IMPACT OF *CARICA PAPAYA* L. FRUIT JUICE ON PLASMA VARIABLES AND TISSUE GLYCOGEN OF INDUCED HYPERGLYCEMIC ALBINO RATS

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Received: *October 20, 2018* ***Revised:*** *March 18, 2019* ***Accepted:*** *June 20, 2019*

ABSTRACT

It was aimed at to evaluate the activity of Carica papaya fruit juice on induced diabetic rats (Rattus norvegicus) with a view of proposing a management scheme. Animals were sacrificed after treatment with unripe and ripe papaya juice. The plasma glucose, cholesterol, protein and tissue glycogen concentrations were estimated. Feeding of papaya juice raised the levels of these parameters more than the controlled value throughout the work. The results showed that the concentrations of these parameters were significantly increased (p<0.05). The rise was more with unripe papaya when compared to ripe papaya. However, papaya intake must be with caution since its consumption increases blood glucose concentration.

Keywords: Papaya, Fruit juice, Albino rats, Plasma variables, Hyperglycemia, Diabetes

INTRODUCTION

Diabetes mellitus affects carbohydrate, fat and protein metabolism due to absolute or relative lack of insulin. It is characterized by hyperglycemia in the postprandial and/or fasting state, and in its severe form is accompanied by ketosis and protein wasting (Ayber *et al.,* 2001).

India has the second largest number of people living with diabetes worldwide. It has 72.9 million diabetic people with an adult diabetes prevalence rate of 10.4 %. Diabetes mellitus type 2 is the most common type of diabetes, accounting for around 90 % of all cases of diabetes (IDF, 2017). American Diabetic Association (ADA) (2016) guidelines for the treatment of type 2 diabetes recommend timely stepwise intensification of therapy by adding one or two drug subsequently. Besides, drugs used for the treatment of diabetes, some 300 plants have been described in the scientific and popular literature as having a hypoglycemic activity (Andrade-Cetto and Heinrich, 2005).

Carica papaya L. (Family: Caricaceae) or papaya is an important plant consumed as a fresh fruit or processed into desserts. Different parts of the papaya plants including fruit, dried fruit and leaves have been used as ingredients in alternative medicine. The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid. Scientific evidences have shown that papaya has anti-diabetic, diuretic, contraceptive and hypoglycemic activities (Sripanidkulchai *et al.*, 2001; Gbolade, 2009; Ezekwe *et al.,* 2014).

Moreover, the cost of modern anti-diabetic drugs is usually beyond the reach of most people in the low income group especially those in the developing world. This has led to the current shift to the use of preparations from various parts of plants because of the current increase in the knowledge of their toxicity, side effects, active constituents and doses. This study was conducted to evaluate the effects of the papaya fruit juice on plasma variables and tissue glycogen in induced diabetic rats.

MATERIALS AND METHODS

Albino Rats: *Rattus norvegicus* were bred in the laboratory so that the age of rats may be taken in to account. For the study, 15 adult albino rats weighing 300 – 500 g kept in the Animal House, Department of Zoology, VKS University, Arrah. The animals approved for the experiment by the local committee and were housed in polypropylene cages under controlled conditions of 12-hour light/dark cycle at 24^0C. They were maintained on standard diet and water *ad-libitum*. The rats were used following the guidelines of Institute for Laboratory Animal Research (ILAR) (Clark *et al.*, 1997). The experiment was conducted using randomized block design of three treatments replicated five times with each animal. The acclimatized experimental animals from the stock were taken and kept in fifteen different cages as per feeding schedule. In each cage, only one animal was kept and regular cleaning of the cage was done to maintain the hygienic condition.

Induction of Hyperglycemia in Rats: Osinubi *et al.* (2006) method of was used to induce diabetes in the rats. 20 g of crystalline alloxan monohydrate was dissolved in 500 ml of normal saline to yield a concentration of 400 mg/ml. Alloxan was given intraperitoneally at 150 mg/kg body weight of rats after overnight fast of twelve hours to make them more susceptible to developing diabetes. Only rats with blood glucose levels between (250 – 400 mg/dl) after one week were considered diabetic and used for the experiment.

