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LONG-TERM HEPATOTOXICITY AND HYPOGLYCAEMIC STUDY OF AQUEOUS EXTRACTS OF *CARICA PAPAYA* LEAVES ON NORMAL RABBITS

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ABSTRACT

The use of *Carica papaya* extracts as an anti-diabetic remedy in Nigeria is well documented. Since these extracts are usually taken for long periods of time, it is important to assess their long term biochemical effects so as to ascertain their safety. In this study, the effects of aqueous extracts of *C. papaya* leaves, orally administered daily at 200mg/Kg body weight, on body weight, blood glucose and liver function tests of normal rabbits were observed at pre-set times in the serum for 24 weeks, and in the tissues. Administration of *C. papaya* aqueous leave extracts caused significant ($p<0.05$) reductions in body weight and fasting blood glucose levels for the 24 weeks of monitoring. The parameters used to assess liver function (alanine transaminase [ALT], aspartate transaminase [AST], alkaline phosphatase [ALP], gamma glutamyl transferase [γ-GT], total and direct bilirubin) revealed that the administration of *C. papaya* extracts caused an initial toxic response from the liver, as well as, bile duct obstruction, that were transient and not severe. Since the hypoglycaemic effects of *C. papaya* was again established by this study, further studies to harness the therapeutic effects and reduce the negative side effects are required; long term consumption of the extracts should be closely monitored by a physician.

KEY WORDS: *Carica papaya*; Hypoglycaemic/anti-diabetic; Liver function tests; Medicinal plants; Toxicity

INTRODUCTION

Carica papaya, popularly known as pawpaw and more commonly known as papaya (Watson, 1997), is common in tropical and sub-tropical countries (Sofowora, 1996). In folkloric medicine, extracts of the fruits are used for a variety of medicinal purposes ranging from treatment of ringworm, malaria and hypertension (Sofowora, 1996), while extracts of unripe fruit have been used in treatment of diabetes (Oke, 1998; Lans, 2006). The hypoglycaemic effect has been reported by Olagunju *et al.* (1995). Some diabetic patients concurrently take leave extracts of *C. papaya* with oral hypoglycaemic prescription drugs (Oyelola, 2005).

The growing acceptance of herbal medicine as an authentic source of health care, especially in developing countries, demands the screening of these remedies for toxicity effects in order to ascertain their safety. This study was therefore intended to evaluate the long term effects of aqueous leave extracts of *C. papaya*, orally administered daily for 24 weeks on body weight, fasting blood glucose and liver function of normal rabbits.

MATERIALS AND METHODS

Collection and preparation of plant materials

The leaves of *C. papaya* were collected locally from Akungba-Akoko, Ondo State, Nigeria. Herbarium specimen, with voucher number UIH 22288 was deposited at the Herbarium of the University of Ibadan, Nigeria. The aqueous plant extracts was prepared by the Onoagbe *et al.* (1999) method.

Animals

Twelve (12) rabbits of the New Zealand strain, weighing between 800-1200g, obtained from Animal Unit of Federal University of Technology, Akure, Ondo State, were used for this study. The rabbits were treated by a veterinary doctor and allowed to acclimatize for three weeks before the commencement of experiments. The animals were placed on commercial feed (Ewu growers from the Bendel Feed and Flour Mill, Ewu, Nigeria) and allowed to drink water freely. Treatment of the animals was in accordance with the Principles of Laboratory Animal Care (NIH Publication 85-93, revised 1985).

Experimental Procedure

The twelve rabbits were distributed into two groups of six rabbits each, group one, which served as control, was given tap water; while rabbits in group two were given aqueous extracts of *C. papaya* leaves at 200mg/Kg body weight for 24 weeks. During the monitoring phase, blood was collected from the ventral vein of the rabbits' ear. At the end of 24 weeks, the rabbits were stunned and in this unconscious state, the thoracic and abdominal regions were opened to expose the heart and other organs. Blood was obtained through heart puncture, the liver was also collected. Blood samples for glucose and biochemical assays were allowed to clot and centrifuge at 5,000 rpm for 5 minutes, the serum was then separated for analysis. The liver was homogenized in ice cold normal saline (1:4 w/v), centrifuged and the supernatant stored in the

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freezer until analysis.

