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Evaluation of hypoglycemic activity and safety of *Carica papaya* seed extracts in alloxan-induced diabetic mice

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***Carica papaya* is used for the treatment of many diseases such as stomach upsets, diarrhoea, bilharzia, diabetes. The study aims to evaluate the safety and hypoglycemic potential effect of *Carica papaya* seeds on alloxan induced diabetic mice. DCM extracts were examined for the presence of terpenoids, flavonoids, steroids, phenols, tannins and alkaloids. Toxicity profile was established at 5, 50, 200, 300 and 2000 mg/kg for both water and methanol extracts. 3 female mice per dosage were given the crude extracts once and observed for 14 days; their weights were recorded twice a week. The mortality rate was 0%. Solvents used to prepare test extracts included methanol, Dichloromethane (DCM) distilled water and phosphate buffer solution. Mice were induced with diabetic by alloxan IP injection 150 mg/kg b.w and fasted overnight; they were allowed to drink 5% glucose solution to overcome drug induced hypoglycemia. The diabetic mice (>8 mmol/dl) were treated with 100 and 500 mg/kg of water and methanol extract, standard drug metformin (100 mg/kg b.w) and normal saline for 10 days, each group consisting of 5 mice each. Blood samples of 0.1 ml were collected from tail tip using strips; glucose concentration was tested using a glucometer. All data were analyzed and computed by SPSS and presented as mean ± standard deviation (SD).**

Key words: Diabetes, hypoglycemic, alloxan, *Carica papaya.*

INTRODUCTION

Global report on diabetes demonstrates that the number of adults living with diabetes has almost quadrupled since 1980 to 422 million adults (Roberts et al., 2012). This dramatic rise is largely due to the rise in type 2 diabetes and factors driving it include overweight and obesity.In 2012 alone diabetes caused rise is 1.5 million deaths. Its

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complications can lead to heartattack, stroke, blindness, kidney failure and lower limb amputation (Kirigia et al., 2009).The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. Diabetes prevalence has been rising more rapidly in middle- and low-income countries. Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation (Kirigia et al., 2009).

In 2015, an estimated 1.6 million deaths were directly caused by diabetes. Another 2.2 million deaths were attributable to high blood glucose in 2012. Almost half of all deaths attributable to high blood glucose occur before the age of 70 years (Roberts et al., 2012).WHO projects that diabetes will be the seventh leading cause of death in 2030. Diabetes can be treated and its consequences avoided or delayed with diet, physical activity, medication and regular screening and treatment for complications. In April 2016, WHO published the Global report on diabetes, which calls for action to reduce exposure to the known risk factors for type 2 diabetes and to improve access to and quality of care for people with all forms of diabetes. According to the International Diabetes Foundation estimates, diabetes mellitus affects about 246 million people worldwide. By the year 2000, approximately 7.1 million Africans were reported to be suffering from diabetes (Motala, 2002). This number is expected to rise to 552 million by 2030 (IDF, 2011). In some rural parts of the country such as Nyeri and Kilifi, the prevalence is as high as 11.6% and above 20% among the richer families in the major urban centers (Chege, 2010).

The estimated total economic cost of diagnosed diabetes mellitus in 2012 was $245 billion. This estimate is a representative of the substantial burden that diabetes mellitus imposes in the world (Yang and Colditz, 2015). There is a steady increase in the prevalence of diabetes mellitus since much of the population cannot afford costs associated with the management of this disease and lack information on symptoms for diagnosis and management (IDF, 2011). Hence, there is a need to search for potential safe and cost effective anti-hyperglycemic agents.

C. papaya, a tropical plant believed to have originated in Southern Mexico and Central America is an evergreen shrub, small tree that grows best in full sun to light shade. Its variety includes: Kamiha, Mexican red, Mexican yellow, solo, sunrise solo, sunset solo, vista solo and Waimanalo solo (Parle and Gurdita, 2011). It has been used locally in the treatment of urinary tract infections among other infections (Aliyu, 2006; Akahet et al., 1997; Anaga and Onehi, 2010; Burkill, 1985; Chinoy et al., 1995; Chinoy and Padman, 1996; Parle and Gurditta, 2011; Sidi-Aliyu, 2006). *C. papaya* is cultivated for its edible ripe fruit; its juice is a popular beverage, and its young leaves, shoots, and fruits are cooked as vegetables (Aravind et al., 2013).This study aimed to establish the hypoglycemic activity of *Carica papaya* seeds extract, test for safety of the extracts and its effects on the blood glucose concentration. This study also aims to conduct the phytochemical screening of the *Carica papaya* seeds extract.