Control of Hyperglyceamia using Papaya Juice: Rats in treatment one (Group 1) were diabetic rats fed with 20 ml of ripe papaya fruit juice daily, rats in treatment two (Group 2) were diabetic rats fed 20 ml unripe papaya fruit juice daily, while rats in treatment three (Group 3) were non-diabetic rats given 20 ml of water daily and serve as control. The 20 ml was given orally in a divided dosage of 10 ml in the morning and 10 ml in the evening for 60 days. All animal were allowing unrestricted access to feed and water.

Blood Biochemical Tests: Blood samples were obtained from the cervical vein into clean, dry and labelled tubes. Blood samples were left to coagulate and then centrifuged at 3000 rpm for 15 minutes. The clear non-haemolysed sera were stored in a deep freezer at -40°C until being used for biochemical evaluation. The tissues of liver and muscle were collected and kept in deep freezer for further analysis. Along with the tissues, blood was also taken out from the experimental animals with the help of a syringe and poured in a sample tube quoted with ETDA and later analyzed for cholesterol.

Quantitative estimations of plasma glucose, plasma protein, plasma cholesterol and tissue glycogen were done by Nelson (1944) method, Biuret (Reinhold, 1953) method, Henly (1957) method and Kemp and Van Heijningen (1954) method respectively.

Data Analysis: Data collected were analysed using analysis of variance (ANOVA) and presented as means and standard errors of means. T-test was used to compare between the ripe and unripe groups. The differences between the control and treatment groups were tested by using Duncan new multiple range tests (Duncan, 1955) at the level of significance $p<0.05$.

RESULTS AND DISCUSSION

The level of plasma variables and tissue glycogen was higher in both unripe and ripe papaya fruit juice fed albino rats when compared to the normal ones (Tables 1 to 5).

According to Norris (2006), when plasma glucose level (PGL) is found above 180 mg/100 ml post pardinal (PP) in a mammal, it may be diagnosed as hyperglycemic. Previous findings suggested the increase in PGL may be due to high glycemic index of *Carica papaya* (Reed, 1976; Guevarra and Panlasigui, 2000; Rahmat *et al.,* 2004).

Alterations in Plasma Variables: The PGL level of experimental unripe papaya fruits juice fed rats increased ($p<0.05$) from (day 0) 97.20 ± 1.019 to (day 60) 160.20 ± 1.092 mg/dl from initial day 0 control values of 82.60 ± 0.547 mg/dl (Table 1). However, the increment in rats fed ripe papaya fruit juice was significantly lower ($p<0.05$) from (day 0) 86.20 ± 1.930 to (day 60) 121.60 ± 0.826 mg/dl (Table 1).

The increase in PGL by eating fruits (papaya, mango, litchi, apple, banana, berry, pine) was earlier reported (Reed, 1976; Wagner *et al.,* 2012). It is also observed that due to presence of some glycosides, hyperglycemic condition develops when papaya is regularly fed to the animals (Yang and Walter, 1992). Study of biochemical aspects also supports that the papaya contains various chemical constituents such as, galactose, glucotropaeolin, glucosinolate, butanol and sucrose which are responsible to elevate the blood sugar level from normal level and thus cause hyperglycemia in animals (Chan and Tang, 1979).

The plasma cholesterol level (PCL) after feeding with unripe papaya fruit juice for 60 days, significantly increased ($p<0.05$) from (day 0) 114.64 ± 0.932 to (day 60) 159.0 ± 0.792 mg/dl in comparison to day 0 control value of 110.04 ± 0.948 mg/dl (Table 2). But when the albino rats were fed with ripe papaya, its value significantly raised ($p<0.05$) from (day 0)

113.20 ± 0.892 to (day 60) 145.33 ± 0.805 mg/dl (Table 2).

Earlier observations and results support the present finding that *C. papaya* juice increased the concentration of total protein and total cholesterol (Reed, 1976; Rahmat *et al.,* 2004). In the experimental control rats, the plasma protein level (PPL) at day 0 was 3.52 ± 0.141 mg/dl. After feeding of unripe papaya fruit juice, its value increased ($p<0.05$) from (day 0) 3.72 ± 0.912 to (day 60) 4.54 ± 1.092 mg/dl (Table 3). Whereas, in the ripped papaya fruit juice fed albino rats the PPL recorded raised ($p<0.05$) from (day 0) 3.68 ± 0.956 to (day 60) 4.32 ± 0.708 mg/dl.