Fasting blood glucose was measured by the glucose oxidase method of Barham and Trinder (1972) as described in the Randox Glucose Kit, product of Randox Laboratory Ltd, Ardmore, Diamond Road, Crumlin, Co. Anrtim, United Kingdom. Serum and tissue ALT and AST activities were measured by the Reitman and Frankel (1957) method, serum and tissue ALP activities were assayed by an optimized standard method according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie (Rec. GSCC DGKC) (1972), while serum and tissue γ-GT activities and total protein levels were assayed by the methods of Szasz (1969) and the Biuret method (Gornall *et al.*, 1949) respectively. Serum albumin levels were determined by the Doumas and Biggs (1972) method, while the amount of globulins was calculated as a difference between total serum proteins and serum albumin. Serum total and direct bilirubin levels were measured by the Jendrassik and Grof (1938) method.

Statistical analysis

The differences between means of control and test group were analyzed by the Independent Samples T-test. The SPSS 11.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis. A value of $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Attention has been given to the medicinal value of herbal remedies for safety, efficacy and economy (Glombitza *et al*., 1993; Mahabir and Gulliford, 1997). The use of *C. papaya* extracts as herbal, as well as anti-diabetic, remedy in Nigeria is well documented (Emeruwa, 1982; Olagunju *et al.*, 1995; Sofowora, 1996; Oke, 1998; Oyelola, 2005). Since these remedies are usually taken for long periods of time, it is important to assess their long term biochemical effects so as to ascertain their safety.

Body weight

C. papaya leaves was found to possess significant weight reducing effects on normal rabbits (Figure 1); upon dissection, rabbits treated with aqueous extracts of *C. papaya* leaves for 24 weeks were found to be almost completely bereft of sub-cutaneous fat, implying an actual loss of fat deposits. Phytochemicals such as tannins and saponins (Kao *et al.*, 2000; Awika and Rooney, 2004) have been shown to promote weight loss by facilitating the metabolism of lipids. The presence of these phytochemicals in *C. papaya* leaves (Omonkhua and Onoagbe, 2010) may be responsible for this anti-obesity effect, This effect could be important in treating type 2 diabetes in obese individuals since loss of weight has been shown to improve insulin sensitivity.

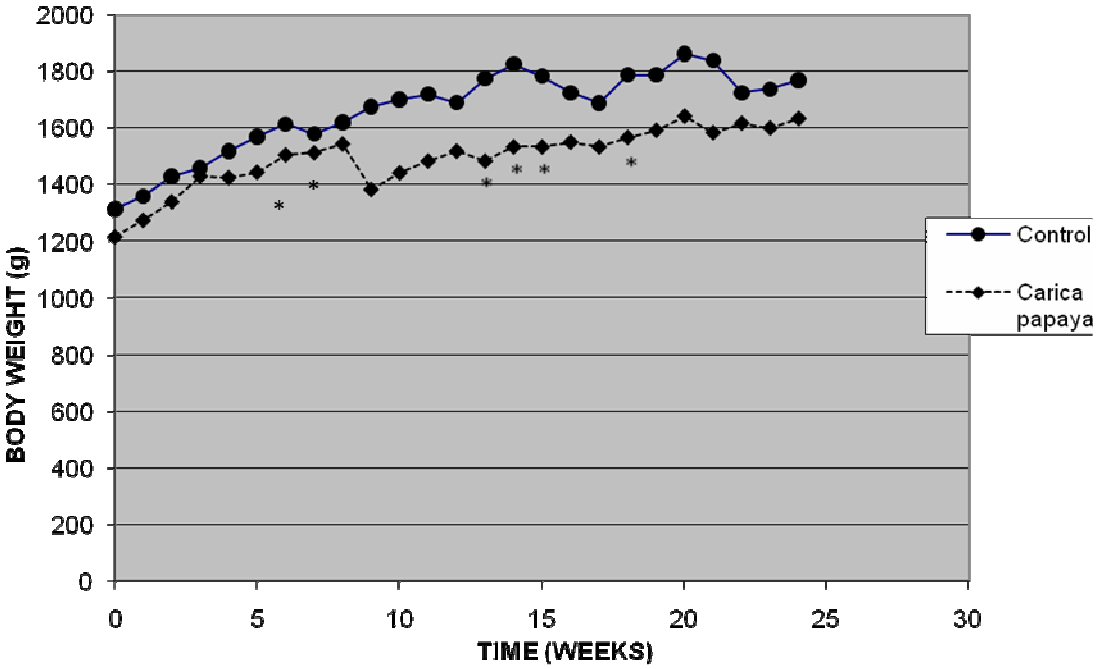

Figure 1: Time-course of body weight gain of control rabbits and rabbits orally treated daily with aqueous extracts of *C. papaya* leaves for 24 weeks at 200 mg/kg body weight. Data was obtained weekly and are means of 4-6 determinations. Values carrying notations are statistically different from control at $p<0.05$.

