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Short Communication

Effect of Aqueous Extract of Matured Unripe *Carica papaya* Seeds on Blood Glucose and Amylase in Wistar Rats

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ABSTRACT
Objective: Healthy adult male Wistar rats were employed to evaluate possible alterations in plasma glucose and intestinal amylase levels to varying doses of aqueous extract of matured unripe *Carica papaya* seeds. The study was carried out in a well-ventilated animal house with standard normal feeds, clean water and ambient temperature.
Methods: The animals were divided into four groups of four animals per group, comprising of three experimental groups, and a control group. Each of the three test groups received a daily dose of one of 50, 100 and 200mg/kg of body weight of the extract, respectively, for 21 experimental days through orogastric route of administration, while the control group was given no treatment with the seeds extract. At the end of the experiments, blood samples from each group were collected from the abdominal aorta using hypodermic syringes and micropipettes. The samples were analyzed for serum glucose and intestinal amylase using automated spectrophotometric analyze. The results were expressed as Mean ±SEM, and values of $p \leq 0.05$, were regarded as statistically significant change.
Results: There was a clear decrease ($p \leq 0.05$) in the serum glucose and amylase levels, in mainly animals treated with the 200mg/kg dose of the extract at the end of the 3 weeks administration period.
Conclusion: The results in this study are strongly suggestive that *Carica papaya* seeds possess hypoglycaemic activity, and therefore a clear demonstration of possible (or potential) anti-hyperglycaemic and anti-diabetic properties of the *Carica papaya* seed.

INTRODUCTION

Carica papaya is known as pawpaw in local parlance in many communities in Nigeria and many parts of Africa and Asia. A herbaceous plant with self-supporting stem, *Carica papaya* belongs to the Family Caricaceae. The plant is believed to have originated in Central America where it was first cultivated for many years, and is now widely distributed in tropical countries including Asia, the Middle East and other parts of the world. *Carica papaya* has a rather complicated means of reproduction as the plants can be males, females or hermaphrodites. The fruit is edible and is used in many countries in different ways. For example the ripe fruit can be eaten raw without the seeds, while the unripe green fruit (known to be a rich source of vitamin A) can be cooked and used in the culinary of salads and stews as used in Thai cuisines[1] and in Southern Asian cooking (Green papaya salad Recipe). The unripe *Carica papaya* fruit is also used in Vietnamese cooking as vegetable, fermented into sauerkraut or candied.[2]

There have been reports of several uses of *Carica papaya* in medicinal applications, especially amongst indigenous populations of Africa, Asia, China and Southern America. Reports have shown that different parts of the plant (the fruit, leaves, seeds, fruit latex and even the flower) have different medicinal applications. For example, the aqueous extract of the unripe *Carica papaya* seeds was reported to possess strong anti-sickling properties.[3] Following some studies that also evaluated the phytochemical composition of *Carica papaya* seeds, the anti-sickling agent was reported to be in the ethyl acetate fraction of the extract.[4] In addition, there have been reports of several medicinal effects attributed to *Carica papaya* seeds which include: anti-diabetic effects believed to be due to the hypoglycemic and hypolipidemic properties[5-7]; its application in the treatment of poisons related renal and hepatic disorders believed to be due to its nephroprotective activities,[8] its general cell protective effects and antioxidant properties and activities.[9-12] *Carica papaya* seeds were also reported to be effective in the treatment of hypertension, diabetes mellitus and hypercholesterolemia [5], effective as a vermifuge used against intestinal worm while the flower infusion is also used to induce menstruation.[13] Other reports noted that chewing of *Carica papaya* seeds helps to clear nasal congestion.[14] From the foregoing accounts, it is clear that *Carica papaya* is a plant with very long history of medicinal uses.

Presently, healthcare delivery appears to be shifting from emphasis on synthetic drugs, to the use of herbal formulations in most parts of the world. Many communities especially in Africa, Asia and South America still hold rigidly on to phyto-medicine and the use of herbs for their healthcare needs. In the light of the growing resistance of parasites and pathogens to synthetic drugs, the use of herbal self-medication and ethno-medicinal practices appear to offer some novel line alternatives. Increasing numbers of patients now use medicinal herbs and prefer to seek the advice of traditional herbal healers regarding their use.[15] It is however a fact that there is very limited knowledge as regards the constituents of the plants employed in herbal medications and their side effects in humans. This is coupled with the fact that there are no documentations on ethno-medicinal data of medicinal plants since traditional healers keep no records, and information on claims of the therapeutic efficacy of medicinal plants are simply passed on verbally from one generation to generation.