Table 1: Evaluation of plasma glucose level (mg/dl) after 60 days of feeding of unripe and ripe papaya fruit juice to experimental diabetic albino rat

Duration of feeding (Days)	Plasma Glucose Level (mg/dl)		
	Control	Unripe papaya fruit juice	Ripe papaya fruit juice
0	82.60 ± 0.547	97.20 ± 1.019 (17.68 %)*	86.20 ± 1.930 (4.36 %)^{NS}
15	82.70 ± 0.281	105.40 ± 0 .927 (27.60 %)*	93.80 ± 1.020 (13.56 %)*
30	81.49 ± 0.191	123.20 ± 0.789 (49.15 %)*	102.20 ± 0.806 (23.73 %)*
45	82.84 ± 0.475	138.60 ± 0.862 (67.80%)*	114.80 ± 0.892 (38.98%)*
60	82.81 ± 0.793	160.20 ± 1.092 (93.95%)*	121.60 ± 0.826 (47.22%)*

*NS=Not Significant, *= Significant*

Table 2: Evaluation of plasma cholesterol level (mg/dl) after 60 days of feeding of unripe and ripe papaya fruit juice to experimental diabetic albino rat

Duration of feeding (Days)	Plasma Cholesterol Level (mg/dl)		
	Control	Unripe papaya fruit juice	Ripe papaya fruit juice
0	110.04 ± 0.948	114.64 ± 0.932 (4.18 %)^{NS}	113.20 ± 0.892 (2.87 %)^{NS}
15	110.88 ± 0.501	121.82 ± 1.048 (10.78 %)*	116.86 ± 0.921 (6.20 %)*
30	110.27 ± 0.419	131.68 ± 1.208 (19.67 %)*	123.32 ± 0.908 (12.07 %)*
45	109.47 ± 0.357	146.24 ± 0.802 (32.92 %)*	133.60 ± 1.092 (21.41 %)*
60	109.70 ± 0.119	159.0 ± 0.792 (44.49 %)*	145.33 ± 0.805 (32.07 %)*

*NS=Not Significant, *= Significant*

Therefore, it appears that in both the experiment, a slow rise in PPL took place in early days of feeding but as the days of feeding advances, the rise in PPL becomes quite pronounced (Table 3).

Rise in PPL after administration of crude papaya latex to growing rabbit in the diet for 15 days indicated that papaya played an essential role in elevating the protein level (Reed, 1976). However, according to Sharma (2001), papaya may be consumed regularly as a nutritional diet and is suitable as diet for the weak, sick and convalescent person which requires enough energy and protein levels.

Antioxidant and antimicrobial properties of unripe papaya has been studied. After treatment with juice of unripe papaya fruit at the rate of 60 mg/day, increases of the PCL between 10 – 20 % and the protein level between 08 – 12 % after 60 days of feeding was noticed (Osato *et al*., 1993). It was found that in addition to some active compounds of papaya may be responsible for increase in PCL. Papaya also contains various types of amino acids and vitamin B-complex which may increase PPL.

Alterations in Tissue Glycogen: The liver glycogen content (LGC) of experimental albino rats fed with unripe papaya fruit juice for different durations exhibited significant increase ($p<0.05$) from the day 0 control value (77.44 ± 0.805 mg/g). The trend for albino rats fed with unripe papaya fruit juice showed a positive correlation with the duration of feeding and ranged from (day 0) 82.54 ± 0.902 to (day 60) 96.92 ± 1.08 mg/g (Table 4). On the other hand, in the albino rats fed with ripe papaya fruit juice, LGC significantly raised ($p<0.05$) from (day 0) 82.36 ± 0.632 to (day 60) 96.40 ± 1.08 mg/g (Table 4).

A gradual increase in muscle glycogen content (MGC) in proportion to the days of feeding of both unripe and ripe papaya fruit juice was observed. The values recorded after feeding with unripe papaya fruit juice were (day 0) 18.85 ± 0.824 to (day 60) 23.80 ± 1.02 mg/g when compared with the day 0 control value (18.04 ± 0.928 mg/g) (Table 5). Whereas, in ripe papaya fruit fed albino rats, the MGC recorded was 18.56 ± 1.24 mg/g in day 0 and 21.88 ± 0.792 mg/g in day 60 (Table 5).