STUDY SITE AND DESIGN

Test drug and chemicals

A single injection of alloxan monohydrate (Alloxan, Sigma Aldrich, USA) was used for diabetic induction in the experimental animals at a dose of 150 mg/kg body weight intraperitoneally. Standard drug used was Metformin (100 mg/kg b.w.). Solvents used to prepare test extracts included methanol, distilled water and phosphate buffer solution.

Animals

A total of 50 mice (15 mice for acute oral toxicity and 35 mice for hypoglycemic studies) were used. Male *Swiss albino* mice (6 weeks old) from the KEMRI animal facility and weighing 20±2 g were moved to an experimental room for acclimatization for one week prior to the experiment. Five groups of mice (5 mice per cage) were housed in 15×21×29 cm transparent cages bedded with wood shaving and equipped with a continuous flow of nipple watering devices. They were fed with pellets (Mice Pellets UNGA feeds) and tap water *ad libitum* throughout the experiment. The wood shavings in the cages were changed twice a week. The animals were handled as humanely as possible and in the same manner as before the onset of the experiment (they were not deprived of feeds and water). All the mice survived throughout the experiment. At the end of the experiment, the animals were immediately euthanized in a CO_2 chamber and incinerated.

Plant material

The seeds were collected from the locally produced papaya (Kiim mountain) bought from Juja, Kalimoni area in Kiambu County, Kenya October, 2016 with the help of a botanist and a voucher sample collected for future reference. Seeds from ripe papaya fruits were used during this experiment.

Preparation of the plant extract

Organic extraction

Mature healthy papaya ripe fruits were cut longitudinally using a knife and all the seeds were removed. The seeds were washed and left to dry. The samples were ground and exactly 50 g of the sample was weighed using a top balance and put in a flat-bottomed flask. Methanol and Dichloromethane DCM at a ratio of 1:1 was then added until the sample was completely immersed. The mixture was then agitated by shaking and then filtered using Butcher funnel: Whatman No 1 filter paper. The residue was re-extracted by adding the solvent and left to extract for 24 h. The extract was then concentrated to obtain a semi liquid mass using a rotary evaporator (0.1 bar) in water bath at 40°C.

Aqueous extraction

Distilled water was used for aqueous extraction. Mature healthy papaya ripe fruits were cut longitudinally using a knife and all the

seeds were removed. The seeds were washed and left to dry. The samples were ground and exactly 50 g of the sample was weighed using a top balance; it was put in a flat-bottomed flask and then mixed with distilled water. The extracts were then filtered using a Whatman paper and the filtrate was concentrated using a freeze drier to obtain the crude extracts

Phytochemical screening

The seed extracts (methanol and aqueous) were screened for flavonoids, flavones, saponins, steroids, alkaloids, phenols and terpenoids using principled laboratory standard methods for each compound.

Flavonoids and flavones

1 g of extract was dissolved in 10 ml distilled water and then filtered using Whatman filter No.1. Magnesium turning of 10 mg was then added into 1 ml of the filtrate, followed by the addition of 0.05 ml concentrated sulphuric acid. The presence of magenta red indicated the presence of flavonoids (Brain and Turner, 1995).

Tannins

Half a gram (0.5 g) of the water extract was dissolved in 2 ml of distilled water and filtered. Two drops of ferric chloride were then added to the filtrate. A blue black precipitate indicated the presence of tannins (Harbone, 1998).

Saponins

The presence of saponins was determined by frothing test. Half a gram (0.5 g) of the plant extract was shaken in 5 ml of distilled water and allowed to stand for 10 min. No froth was formed

Steroids

Two milliliters of acetic anhydride was added to 0.5 g methanol extract of each sample with 2 ml H_2SO_4. The color changing from violet to blue/green indicated the presence of steroids (Brian and turner, 1995).

