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ANTIDIABETIC ACTIVITY OF *Annona squamosa* RAW FRUIT PEEL EXTRACTS IN ALLOXAN INDUCED DIABETIC RATS

Diabetology

KEYWORDS: *A.squamosa* raw fruit peel aqueous extract, Diabetes

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ABSTRACT

Diabetes mellitus (DM) is metabolic disorder showing multiple network associations with aging diseases. A.squamosa raw fruit peel aqueous extract was tested for antidiabetic activity. Wister albino rats weighing about 150-200 g were used in the present study. The aqueous raw fruit peel extract of A.squamosa caused reduction in fasting blood glucose level. The decrease in cholesterol, serum triglycerides and HDL and increase in increase in LDL shows significant effect of aqueous raw fruit peel extract of A.squamosa as antidiabetic agent.

INTRODUCTION

Diabetes mellitus (DM) is metabolic disorder of multiple network associations characterised by chronic hyperglycaemia resulting from defects in insulin secretion or action with disturbances of carbohydrate, lipid and protein metabolisms (Saltiel & Kahn, 2001; Goldberg, 1981; Wilcox, G. 2005). Insulin Dependent Diabetes Mellitus [IDDM] such as Alloxan induced diabetes act as essential tool for investigating metabolic changes during the evolution of diabetes in rats (*Sunanda and Anand,* 2003). An investigation was to ascertain the scientific basis for the use of A. squamosa in the management of diabetes using Alloxan - induced diabetis (Sharma *et al.*, 1997).

Phytochemical studies from raw fruit peel solvent extracts of *Annona squamosa* like Methanol, Ethanol, Ethyl acetate and Aqueous reported in showing alkaloids, flavonoids, phenols, and saponins (Kaladhar et al., 2015). Risk of diabetic foot can be determined by protein thiols, plasma levels of TAC and plasma levels of TAC were associated with the foot ulcerations and loss of sensitivity (Ana et al., 2014). Treatment of nano-MgO for effects on triglyceride (TG), cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) in placebo group showed decreased glucose concentration (Noushin and Shaghayegh, 2014).

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Prevention and treatment of aging diseases like Diabetes is providing good results with Antioxidant therapy (Amel, 2016). Hypolipidemic, hypoglycemic, and antioxidant potency are the are the potential parameters for the studies of diabetic rats. Modification in liver enzyme levels is a major nutritional changes that are associated with glucose and lipid metabolism changes (Badr et al., 2006).

MATERIALS AND METHODS

Plant material

The *Annona sqamosa* (AS) raw fruit peel materials were collected during June to September 2013 in the Appannapalem-Jami, Vizianagaram Distict, state of Andhra Pradesh, India. AS raw fruit peel part was dried in the shade and grind into a powdered material using an appropriate heavy stone grinding vessel (Telugu: rolurokali). Nearly 1000 fruits are taken for the experimentation. The identity was confirmed based on Voucher specimens (No.6) by Dr. PV Arjun Rao, Ethanobotanist, Visakhapatnam, India.

Aqueous extracts: Each dry pure powdered raw fruit peel extract of AS (100 g) was infused in distilled water until complete exhaustion of peel. The extract was then filtered using Whatman filter paper no.1, and the filtrate was evaporated in vacuum and dried using freeze drier or lyophilisation. The final dried material was stored in labelled sterile borosil 250ml screw capped bottles and kept in the freezer at 20ºC.

Experimental animals

Male Wister albino rats weighing about 150-200 g were used in the present study. Animals were housed in individual polypropylene cages and were maintained on rat pellet food and tap water.

Figure 1: Treatment with Aqueous raw fruit peel extract of *A, squamosa* in Wistar albino rat

2 Extract and drug administration

Before use, the extracts and standard drug glibenclamide (at a dose of 0.5 mg/kg) were reconstituted in normal saline (vehicle) and administered orally via gastric intubation. The raw fruit peel extracts from *Annona squamosa* used at a dose of 50 mg/kg and 350 mg/kg *b.w.* respectively for single extract treatment. The controls received normal saline.