Table 3: Evaluation of plasma protein level (mg/dl) after 60 days of feeding of unripe and ripe papaya fruit juice to experimental diabetic albino rat

Duration of feeding (Days)	Plasma Protein Level (mg/dl)		
	Control	Unripe papaya fruit juice	Ripe papaya fruit juice
0	3.52± 0.141	3.72 ± 0.912 (5.68 %)*	3.68 ± 0.956 (4.55 %)NS
15	3.53 ± 0.032	3.98 ± 0.937 (13.07 %)*	3.84 ± 0.908 (9.09 %)*
30	3.57 ± 0.088	4.02 ± 0.789 (14.20 %)*	3.94 ± 0.084 (11.93 %)*
45	3.50 ± 0.047	4.28 ± 0.432 (21.60 %)*	4.14 ± 0.692 (17.61 %)*
60	3.52 ± 0.046	4.54 ± 1.092 (22.73 %)*	4.32 ± 0.708 (22.73 %)*

*NS=Not Significant, *= Significant*

Table 4: Evaluation of liver glycogen content (mg/dl) after 60 days of feeding of unripe and ripe papaya fruit juice to experimental diabetic albino rat

Duration of feeding (Days)	Liver Glycogen Content (mg/dl)		
	Control	Unripe papaya fruit juice	Ripe papaya fruit juice
0	77.44 ± 0.805	82.54 ± 0.902 (6.59 %)*	82.36 ± 0.632 (6.35 %)*
15	76.97 ± 0.736	85.32 ± 0.624 (10.18 %)*	84.08 ± 1.052 (8.57 %)*
30	78.10 ± 1.303	89.04 ± 1.045 (14.98 %)*	88.12 ± 0.864 (13.79 %)*
45	76.90 ± 0.412	93.64± 0.724 (20.92 %)*	92.72 ± 0.902 (19.57 %)*
60	77.58 ± 0.091	96.92 ± 1.08 (25.15 %)*	96.40± 1.08 (24.48 %)*

*NS=Not Significant, *= Significant*

Table 5: Evaluation of muscle glycogen content (mg/dl) after 60 days of feeding of unripe and ripe papaya fruit juice to experimental diabetic albino rat

Duration of feeding (Days)	Muscle Glycogen Content (mg/dl)		
	Control	Unripe papaya fruit juice	Ripe papaya fruit juice
0	18.04 ± 0.928	18.85 ± 0.824 (4.59 %)NS	18.56 ± 1.24 (2.88 %)NS
15	18.36 ± 0.229	20.64 ± 1.042 (14.41 %)*	19.20 ± 0.642 (6.43 %)*
30	18.50 ± 1.091	21.56 ± 0.645 (19.51 %)*	20.28 ± 1.085 (12.42 %)*
45	18.30 ± 0.632	23.08 ± 0.762 (27.94 %)*	21.32 ± 0.942 (18.18 %)*
60	17.04 ± 0.160	23.80 ± 1.02 (31.93 %)*	21.88 ± 0.792 (21.29 %)*

*NS=Not Significant, *= Significant*

The increase in LGC and MGC by eating fruits like papaya, mango, litchi, apple, banana, berry, pine etc. is earlier reported (Ogata *et al.,* 1972; Reed, 1976; Wagner *et al*., 2012). The increase in PGL caused by feeding of papaya in present study may be attributed to the fact that the free D-glucose is one of the ingredients of papaya and probably it passes into the blood to elevate the PGL in absence of proper insulin concentration. The above literatures established that the ingredients of papaya fruit were potent constituents, causing increase in PGL, LGC and MGC in rats. Furthermore, regular feeding of ripe papaya fruit caused little enhancement in plasma cholesterol level. Papaya fruit is a rich source of glucose and is very suitable for feeding to hypoglycaemic persons (Greenhalgh, 2003).

Conclusion: The study suggests that papaya fruit juice may be beneficial in the management of diabetes, but the consumption of ripe and unripe papaya fruit juice by diabetic patients should be regulated and closely monitored since its consumption increases PGL, PCL, PPL, LGC and MGC concentrations. Its consumption may benefit for patients with hypoglycemia.

ACKNOWLEDGMENTS

The second author is highly grateful to the Head, Department of Zoology, Veer Kunwar Singh University, Arrah 802 301, Bihar, India for providing laboratory facilities to conduct the experiment as no financial support was received from any funding agency for this study.

REFERENCES

ADA (2016). Approaches to glycemic treatment. *Diabetes Care*, 39: S52 – S59.

