet patients (Tandon and Prasad, 1989).

Blood examination provides means of establishing the nutritional, health, physiological and pathological state of an organ (Levene and Gorgen, 2003; Karnish, 2003). Table 3 shows the blood cells parameters of the rats administered with aqueous extracts of mesocarp of *Balanites aegyptiaca* fruit following CCl_4 - induced liver damage. The data indicated a significant increase ($p<0.05$) in red blood cells as from the 2nd day of administration in groups 3 and 4 rats, which may have developed to prepare the body to fight the effect of the CCl_4– induced liver damage in the rats. However, there was significant reduction ($p<0.05$) in the red blood cells counted in the test control (i.e. the group 2 rats) compared to the treated animals (group 3 and 4). The increased red cell counts fluctuates with days of treatment, but the groups of rats treated with extracts showed a slight increase ($p<0.05$) than those untreated with extracts. This may be due to more of the red cells constituents being used to carry oxygen for tissue respiration in the CCl_4 – induced liver damage rats (Aduloju, 2000). The haemoglobin counts of the treated animal indicated that there was significant increase ($p< 0.05$) in the CCl_4 - induced liver damage animals treated with the 0.08mg/kg body weight while those treated with the 0.19mg/kg body weight are not significantly different from the control with the increasing days of the experiment. The same observation was also noted for other red cell haemoglobin parameters like the MCH and MHCH counts of the treated rats with days of treatment compared with the normal control (Nc) and test control (Tc) rats (Table 3).

Furthermore, for the packed cell volumes of the rats after the administration of aqueous extract of mesocarp of *Balanites aegyptiaca* fruit on the treated rats shows a significant increase ($p<0.05$) in the treated animals compared with the test control (group 2) but the values are not significantly different ($p<0.05$) from that of the normal control (group 1) but the (group 4) rats have a significantly higher values than those of the (group 3) groups of rats.

Table 4 shows the effects of administration of aqueous extract of *Balanites aegyptiaca* fruit mesocarp on the serum total protein in the treated rats compared with the control. The concentration of liver fubction indices like protein, albumin, bilirubin, conjugated bilirubin in the serum can be use to ascertain the state of the liver (Naganna, 1989). There was a fluctuation in the serum total protein in the CCl_4 – induced animals compared

Table 4: Serum liver function indices of the CCl_4-induced experimental rats treated with aqueous extract of the mesocarp of *Balanites aegyptiaca*