Fasting blood glucose

Significant ($p<0.05$) decreases in fasting blood glucose levels were observed from week 4 to the end of the monitoring phase for the *C. papaya* treated rabbits (Figure 2), indicating that this plant possesses hypoglycaemic properties. This observation correlates with that obtained by Olagunju *et al.* (1995) and shows that the hypoglycaemic effect of *C. papaya* is sustained. The presence of tannins and saponins, and other phytochemicals, as well as the presence of plant carbohydrates (Omonkhua and Onoagbe, 2010), which have recognizable anti-diabetic effects (Welsh *et al.*, 1989; Murakami *et al.*, 1996; Yuan *et al.*, 1998), may contribute this observation.

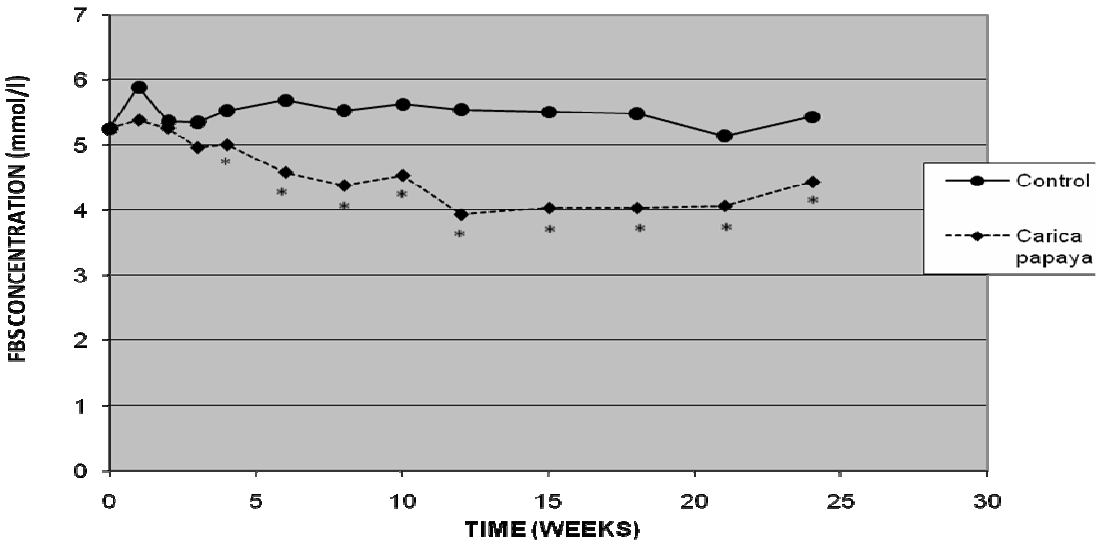

Figure 2: Effects of repeated daily oral administration of aqueous extracts of *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on the fasting blood sugar concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4-6 determinations. Values carrying notations are statistically different from control at $p<0.05$

Liver function tests

Serum aminotransferases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are sensitive tests of hepatocyte injury (Wrobleski, 1959; Ellis *et al.*, 1978). Although often referred to as "liver function" tests, they do not measure hepatocyte function but instead hepatocyte damage. Hepatocyte necrosis in acute hepatitis, toxic injury or ischemic injury results in the leakage of these enzymes into the circulation (Haber *et al.*, 1995; Healey *et al.*, 1995). ALT/AST ratio higher than control values were observed in weeks 1, 3, 4, 6 and 8 in the *C. papaya* treated rabbits (Figure 3). None of the increases observed in serum ALT activities was more than 1.5 times the value of control. Since a high ALT/AST ratio is indicative of liver damage, these results imply that though a toxic response occurred, the increases were not high enough to indicate severe (necrotic) liver damage (Sherman, 1991). Since the serum ALT and AST activities were subsequently restored to normal, as well as the fact that liver enzymes were not depleted, shows that this event was not sustained.