Alkaloids

Three drops of Mayer's reagent were added to 2 ml of the extract. Formation of a yellow colored precipitate indicated the presence of alkaloids.

Phenols

Three drops of ferric chloride solution were added to the extract. Formation of a blue black color indicated the presence of phenols.

Terpenoids

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of Terpenoids.

Acute oral toxicity testing

Extracts at the dose range of 5, 50, 300 and 2000 mg/kg body weight were administered using oral gavage to different groups of mice comprising 3 female mice in each group. On the day of the experiment, animals were starved of food for 2 h before drug administration. Animals were kept under close observation for 4 h after administering the fraction for behavior, neurological and autonomic profile and then observed for any change in the general behavior and/ or physical activities; any mortality was recorded within 72 h (OECD Guidelines, 2001).

Hypoglycemic studies

Induction of experimental diabetes

Mice aged 6 weeks or older were fasted overnight and received a single injection of alloxan monohydrate (Alloxan, Sigma Aldrich, USA) at a dose of 150 mg/kg body weight intraperitoneally. The induction of alloxan-induced diabetes was confirmed by measuring blood glucose levels of each mouse after 7 days by following the blood glucose monitoring after alloxan administration. Mice with glucose levels above 150 mg/dl were considered diabetic and used for the efficacy study (Xu et al., 2008).

Blood glucose determination

Blood was obtained by a prick on the lateral tail vein and blood glucose determined using a glucometer based on the glucose oxidase method for normal and diabetic mice after an overnight fasting for the determination of the glycaemia (0 time). Treatments were administered orally and the blood samples approximately 0.5 ml per mouse were collected for blood glucose level estimation at specific time intervals. Daily treatment took place post-induction of diabetes and assessment of the glucose level was determined after 72, 96, 120 and 144 h post diabetes induction (Benedict, 1911).

Statistical analysis

All data were analyzed and computed by SPSS and presented as mean ± standard deviation (SD). A significant difference between control and experimental groups was assessed by the use of Student's t-test. Data from different treatments in the groups were compared using a one- way analysis of variance (ANOVA). Box plot was also generated to compare the groups. The level of significance was set at p values less than 0.05.

Ethical considerations

This study was carried out at Kenya Medical Research Institute (KEMRI). Permission to carry out the study and ethical clearance was sought from KEMRI's Scientific and Ethics Review Unit (SERU) and KEMRI Animal Care and Use Committee (ACUC). The research was conducted in accordance with KEMRI guidelines on animal care and internationally accepted principles for laboratory animal use and care as found in WHO guidelines.

RESULTS

Acute oral toxicity studies

Table 1 shows the variation of weight measured in animals administered with methanol seeds extracts during the oral acute toxicity test. The weight of the mice

Table 1. Phytochemical analysis.

Test	Observation	Aqueous extract	Methanol extract
Saponins	No froth formation	-	-
Alkaloids	Yellow precipitate formed	+	+
Flavonoids	Magenta red color present	+	+
Tannins	Blue black precipitate formed	+	+
Phenols	Blue black color formed	+	+
Steroids	Color change: violet to blue green	+	+
Terpenoids	Brown coloration at the interface	+	+

Key: - Absent, + Present.

Table 2. Methanol extracts.

Groups	No. of animals	Weight at day 0(g)	Weight at day 7(g)	Weight at day 14(g)
Control	3	18.33 ± 0.577	21.67 ± 1.155	23.33 ± 1.154
5 Mg/Kg	3	20.00 ± 1.000	22.67 ± 1.528	26.00 ± 1.000
50 Mg/Kg	3	17.67 ± 0.577	21.33 ± 0.577	23.33 ± 0.577
300 Mg/Kg	3	18.00 ± 1.732	22.33 ± 1.528	23.67 ± 0.577
2000 Mg/Kg	3	18.33 ± 0.577	21.33 ± 0.577	22.67 ± 0.577

Table 3. Water extracts.