Parameter	Animals Grouping	2nd day	4th day	8th day	14th day	21st day
Total bilirubin (μmol/l)	Group 1	0.9 ± 0.08 [a]	0.7 ± 0.10 [a]	0.9 ± 0.10 [a]	0.8 ± 0.07 [a]	0.9 ± 0.10 [a]
	Group 2	1.0 ± 0.06 [b]	1.0 ± 0.09 [b]	1.2 ± 0.06 [b]	2.0 ± 0.08 [c]	2.1 ± 0.08 [c]
	Group 3	0.8 ± 0.05 [a]	0.9 ± 0.05 [b]	1.1 ± 0.03 [b]	1.2 ± 0.10 [b]	1.4 ± 0.12 [b]
	Group 4	0.9 ± 0.01 [a]	0.9 ± 0.04 [b]	1.0 ± 0.07 [a]	1.1 ± 0.07 [b]	1.2 ± 0.11 [b]
Conj. Bilirubin (μmol/l)	Group 1	0.03± 0.01 [b]	0.03± 0.00 [c]	0.03± 0.01 [c]	0.02± 0.00 [c]	0.02± 0.01 [c]
	Group 2	0.02± 0.00 [ab]	0.01± 0.01 [a]	0.01± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]
	Group 3	0.02± 0.00 [a]	0.03± 0.01 [c]	0.02± 0.01 [b]	0.01± 0.00 [b a]	0.01± 0.00 [ba]
	Group 4	0.02± 0.00 [a]	0.02± 0.00 [b]	0.02± 0.00 [b]	0.01± 0.00 [b a]	0.01± 0.00 [ba]
Total protein (g/l)	Group 1	57.0± 4.03 [a]	54.0 ± 5.02 [b]	55.3 ± 2.01 [a]	58.4± 6.33 [a]	56.2± 6.03 [a]
	Group 2	60.0± 0.98 [d]	56.0 ± 3.42 [c]	60.7 ± 7.11 [b]	69.2±11.25 [c]	70.1± 11.32 [c]
	Group 3	58.0± 2.41 [b]	60.0 ± 9.51 [b]	63.3 ± 3.21 [c]	62.1± 3.06 [b]	61.2± 9.57 [b]
	Group 4	59.3± 10.01 [c]	49.3 ± 4.42 [a]	59.0 ± 2.91 [b]	60.6± 5.13 [ab]	63.1± 11.17 [b]
Albumin (g/l)	Group 1	32.3 ± 6.01 [b]	33.0 ± 3.22 [b]	33.0 ± 7.32 [d]	43.7 ± 5.14 [c]	45.6± 5.30 [c]
	Group 2	31.0 ± 2.14 [a]	30.0 ± 1.69 [a]	27.3 ± 5.01 [a]	35.3 ± 3.12 [a]	35.8± 4.11 [a]
	Group 3	35.7 ± 4.01 [c]	36.7 ± 5.72 [c]	29.7 ± 4.12 [b]	48.3 ± 7.13 [d]	49.7 ± 6.23 [d]
	Group 4	36.7 ± 1.09 [c a]	36.3 ± 0.89 [c a]	30.3± 6.92 [ca]	41.7 ± 1.41 [b]	40.1 ± 7.22 [b]

Each value is a mean ± SEM (n = 5 in a group) Values along the same column and row with different superscripts are significantly different ($p < 0.05$)

with the normal control. The fluctuation in total protein level indicates pathological damage in the liver. Earlier report of Tapley (1956) indicated that any condition in which liver is diseased, as induced in this study, there was reduction in protein synthesis. Also, the albumin concentration in the experimental rats showed that there was significant reduction ($p<0.05$) in the 0.19mg/kg body weight sample administered rats compared with the other groups but the 0.08mg/kg body weight rats. The effects of the administration of aqueous extract of *Balanites aegyptiaca* fruit mesocarp on the serum total bilirubin on the rats, showed there was significant increase ($p<0.05$) with days of treatment in the test control animals while those for the raw extracts gradually became significantly high ($p<0.05$) with days of treatment. Evidence is accumulating that suggests bilirubin can protect tissues against oxidative damage caused by free radicals and other reactive oxygen species. Statistical analysis of people with high normal or slightly elevated bilirubin levels in blood shows that they have a lower risk of developing cardiovascular diseases (Wikipedia, 2006).

CONCLUSION

As could be observed from the results, the mesocarps of fruits contain nutrients which can assist to restore and maintain biological functions of the body, if regularly consumed in a diseased state.