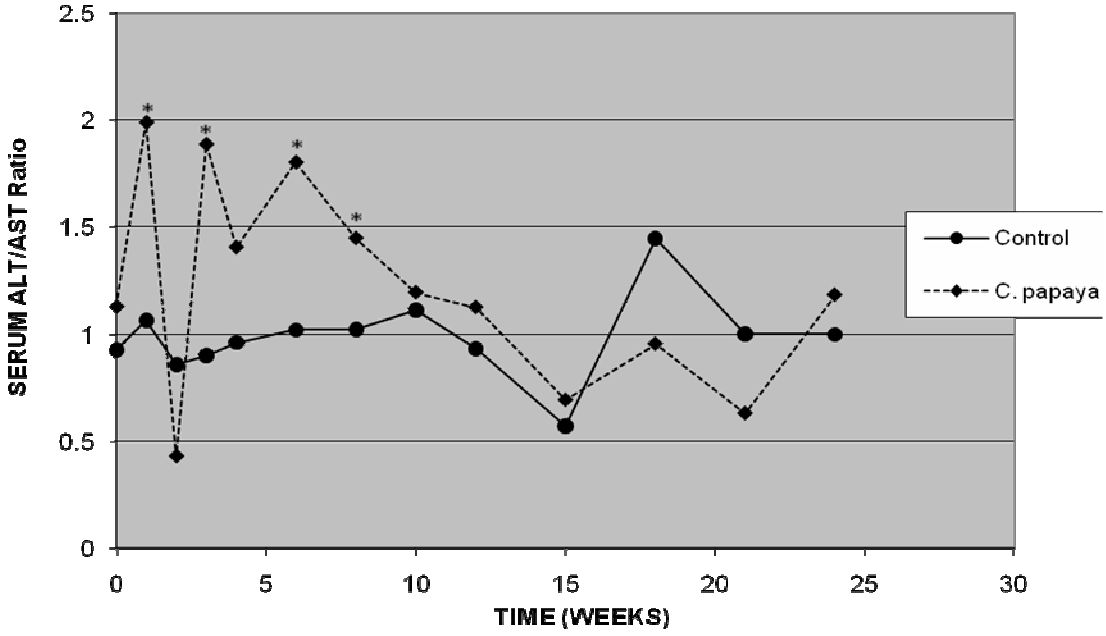

Figure 3: Effects of repeated daily oral administration of aqueous extracts of *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on serum ALT/AST ratio of normal rabbits. Data was obtained by dividing the serum ALT values by serum AST values and are means of 4-6 determinations. Values carrying notations are statistically different from control at $p<0.05$

Increase in serum alkaline phosphatase, ALP and γ-glutamyl transferase (GGT) levels is indicative of bile duct obstruction or intra-hepatic cholestasis (Johnston, 1999). A nearly four-fold increase was observed in week 3 in the serum ALP activities of *C. papaya* treated rabbits (Figure 4). The same degree of increase was observed in serum GGT activities in week 4 (Figure 5), suggesting that the increased serum ALP originated from the liver. These results indicate cholestasis at this point of monitoring (from week 3 to 4). The fact that most of the other points of monitoring showed values similar to control, in addition to the fact that liver ALP and GGT levels were similar to control, shows that this effect was fleeting.