Groups	No. of animals	Weight at day 0(g)	Weight at day 7(g)	Weight at day 14(g)
Control	3	20.33 ± 1.155	25.00 ± 1.000	25.67 ± 0.577
5 Mg/Kg	3	21.33 ± 1.528	26.33 ± 1.528	27.33 ± 1.528
50 Mg/Kg	3	22.67 ± 1.528	26.67 ± 1.155	28.33 ± 1.528
300 Mg/Kg	3	21.33 ± 1.528	24.67 ± 1.155	26.00 ± 1.000
2000 Mg/Kg	3	24.67 ± 1.155	25.00 ± 1.732	29.00 ± 1.732

increased as the number of experimental days increased because of the regular uptake of food. Table 2 shows the variation of weight measured in animals administered with water seeds extracts during the oral acute toxicity test. The weight of the mice increased as the number of experimental days increased due to regular uptake of food, with the results almost similar to that of Table 3.

Hypoglycemic studies

In Table 4 it was observed that the blood glucose concentration decreased as the number of days of experiment increased. Table 5 shows the *p* and sig. value. There was no statistically significant difference between the experimental crude *Carica papaya* seeds extract and the control drug which was metformin since the sig. value calculated was >0.05. Table 6 indicates the summary of the analysis of variance between and within the experimental groups and the positive control group. There was no significant difference since the *sig*. >0.05 (Figure 1).

DISCUSSION

Phytochemical analysis

The methanol and water extracts from the seeds of *C. papaya* plant contain a number of phytochemicals such as flavonoids, phenols, steroids and tannins. All these compounds were present in both water and methanol extracts. The presence of flavonoids in the extracts indicates the naturally occurring phenolic compound with beneficial effects on the human diet such as antioxidant activity and neutralizing free radicals. Flavonoids present

Table 4. Blood glucose concentration.

Groups	Blood glucose concentration (mmol/dl)		
	Day 0	Day 5	Day 10
100 Mg/Kg water extract	19.325 ± 10.251	17.200 ± 6.784	17.993 ± 7.591
500 Mg/Kg water extract	24.920 ± 8.607	21.832 ± 7.009	21.728 ± 6.883
100 Mg/Kg methanol extract	12.620 ± 6.325	12.876 ± 6.816	12.725 ± 7.505
500 Mg/Kg methanol extract	15.940 ± 11.453	13.804 ± 8.477	10.831 ± 4.817
100 Mg/Kg metformin(positive control)	16.960 ± 11.925	15.936 ± 10.455	15.991 ± 10.476
Untreated mice	22.660 ± 10.127	20.688 ± 8.478	21.613 ± 7.922
Uninduced mice	6.825 ± 1.284	7.180 ± 0.700	7.069 ± 0.431

Table 5. Analysis of variation after the experiment.

Comparison between groups	Sig. level	Sig. value
100 Mg/Kg H_20 **vs.** 100 Mg/Kg metformin	0.05	1.000
500 Mg/Kg H_20 **vs.** 100 Mg/Kg metformin	0.05	0.873
100 Mg/Kg methanol **vs.** 100 Mg/Kg metformin	0.05	0.991
500 Mg/Kg methanol **vs.** 100 Mg/Kg metformin	0.05	0.937

Table 6. Annova.

Duration	*F* value	*Sig*. value
Day 1	1.063	0.402
Day 5	0.949	0.458
Day 10	1.402	0.273

a variety of biochemical and pharmacological actions that may affect the function of various mammalian cell systems. Several studies have shown that flavonoids, which are naturally occurring phenolic compounds and are widely distributed in plants, can act in lowering blood glucose in experimental models of diabetes. Such studies using isolated flavonoids like quercetin and myricetin, showed a reduction in the fasting blood glucose of diabetic mice induced by streptozotocin. (Goycheva et al., 2006)

Terpenoids and steroids were also present. Alkaloids have been used in medicines for reducing headaches and fever hence portraying antibacterial and analgesic properties. *C. papaya* seeds extract is a primary source of some of the secondary metabolites responsible for medicinal properties. This indicates that the plant can play a vital role in treating and managing some of the most severe diseases such as cancer, malaria, diabetes etc.

Acute oral toxicity

It was clearly observed that the mortality rate among the groups after 72 h and even after 14 days for bothmethanol and water seed extracts was 0%. The mice maintained their normal activity *viz* being active, normal response to stimuli, normal appetite, and normal body temperature of 35^{o}C. There was no isolation of any individual mice from the rest hence indicating no signs of stress and abdominal pain among the mice. The coat of the mice was also observed to be smooth and kempt. There was significant increase in weight from day 1 to day 14 with an average increase of 5.2 gms per mice in water and methanol seeds extract. This indicated that the water seed extracts had no effect on the uptake of food and the digestive system of the mice.

