Phytochemical Screening and Effects of Methanol Extract of Carica papaya Stem bark in alloxan induced Diabetic Rats

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Abstract
The phytochemical screening and hypoglycemic effect of Carica papaya stem bark in alloxan-induced diabetic albino rats was investigated. Phytochemical analysis of the methanolic extract of Carica papaya revealed the presence of steroids, phenolics, glycosides, alkaloids, saponins, flavonoids and tannins. The hypoglycemic activity of the same extract of Carica papaya stem bark was compared with that of a known antidiabetic standard drug-glibenclamide in alloxan induced diabetic rat models. There was a significant (p<0.05) reduction in blood glucose concentration (mg/dl) by the methanolic extract of Carica papaya stem bark (500mg/kg bw) and glibenclamide (0.6mg/kg bw) from 388.21±17.40 to 103.25±6.79 and 365.00±15.98 to 95.50±3.96 respectively. The blood glucose of the induced diabetic but non treated rats increased from 307.50±22.35 to 509.45±50.57 while for non induced rats, no significant changes were observed. The extract and the standard drug also prevented further body weight loss in treated diabetic rats compared to the untreated. The purpose of this study is to ascertain scientifically, the claim that the plant part has antidiabetic potentials. The results obtained from this study indicate that the methanolic extract of Carica papaya contains bioactive substances with hypoglycemic potency. Thus, providing scientific link for the plant part to be processed for oral hypoglycemic remedy.

Keywords: Hypoglycemic, phytochemical, induced, extract, diabetic

INTRODUCTION
Diabetes is a metabolic disorder in which the body is unable to process enough insulin or cannot use insulin properly. This can cause accumulation of glucose in blood, leading to potential complications which continue to be a mystery. Although, both genetic and environmental factors, such as obesity and lack of exercise appear to play a part (Tierney and Papadakis, 2002). The disease has been considered as one of the major health concerns worldwide today (Stoler et al., 2008). The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly diet. This has in a way suggested an environmental effect but there is little understanding of the mechanism (Wild et al., 2004).

The current agents used for diabetes mellitus control are: Biguanides, Thiazolidione derivatives and insulin injection. Although these drugs have been extensively used because of their beneficial effects in the control of hypoglycemia, they are unable to establish adequate control of the disease due to their inability to suppress the associated chronic and acute complications, in addition to specific effects for each of the drugs. Their administration and dosage problem has led to search for alternative therapies with similar degree of efficacy without the undesirable side effects. Hence, hypoglycemic agents of plant origin (Okigbo and Mmeka, 2006).

Therefore, study of plants with emphasis on hypoglycemic activities may give a novel approach in the treatment of diabetes mellitus. In Nigeria, the use of medicinal plants in the control and treatment of several diseases including diabetes mellitus are common practices in the rural areas where people depend solely on herbal treatment. The effectiveness of some of the medicinal plants has only been a mere claim, while some have been scientifically proved to be either active or inactive hypoglycemic agents. In order to reduce the number of diabetes complications and to delay their development, Savickiene et al., 2002 recommended the use of biologically active components and plants. This study tends to highlight the hypoglycemic potentials in stem bark of Carica papaya for a possible drug development towards the management of diabetes mellitus. Carica papaya is a tropical tree widely cultivated in tropical regions of the world. The fruits are rich in vitamins A and C. The fruit leaf, latex are used for the treatment of various ailments which include typhoid fever, wound infection, asthma, fever, diarrhea, hypertension and diabetes. The juice is used for curing cancer and tumors. The antihypoglycemic effect of unripe mature fruits and seeds of Carica papaya have been widely reported (Olagunja et al., 2009).

Alloxan is a urea derivative which causes selective necrosis of the β-cells of pancreatic islets. It has been
widely used to induce diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose (Iranloye, et al., 2011). The toxic action involves oxidation of essential sulphydryl groups, inhibition of glucokinase, generation of free radicals and disturbances in intracellular calcium homeostasis (Szkudeiski et al., 2001).

MATERIALS AND METHODS

Sample Collection and Preparation
The stem bark of Carica papaya was collected from Federal University of Technology, Minna botanical garden located at Bosso Campus. The sample after collection was washed with water and dried at room temperature for a period of two (2) weeks. Dried stem bark was then pounded using a clean mortar and pestle. The dried product was then blended into fine powder and kept in air tight sample containers.

Extraction of Sample
50.0g of the powdered stem bark Carica papaya was weighed into 400ml of methanol in a round bottom flask and refluxed at 65°C for a period of 3 hrs. The extract was then dried on a water bath to ensure the complete evaporation of methanol.

Animals Used
Twenty (20) albino rats of both sexes weighing between 175-230g were obtained from National Veterinary Research Institute, Vom in Jos. They were kept in animal cages to acclimatize in the laboratory for 2 weeks. In all, sixteen (16) rats were randomly grouped into four groups of four rats each as shown below:-

Group A: Non induced rats
Group B: Induced diabetic rats but not treated
Group C: Induced diabetic rats but treated with 500mg/kg BW of extract
Group D: Induced diabetic rats but treated with 0.6mg/kg BW glibenclamide.