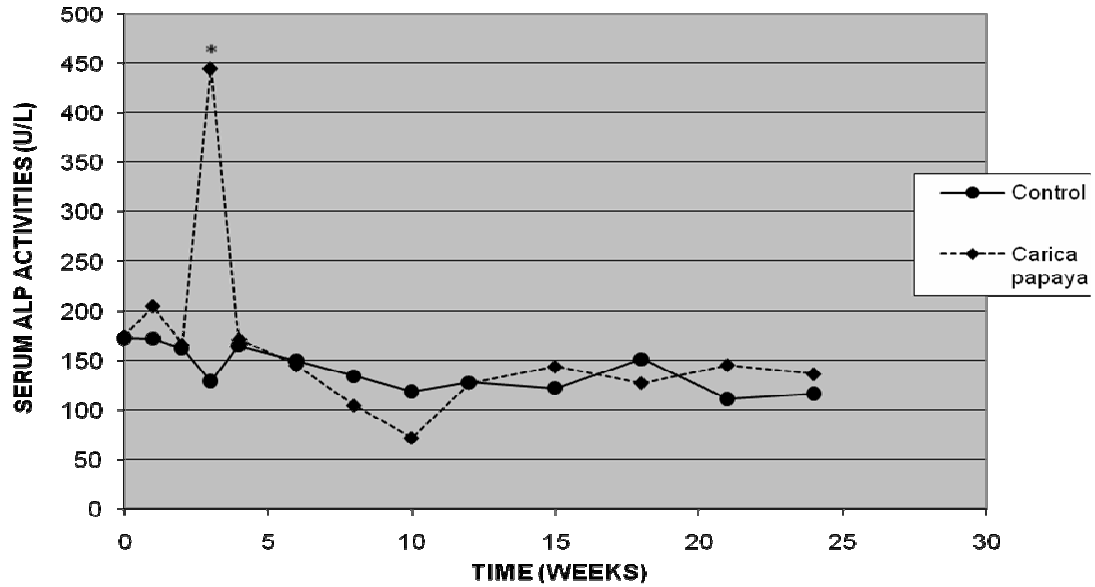

Figure 4: Effects of repeated daily oral administration of aqueous extracts of *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on serum ALP activities of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4-6 determinations. Values carrying notations are statistically different from control at $p<0.05$

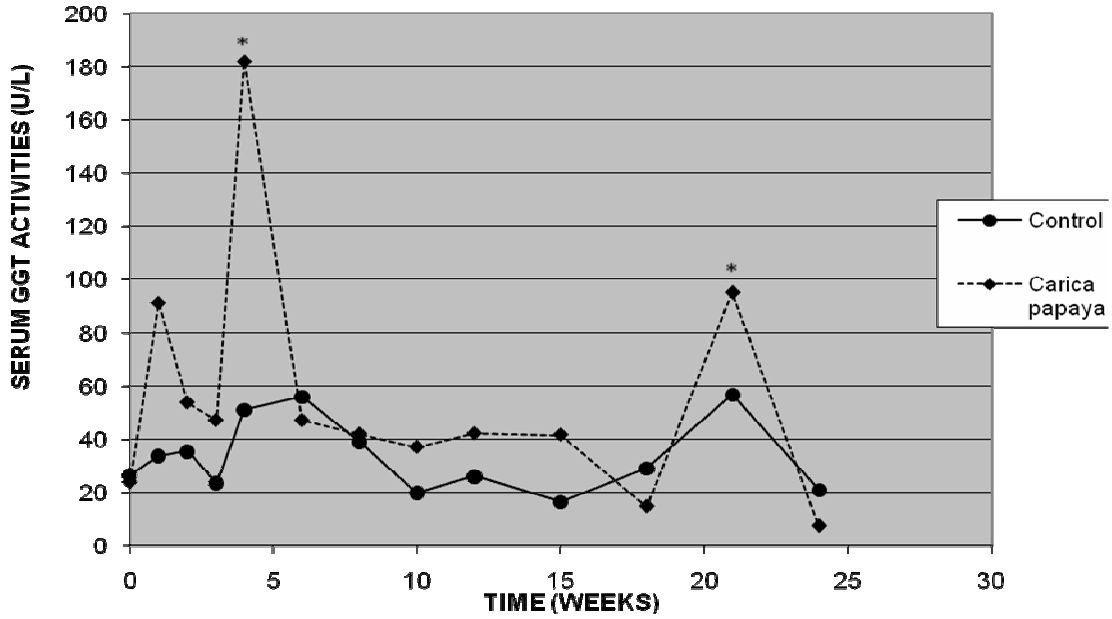

Figure 5: Effects of repeated daily oral administration of aqueous extracts of *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on serum GGT activities of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4-6 determinations. Values carrying notations are statistically different from control at $p<0.05$