Chemical composition analysis of *Carica papaya* show that the fruit contains water, carbohydrate low in calories, dietary fiber, folate, vitamins A and C, ascorbic acid and potassium. Analysis of the seed revealed that, per 100g, it contains: protein (24.3%), fatty oil (25.5%), carbohydrate (17.0%), crude fiber (8.8%), volatile oil, glycoside, caricin, the enzyme myosin, saturated acids (palmitic, stearic and arachidic), unsaturated acids (oleic and linolic). The seeds also yielded 660-760mg bactericidal aglycone of glucotropeaolin benzyl isothiocyanate (BTIC), glocoside, sinigrin, and carpasemine. Fermentation with brewer's yeast and distillations identified 106 volatile compounds in *carica papaya* seeds and yielded 4% alcohol, 91.8% ethanol, 4.8% methanol, 2.2% N-propanol and 1.2% unknown non-alcohol compounds. Some earlier phytochemical analysis of *Carica papaya* showed that the seed also contains alkaloids, flavonoids, saponin, tannin, anthraquinones and anthacyanosides (16a), while benzyl isothiocyanate, a natural toxin with potent diverse biological activities, is contained in both the fruit and seeds.[16]

Carica papaya fruit and seed extract, like any other plant material or drug ingested orally, passes into the intestine and is absorbed into blood from where it eventually reaches the liver. The liver contains diverse enzymes and it receives blood from the intestine through the portal vein. With a wide variety of functions, the liver metabolizes whatever active molecules reaches it, and carrying out detoxification function, it removes many active molecules by excretion in the bile, phagocytosis by Kupffer cells and by alteration of the molecules within the hepatocytes. This study aimed to evaluate possible alterations or changes in the serum blood glucose and intestinal amylase levels in doses of aqueous extract of *Carica papaya* seeds in Wistar rat.

MATERIALS AND METHOD

The seeds of matured unripe fruits of *Carica papaya*, obtained from Faculty of Agriculture Co-operative Farms in Benin City, Edo State, Nigeria, were prepared by drying in the sun for a few days. Care was taken to ensure that no particles of dust or dirt mixed with the seeds during the drying. The dried seeds were pulverized into fine powder using a mortar. The powder was soaked in distilled water to allow for the extraction of the constituents. The Soxhlet extractor used for the aqueous extraction was assembled with distilled water used as the solvent extractor.

The fine powder of the seeds was placed in a thimble and inserted into the Soxhlet extractor, and distilled water was allowed to enter constantly into the extractor through the inlet. The extract was obtained in solution and was placed in the evaporator at about 600c. The extraction yield was recovered in a fine paste weighing about 38gm. The extract was kept in clean sample containers and stored in a refrigerator for proper preservation until use. Determination of the various doses of the extract was based on the methods described by earlier workers, in mg/kg per body weight of the animal.[7]

Twenty adult male Wistar rats of age in the range of four to five months, of comparable size and weighing between 200-250gm, were kept to acclimatize in the laboratory for a period of one week. The animals were adequately fed with livestock feeds and clean water throughout the period of the experiments. Meanwhile, baseline values of the parameters which included: the serum glucose level (mg/dL), the serum intestinal amylase level (iu/L), and mean body weight in the various groups of animals were measured using spectrophotometer and weighing balance and recorded.

The rats were organized into four groups of four animals per group. One group of four animals served as control. The test groups received the doses of the extract daily for a period of three weeks as follows:

Group 1 (Control) were given no treatment with the seeds extract throughout the experiments; Group 2 received 50mg/kg per body weight; Group 3 received 100mg/kg, and Group 4 received 200mg/kg per body weight of the extract, respectively.

The animals were given the extract by orogastrictube administration. Each animal in a test group received a daily dose administration of one of the doses 50, 100 or 200mg/kg per the body weight, for the period of 21 days. At the end of the administrations, blood samples were collected from the abdominal aorta using hypodermic syringes, needles and micropipettes. The samples were thereafter analyzed for the serum glucose and intestinal amylase level using automated spectrophotometric analyzer (Zhejiang Top Instruments C Ltd, China).

Data from the various animal groups were pooled and subjected to statistical analysis, employing the Student t- test, and values of $p<0.05$ were regarded as statistically significant.

RESULTS

At the end of the experiments with the various doses of the *Carica papaya* seeds extract, there were clear observable (but not statistically significant) decreases in the serum glucose and amylase levels in animals of Group 2 (treated with the 50mg/kg), and Group 3 (treated with the 100mg/kg) dose of the seeds extract (Table 1). At increases in dose of the extract, the serum blood glucose and amylase levels showed significant decreases($p<0.05$) in animals of Group 4 that received the 200mg/kg (Table 1, Figure 1). As compared to the baseline control, there was no appreciable change in mean body weight of the animals in all the test groups at the end of the experiments (Table 1).

Comparative analysis of the plasma glucose and serum amylase levels presented in Figure1 showed that the nature of the alteration in both compounds was relatively similar; the

pattern of the decrease was fairly uniform in both compounds and which also corresponded well with baseline control values at the end of the experiments. Similarly, there was comparatively no clear change in the mean body weight in the animals of the different groups at the end of these experiments (Table 1).