3 Oral glucose tolerance test (OGTT)

Twenty-four albino rats of the Wistar strain weighing between (150-200 g) were obtained from the Animal House Unit, Department of Biochemistry, GITAM University, India . The animals are be divided into four experimental groups of 6 rats per group, housed in standard rat cages and will left to acclimatize to laboratory conditions for two weeks before the commencement of the experiment.

The oral glucose tolerance test was performed in overnight fasted normal animals. Rats divided into four groups each consists six animals. The different groups were administered with normal saline, diabetic control, glibenclamide and *Annona squamosa* aqueous raw fruit peel extract. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation at 0, 12, 24, 48 and 72 hours of extract administration and blood/ serum glucose levels were estimated

4 Alloxan-Induction of experimental diabetes

Animals were fasted overnight and diabetes was induced by the intraperitoneal injection of alloxan monohydrate dissolved in normal saline at a dose of 120 mg/kg body weight. The animals were kept under observation and the fasting blood glucose level was determined after 48 h of alloxan injection. The animals showing hyperglycemia were used for the study

Alloxan-induced diabetic rats (mentioned above) were divided into four groups each consists eight animals,

Group 1: Normal control, normal rats were treated with normal saline.

Group 2: Diabetic control, diabetic mice treated with normal saline.

Group 3: Standard drug treated group, diabetic mice treated with 0.5 mg/kg glibenclamide.

Group 4: Aqueous raw fruit peel of *Annona squamosa* treated group with 350 mg/kg.

5 Treatment

Treatment of the rats with the raw fruit peel of custard apple extract will begin on day 5, post-alloxan treatment. Group 4 rats receive oral doses of 350 mg/kg body weight of the stock crude once a day for twenty one days using a gavage. The diabetic control rats (Group 1) will be given distilled water (orally) in place of the raw fruit extract. Group 3 rats review oral doses of 0.5 mg/kg body weight of Glibenclamide once a day for twenty one days using a gavage. The blood samples were drawn on 1st, 7th, 14th, and 21st days from the retro orbital venous plexus of rats under ether anesthesia and the blood was centrifuged at 2,500 rpm for 10 min. The serum thus obtained was used for biochemical estimation of blood glucose, total cholesterol (CHL), triglycerides (TG), high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL)

6 Biochemical parameters

Various biochemical parameters like Blood glucose, CHL, TG, HDL and LDL levels in serum were measured for finding anti-diabetic activity (Allain et al., 1974).

RESULTS AND DISCUSSION

A marked rise in fasting blood glucose level was observed in diabetic control compared to normal rats. The aqueous raw fruit peel extract of *A.squamosa* caused reduction in fasting blood glucose level (Table 1). The decrease in cholesterol, serum triglycerides and HDL and increase in increase in LDL shows significant effect of aqueous raw fruit peel extract of *A.squamosa* as antidiabetic agent (Table 2 to 6).

TABLE 1: Effect of *A. squamosa* on fasting serum glucose level

Treatment	Serum glucose (mg/dl) (mean±SEM) Time after glucose administration in hours				
	0	12	24	48	72
Normal Control	66±0.86	91.17±0.94	95.33±0.61	103.17±0.94	112.33±0.61
Glibenclamide (0.5 mg/kg)	64±0.73	72±0.86	66±1.5	71.83±0.6	66.67±1.28
Aqueous extract of raw fruit peel from A. *squamosa*(350 mg/kg)	61±0.97	84.33±1.14	85.17±0.94	74.17±0.94	69.83±0.48