REFERENCES

1. Abdel-Azim, A. M., Abdel-Halim, N. and Ahmed, R. (1985): A chemical study of *Balanites aegyptiaca* L. (Lalob) fruits grown in Sudan.
2. Aduloju, Y. O (2000): Blood composition of broiler hicks fed raw or processed full fat soybeans supplement with *Saccaromyces cereviae.* Bsc. Project, department of animal production, University of Ilorin, Nigeria.
3. Akanji, M.A. (2002):"Eat and die by little ", University of Ilorin, the sixth inaugural lecture (61).
4. Anon, A. (1977):"An All-Purpose Tree for Africa Offers Food and Income", UNIDO. Under Exploited Tropical Plants with Promising Economic Value", Pg.100- 102, National Academy of Sciences, 3rd Print.
5. Barker, H. M. (2002): Nutrition and Diabetics for Health Care, Churchill Livingstone Pg. 3 - 6, 27 - 58, 95 - 113.
6. British Nutrition Foundation (BNF) (2005): A note on occurrence of treatment and prevention of cancer, diabetes, cardiovascular diseases.
7. Creach, P. (1943): *Balanites aegyptiaca* and its various uses in Tchad territory Rev. Institute of Botanical application in Tropical agric. 20: 578 – 582.
8. Dauthy, M. E. (1995): Fruit and vegetable processing, FAO AGRICULTURAL SERVICES BULLETIN No. 119**.** Food and Agriculture Organization of the United Nations, Rome.
9. Dacie, J. V. and Lewis, S. M. (1995): Practical Haematology. 7th Edition. Churchill Livingstone, Edinburg, London. Pg. 12-17.
10. Doumas, B. T., Watson, W.A and Biggs, H. G. (1971): Albumin standards and measurement of serum albumin with Bromocresol green. Clin. Chim. Acta. 31: 87 - 92.
11. Firley, J. N. and Feller, O. A. (1973): Sucrose determination by mordified anthrone method. Cereal Chemistry. 50: 210 – 215.S
12. Fetuga, B. L., Babatunde, G. M. and Oyenuga, V. A. (1973): Protein quality of some Nigerian feedstuff I, Chemical assays of nutrients and amino acids composition. J. Sci. Fd. Agric., 24: 1505 - 1514
13. Gills, L. S. (1992): The Ethnomedical Used plants in Nigeria, Uniben Press, Benin, Nigeria.

14. Hulme, H. B. (1987): soil and Plant Analysis. Interscience Publisher. Inc. New York.
15. Hutchinson, J. and Dalziel, J. M. (1958): Floral of West Africa, Vol. I, part 2, by Crown Agents for Overseas Govt. and Administration, Milbank, London. Pg. 363.
16. Iwu, M. M. (1993): Handbook of African Medicinal Plants, Pharmacological, Profile of selected plants. CRC Press New York
17. Johnson, G., Lambert, C., Johnson, D. K. and Sunderwith, S. G. (1964): Colorimetric determination of glucose, fructose in plant materials using a combination of enzymatic and chemical methods. Journal of agric. Food Chem. Vol. 12: 216 - 219.
18. Johnson, I. T., Gee, J. M., Price, K., Curl, C. and Fenwick, G. R. (1986): Influence of saponins on gut permeability and active nutrient transport in vitro. Journal of Nutrition 116. Pg. 2270 – 2277.
19. Karnish, A.R. (2003): Immune regulation in health and disease. Academic Press. San Diego. Pg. 140 – 152.
20. Levene, W. H. and Gorgen, B. (2003): Fundamental Immunology. Raven Press, New York. Pg. 256 – 257.
21. Naganna, B. (1989): Plasma proteins. In: Textbook of Biochemistry and Human Biology. G.P. Tawlar, L.M. Srivastava and K.D. Moudgils (eds). Second edition, Prentice-Hall of India Private Ltd., Pg. 172-181.
22. Nelson-Somogyl, M. A. (1945): A reagent for the copper-iodometric determination of very small amount of sugars. Vol.153: Pg. 375 – 380.
23. Oloyede, O. B. (2005): "All for the love of the nutrients", University of Ilorin, the seventy-eight inaugural lecture (78)
24. Biochemistry, University of Benin, Nigeria. Pg. 221 – 224.
25. Plummer, D. T. (1978): An Introductory to practical Biochemistry, 2nd. Ed. McGraw Hill, London. Pg. 144 – 145.
26. Shryock, H. (1992): A textbook of modern medical guide. Macmillan Publishing Company. Inc. New York.
27. Southgate, S. C. A. (1978): Tropical root crops and Africa food crisis. Pg. 17 - 23.
28. Tandon, B. N. and Prasad, N. (1989): Diet in the management of diseases in textbook of Biochemistry and Human Biology.
29. Tapley, D.F (1956): The effect of thyroxine and other substances on the swelling of isolated rat liver mitochondria. J. Biol. Chem. 222:325.