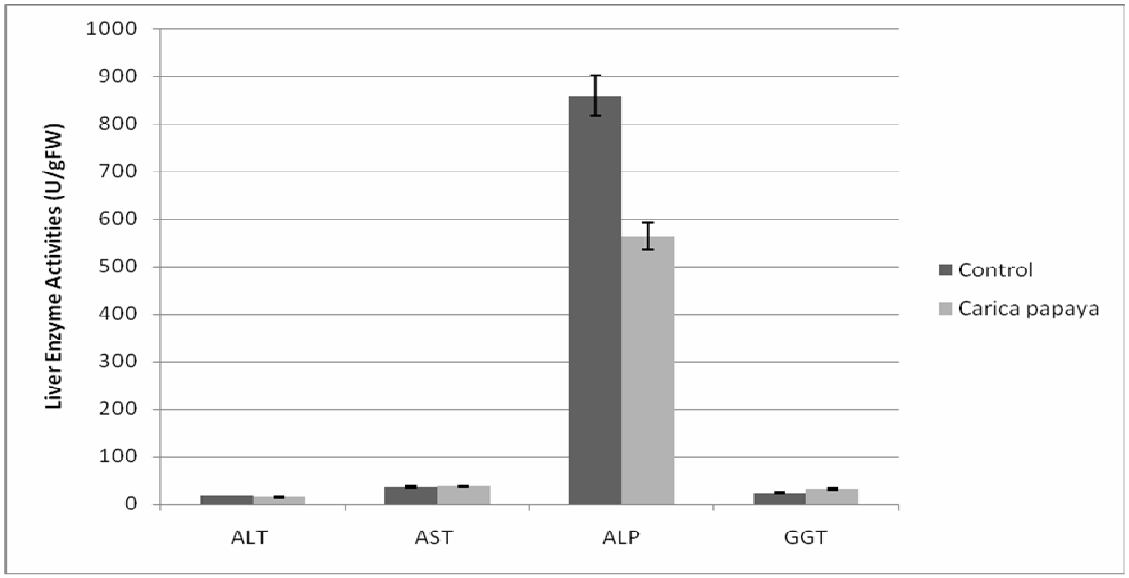

Figure 6: Effects of repeated daily oral administration of aqueous extracts of *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on liver ALT, AST, ALP and GGT activities of normal rabbits. Data was obtained from tissue homogenates at the end of 24 week of monitoring and are means of 4-6 determinations.

Any change in the concentration of serum protein indicates a change in the normal liver functions. Serum total proteins, albumin and globulins concentrations of rabbits were not significantly altered by *C. papaya* treatment (Figures not shown). Low protein level results when there is extensive liver damage (Wada and Snell, 1962), however, determination of serum albumin is a preferred assay for the synthetic liver function (Corless and Middleton, 1993). Serum albumin does not change in mild liver injury but readily declines in the face of sub-massive liver necrosis. Albumin synthetic capacity is typically preserved even in severe cirrhosis (Rosenthal, 1997). Taken together, the implication of these results is that the medicinal plants did not diminish the protein synthetic capacity of the liver. It is well-established that the serum level of bilirubin is raised when the hepatic biliary system is affected (Ingram, 2003). Serum total bilirubin concentration was transiently increased in week 3 (Figure 7) while serum direct biliribin was increased in weeks 4, 6, 8 and 10 (Figure 8). Increase in serum direct (conjugated) bilirubin is indicative of bile duct obstruction. This situation was however resolved before the end of the monitoring period.