TABLE 2: Effects on serum glucose level after the administration

Treatment	Serum glucose (mg/dl) (mean±SEM) Time after administration			
	1st day	7th day	14th day	21st day
Normal Control	83±0.82	77±0.63	74.67±0.99	71±0.36
Diabetic Control	256±1.34	261.67±0.33	270.33±0.67	280.83±0.31
Glibenclamide (0.5 mg/kg)	244.17±1.56	212.5±0.5	191.83±0.70	171.33±0.61
Aqueous extract of raw fruit peel from A. *squamosa*(350 mg/kg)	252.5±0.99	221.67±0.33	207.83±2.51	191±0.36

TABLE 3: Effect of aqueous raw fruit peel extract of *A.squamosa* on serum total cholesterol

Treatment	Serum cholesterol (mg/dl) (mean±SEM)			
	1st day	7th day	14th day	21st day
Normal Control	55.33±0.8	63.67±0.49	54.83±1.01	61.17±0.60
Diabetic Control	250.83±2.13	261.67±0.84	265.67±1.65	271.33±1.02
Glibenclamide (0.5 mg/kg)	253±1.41	170.83±3.6	109.83±2.33	83.17±0.79
Aqueous extract of raw fruit peel from A. *squamosa* (350 mg/kg)	250.17±2.49	192.33±1.47	163.5±1.57	103.17±0.79

TABLE 4: Effect of aqueous raw fruit peel extract of *A.squamosa* on serum triglycerides

Treatment	Serum triglycerides (mg/dl) (mean±SEM)			
	1st day	7th day	14th day	21st day
Normal Control	152.33±0.99	163.17±1.42	163±1	168.17±0.31
Diabetic Control	181.67±0.21	182.83±0.7	194±1.29	206.5±0.62
Glibenclamide (0.5 mg/kg)	166.5±0.34	165±0.52	161.33±0.33	158±0.26
Aqueous extract of raw fruit peel from A. squamosal (350 mg/kg)	177.67±0.21	170.33±0.61	164.83±1.9	162.5±1.15

TABLE 5: Effect of aqueous raw fruit peel extract of *A.squamosa* on serum HDL cholesterol

Treatment	HDL cholesterol (mg/dl) (mean±SEM)			
	1st day	7th day	14th day	21st day
Normal Control	52±0.86	53.83±0.48	56.83±0.54	60±0.52
Diabetic Control	49.83±0.60	47.17±0.40	43±0.45	36.83±0.98
Glibenclamide (0.5 mg/kg)	54.33±0.61	58.67±0.49	60.83±0.40	63.67±0.49
Aqueous extract of raw fruit peel from A. squamosa(350 mg/kg)	50.33±0.49	55.17±0.60	59.83±0.60	62.17±0.54

TABLE 6: Effect of aqueous raw fruit peel extract of *A.squamosa* on serum LDL level after administration

Treatment	LDL level cholesterol (mg/dl) (mean±SEM)			
	1st day	7th day	14th day	21st day
Normal Control	43.67±0.49	49.83±0.6	53.67±0.76	55.67±1.17
Diabetic Control	85.83±1.54	102.67±0.95	107.83±0.48	111.17±0.70
Glibenclamide (0.5 mg/kg)	78.17±1.17	73.17±1.19	71±0.36	67.33±0.61
Aqueous extract of raw fruit peel from A. squamosa(350 mg/kg)	80.67±0.33	77.33±0.61	72.5±1.15	70.83±0.4

Although *A. squamosa* is reported to possess varied biological properties such as insecticidal, antiovulatory and antitumour, activities, there is limited previous report about the hypoglycaemic and antidiabetic activities from raw fruit peel extract of *Annona squamosa.* The observation and preliminary idea of action of aqueous extract of raw fruit peel extract of *Annona squamosa* reported here offer scientific explanation for the potential use for the treatment of diabetes mellitus. These results indicate that it is worth undertaking further studies on possible usefulness of the water extract of raw fruit peel extract of *Annona squamosa* in diabetes mellitus.

4.4 CONCLUSION

The in vivo results showed good understanding for aqueous raw fruit peel extract of *A.squamosa* as antidiabetic agent.

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