Induction of Diabetes
The rats were induced to diabetic status by Alloxan monohydrate (100mg/kg.BW). After one week of induction, the rats were tested to confirm their diabetic status. Treatment with extract and standard drug-glibenclamide was carried out for a period of four weeks.

Phytochemical Screening
The Carica papaya stem bark extract was screened for phytoconstituent properties by Treese et al., (1983) method.

Administration of Extract
500mg/kg,BW of the extract was administered to group C rats while 0.6mg/kg,BW of standard drug-glibenclamide to group D. The group B was administered dimethylsulphoxide (DMSO) in distilled water and group A was given distilled water only.

RESULTS

Table 1 indicates the phytochemical constituents of the stem bark extract of Carica papaya while fig. 1 depicts the fasting blood glucose concentration (mg/dl) of diabetic rats treated with methanolic extract, standard drug-glibenclamide non treated and non induced (control). Fig. 2 reveals the weight changes in diabetic rats treated with extract, standard drug-glibenclamide non-treated and non induced (control)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inference</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
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<tr>
<td>Tannins</td>
<td>+</td>
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<tr>
<td>Phenol</td>
<td>+</td>
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<tr>
<td>Steroid</td>
<td>+++</td>
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<tr>
<td>Terpene</td>
<td>+</td>
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<tr>
<td>Anthraquinones</td>
<td>-</td>
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<tr>
<td>Cardiac glycosides</td>
<td>+++</td>
</tr>
</tbody>
</table>

Keys: (Slightly present)+,  + + (Moderately present) + + + (Highly present) - (Absent)
DISCUSSION
Phytochemicals occur naturally in plants and they are responsible for colour and organoleptic properties, such as the deep purple of blue berries and red for tomatoes. Previous reports have indicated that phytoconstituents in fruits and vegetables may reduce the risk of cancer possibly due to dietary fibers, polyphenols, antioxidants and anti-inflammatory effects. The phytochemical analysis of the methanolic extract as shown in Table 1 was found to contain steroids, phenolic substances, glycosides, alkaloids, saponins, flavonoids and tannins. These substances have been reported to be responsible for hypoglycemic activities of the extract as most plants that had potentials for blood glucose reduction contains similar phytochemicals as found in the methanolic extract of Cajanus cajan leaf (Adeobii et al., 2010), ethanolic extract of Pongamia pinnata leaf (Mukesh et al., 2010) and aqueous extract of Gaaruga pinnata stem and root barks (Shirwaikar et al., 2006). All the mentioned extract had hypoglycemic activities in experimental diabetic animals.

The oral administration of 500mg/kg BW of the extract and 0.6mg/kg BW standard drug significantly (p<0.05) reduced the blood of glucose levels in alloxan-induced diabetic rats from 388.21±17.40 to 103.25±6.79 and 365.00±15.98 to 95.50±3.96 respectively. The induced but not treated animals had increased blood glucose levels from 307.50±22.35 to 509.25±30.73 as depicted in figure 1. The percentage glycemic change for the extract was 73.20% and 74.84% for glibenclamide indicating that the drug was slightly more effective than the methanolic extract. This finding is in agreement with the effect of ethanolic extract of Rubus ellipticus (Sharma et al., 2011) and effect of methanolic extract of Diospyros melanoxylon root and stem barks (Jadhav, et al., 2009) which had similar hypoglycemic activity against alloxan-induced diabetic rats. The induced but not treated group showed a steady blood glucose concentration.

The body weight change results depicted in figure 2 showed a significant (p<0.05) reduction in body weight in alloxan-induced diabetic rats treated with Carica papaya stem barks extract and those rats treated with standard drug-glibenclamide when compared to the untreated diabetic rats. The diabetic rats treated with extract and standard drug-glibenclamide began to regain weight lost in week three of treatment while the diabetic not treated kept on losing weight. This might be due to impaired glucose metabolism and excessive breakdown of tissue protein which is an indication of diabetes mellitus. This finding is in agreement with Shalini et al., 2009, aqueous extract of Trichosanthes dioica leaves and methanolic extract of Berberis aristata stem bark (Semwal et al., 2008). The hypoglycemic activity exhibited by methanolic extract of Carica papaya stem bark might be due to enhanced glucose utilization by peripheral tissues.

*Statistical Analysis: The results were analyzed by one-way ANOVA treatment

CONCLUSION
This study reveals the hypoglycemic potentials of the methanolic extract of Carica papaya stem bark in diabetic rats which in a way confirms its use as tradomedical remedy for diabetes mellitus.

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LIMITATION OF THE RESEARCH
Further research work which will cover purification and characterization of the active principles contained in the extract is suggested since it's not covered in this study.

REFERENCES