Table 1: Serum Glucose, Intestinal amylase level and Mean body weight in various groups of animals

GROUP	SERUM GLUCOSE (mg/dL)	SERUM AMYLASE (iu/l)	MEAN WEIGHT (gm)
1(Control)	117.40±5.1	1273.0±173.6	203±7.0
2	101.0±8.3	1084.2±215.8	197±3.4
3	96.80±7.5	1005.8±201.4	192±5.1
4	86.0 ±8.9*	976.4±214.3*	191±2.9

*Significant values ($p<0.05$) are compared to the baseline control. Group 1 (no extract); Group 2 (50mg/kg); Group 3 (100mg/kg); Group 4 (200mg/kg), respectively. (n=4)

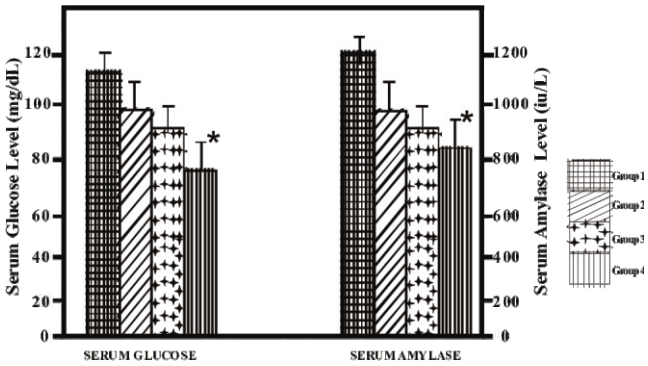

Figure 1: Comparative values of Serum Glucose and Intestinal Amylase in the various groups of rat at the end of the experiments.
*Significant values are Mean±SEM, compared to baseline control.

DISCUSSION

The results of this study revealed clear alterations in both serum glucose and intestinal amylase levels at the end of the administrations with doses of *Carica papaya* seeds extract in Wistar rats. From the results, it can be inferred that *Carica papaya* seeds may have some definable effects or modulating properties influencing the serum glucose and amylase concentrations in Wistar rat. Thus, data from this study appear indicative of possible hypoglycemic properties of *Carica papaya* seeds. Some earlier reports have also speculated on possible hypoglycemic properties of *Carica papaya* seeds.[7] In another related report, the various phytoconstituents of *Carica papaya* seeds were determined along with analysis of their chemical compositions. Subsequently, the phytochemical analysis yielded various constituent compounds, most of which were also implicated in various metabolic processes in some organs (e.g. liver) of these animals.

It has been reported that phytoconstituents of the *Carica papaya* seed like the alkaloids, inhibited alpha-glucosidase and decreased glucose transport through the intestinal epithelium.[17] It was also reported that flavonoids significantly suppressed serum glucose and reduced plasma cholesterol and triglycerides, while it increased hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets.[18] In addition, a number of known phytoconsituents of *Carica papaya* seeds have been implicated in the mechanism of insulin release, transport and degradation. For example, it was observed that saponin stimulates insulin release and blocks the formation of glucose in the bloodstream, while ferulic acid stimulates insulin secretion generally.[18]

Earlier reports show that *Carica papaya* seeds area rich source of proteins, lipids and crude fibres, among others.[19,20] A number of workers independently demonstrated that hypoglycaemic effects of a plant material may be as the result of negative effect of the plant material on pancreatic tissues.[21,22] Also according to some earlier reports, hypoglycaemic effect of a plant material may be as the result of the level of fibers in the plant which may interfere with carbohydrate absorptive processes.[23] It is also noteworthy from earlier reports that hypoglycaemic effect could be as the result of mutual interference of aggregates of the fibers in the plant material with absorptive processes. In the light of these reports and in view of the results of the present study, it is possible that *Carica papaya* seed possesses hypoglycaemic properties in rats by some mechanism of its biologically active constituents on organs such as the pancreas. Recently, it was learnt that dietary fibers effectively absorbed glucose, retarded glucose diffusion and inhibited the activity of alpha-amylase and may also be responsible for decreasing the rate of glucose absorption and concentration of postprandial serum glucose.[18]

Meanwhile, it is suggested that further studies be conducted to further elucidate the present observations and ascertain the mechanism of action for the observed lowering effects of both blood glucose and intestinal amylase observed in this study. It is hoped that data from the present study may provide some scientific basis for the possible use of *Carica papaya* seeds as anti-hyperglycaemic and anti-diaberic agent in polyherbal formulations by traditional healers in many communities in different parts of the world.

CONCLUSION

The result of this study is strongly suggestive of hypoglycaemic effects. It is therefore possible that Carica papaya seeds may have some clearly definable anti-diabetic properties in view of the definite decrease in the serum glucose and amylase levels in the test animals of, especially Groups 3 and 4, at the end of the 21-days' administration with the seeds extract. It is hoped that future studies will help to further elucidate the present observation.

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