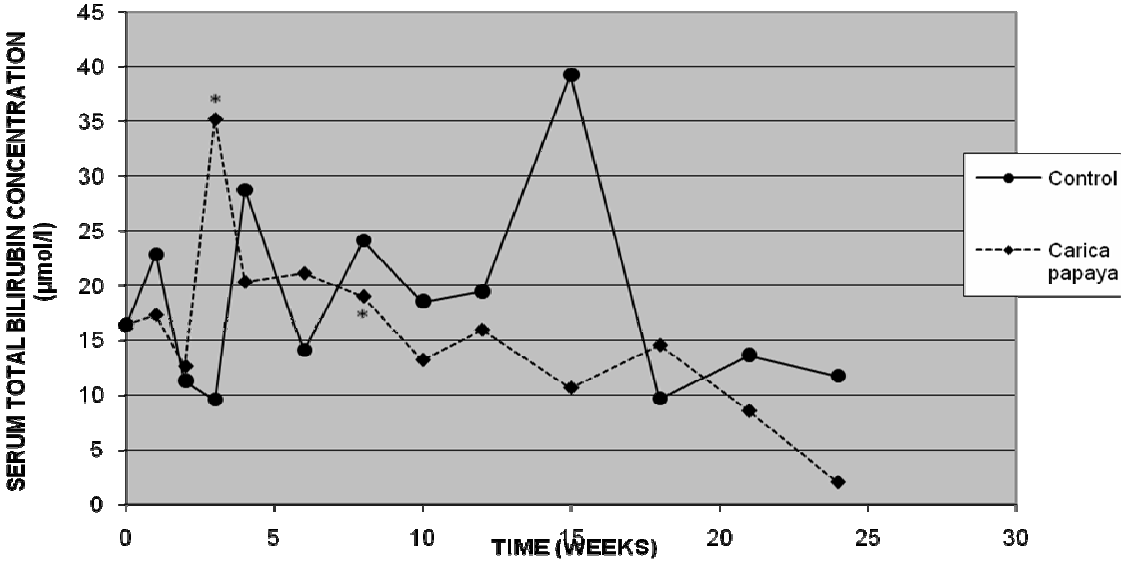

Figure 7: Effects of repeated daily oral administration of aqueous extracts of *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on serum total bilirubin concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4-6 determinations. Values carrying notations are statistically different from control at p<0.05

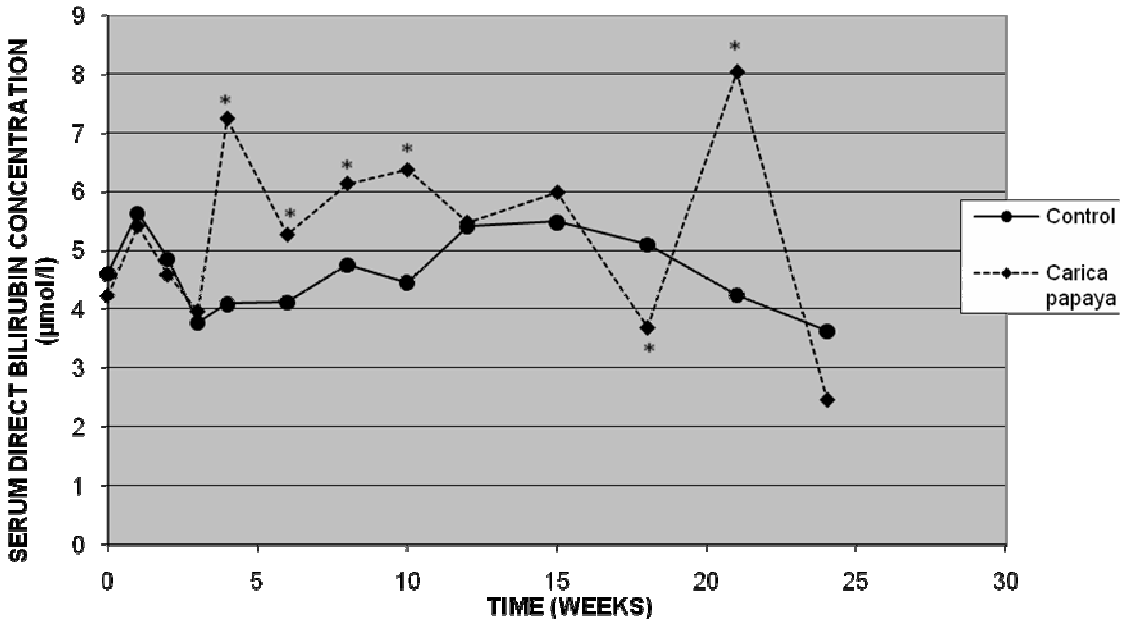

Figure 8: Effects of repeated daily oral administration of aqueous extracts of *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on serum direct bilirubin concentration of normal rabbits. Data was obtained from serum at pre-determined intervals and are means of 4-6 determinations. Values carrying notations are statistically different from control at p<0.05

The results obtained from this study revealed that administration of *C. papaya* aqueous extracts to normal rabbits for 24 weeks caused an initial toxic response from the liver, as well as, bile duct obstruction, that were short-lived and not severe. Since the therapeutic effects of *C. papaya* on diabetes mellitus was again established by this study, further studies to harness the therapeutic effects and reduce the negative side effects are required; in the mean time, long term consumption of *C. papaya* extracts by diabetes sufferers must be closely monitored by a physician.

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