Hypoglycemic studies

During the hypoglycemic study, it was observed that there was a significant decrease change in the groups treated with both the extracts and the control drug, especially in the first five days. After that, there were still some significant changes observed but not as consistent as the first five days. The body weight of the animals increased significantly as the treatment continued with an

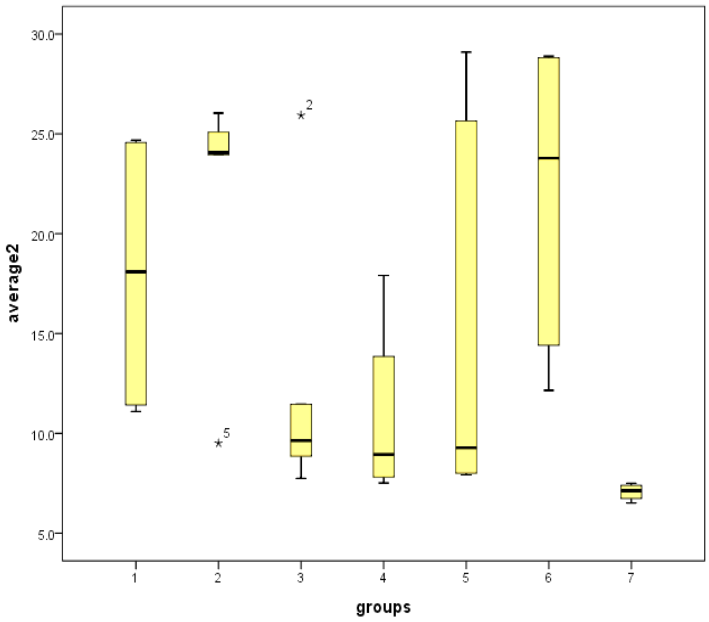

Figure 1. Box plot.

Table 7. Turkey's HSD results.

Groups	N	Subset for alpha=0.05
		1
4	5	10.825
3	5	12.720
5	5	16.000
1	5	18.000
2	5	21.740
Sig.		0.259

N- Number of animals per group (hypoglycemic studies was 5 mice per group, acute oral toxicity was 3 mice per group, OECD guidelines, 2001), *Sig* – significance value.

average of 6 gm for the ten days indicating that the treatment did not interfere with uptake of food or any alimentary activities of the animals. During the start of the experiment, all the seed extracts had no significant difference (sig. >0.05) as compared to metformin which was the positive control group; this indicates that they had effect the same way as the reference drug on the diabetic mice. Table 7 (Turkey's HSD results) indicated clearly that 500 mg/kg methanol extract dosage and metformin (positive control) 100 mg/kg dosage had the same mean variation of ≤ 9 mmol/dl on the blood glucose concentration; this indicated that they had the same effect in lowering the blood glucose concentration followed by 100 mg/kg methanol extract dosage, whose mean variation was ≤10 mmol/dl. 100 mg/kg H_20 extract dosage and 500 mg/kg

H_20 extract dosage, even though had significant effect on lowering blood glucose concentration, presented the highest mean variation of all the groups that were treated. Negative control (untreated diabetic mice) also had a higher mean variation of blood glucose ≥25 mmol/dl since there was no treatment administered to them. Normal control (un-induced diabetic mice) recorded the lowest mean value of ≤6 mmol/dl, as they were not injected with alloxan and hence not diabetic.

Conclusion

In conclusion, the crude extract of the *C. papaya* seeds

plays a role in the reduction of blood glucose through mechanisms that involve the reduction of hepatic glucose production and/or that promote the storage of glucose in the liver. The molecular mechanisms by which *C. papaya* seed extracts stimulate the hypoglycemic effects are still unknown. The characterization of the extracts of *C. papaya* seeds as hypoglycemic agent opens an interesting field of investigation requiring further studies about the mechanisms involved; this is because apart from hypoglycemic activities related to it, *C. papaya* seed extracts have been reported to have contraceptive effects on male mice among other pharmaceutical values.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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