ANDRADE-CETTO, A. and HEINRICH, M. (2005). Mexican plants with hypoglycaemic effect used in the treatment of diabetes. *Journal of Ethnopharmacology*, 99(3): 325 – 348.

AYBER, M. J., RIERA, A. N., GRAU, A. and SANCHHEZ, S. S. (2001). Hypoglycemic effect of *Smallanthus soncitolius* water extract of leaves in normal and diabetic rats. *Journal of Ethnopharmacology,* 74: 125 – 132.

CHAN, J. R. H. T. and TANG, C. S. (1979). The chemistry and biochemistry of papaya. *Tropical Foods: Chemical Nutrition*, 1: 33 – 53.

DUNCAN, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(1): 1 – 42.

EZEKWE, A. S., ELEKWA, I. and OSUOCHA, K. U. (2014). Hypoglycemic, hypolipidemic and body weight effects of unripe pulp of *Carica papaya* using diabetic Albino rat model. *Journal of Pharmacognosy and Phytochemistry*, 2(6): 109 – 114.

GBOLADE, A. A. (2009). Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria. *Journal of Ethnopharmacology,* 121(1): 135 – 139.

GREENHALGH, D. G. (2003). Wound healing and diabetes mellitus*. Clinics in Plastic Surgery,* 30(1): 37 – 45.

GUEVARRA, B. M. T. and PANLASIGUI, N. L. (2000). Blood glucose responses of diabetes mellitus type II patients to some local fruits. *Asia Pacific Journal of Clinical Nutrition*, 9(4): 303 – 308.

HENLY, A. A. (1957). The determination of serum cholesterol. *Analyst*, 82: 286 – 287.

IDF (2017). *International Diabetes Federation Diabetes Atlas*. 8th Edition, International Diabetes Federation (IDF), Brussels.

KEMP, A. and VAN HEIJNINGEN, A. J. M. K. (1954). A colorimetric micro method for the determination of glycogen in tissue. *Biochemistry Journal,* 56: 646 – 648.

NELSON, N. (1944). A photometric adaptation of the Somogyi method for determination of glucose. *Journal of Biochemical Chemistry*, 153(2): 375 -– 380.

NORRIS, D. O. (2006). *Vertebrate Endocrinology.* 3rd Edition, Elsevier Publishers, United Kingdom.

OSATO, J. A., SANTIAGO, L. A., RENO, G. M., CAUDRA, M. S. and MORI, A. (1993). Antimicrobial and antioxidant activity of unripe papaya. *Life Science*, 53(17): 1383 – 1389.

OSINUBI, A. A., AJAYI, O. G. and ADESIYUN, A. E. (2006). Evaluation of the anti-diabetic effect of aqueous leaf extract of *Tapianthus butungii* in male Sprague-Dawley rats. *Medical Journal of Islamic World Academy of Sciences,* 16(1): 41 – 47.

RAHMAT, A., ABU-BAKER, M. F., FAEZAH, N. and HAMBALI, S. (2004). The effect of consumption of guava, (*Psidium guajava*) or papaya (*Carica papaya*) on total antioxidant lipid profile in normal male youth. *Asia Pacific Journal of Clinical Nutrition,* 13: 5106 – 5112.

REED, C. F. (1976). Information summarizes on 1000 economic plants. Pages 102 – 103. *In:* Typescripts submitted to the USDA.

REINHOLD, J. G. (1953). *Biuret method for determination of plasma protein*. *In:* VARLEY, H. (1976). *Practical Clinical Biochemistry*. Arnold-Hinemann Book Publishers, India.

SHARMA, P. V. (2001). *Dravyaguna Vignyan*. 4th Edition, Volume II. Chaukhambha Vishwabharti, Varanasi.

SRIPANIDKULCHAI, B., WONGPANICH, V., LAUPATTARAKASEM, P., SUWANSAKSRI, J. and JIRAKULSOMCHOK, D. (2001). Diuretic effects of selected Thai indigenous medicinal plants in rats. *Journal of Ethnopharmacology*, 75(2-3): 185 – 190.

WAGNER, H., HIKINO, H. and FARNSWORTH, N. R. (2012). *Economic and Medicinal Plant Research.* Academic Press, United Kingdom.

YANG, S. L. and WALTER, T. W. (1992). Ethnobotany and the economic role of the Cucurbitaceae of China. *Economic Botany*, 46(4): 349 – 367.