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EFFECT OF FRUIT EXTRACT OF *ARTOCARPUS HETEROPHYLLUS* IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The present study was aimed at evaluating the antidiabetic activity of aqueous extract of fruits of *Artocarpus heterophyllus* (Moraceae) in alloxan induced type- II diabetes in rats. The diabetic rats were administered aqueous fruit extract (250 and 500 mg/kg, p.o., n = 6) or vehicle (gum acacia solution) or standard drug glibenclamide (0.25 mg/kg) for 15 days. Blood samples were collected by retro-orbital puncture and were analyzed for serum glucose on 0, 5, 10 and 15th day by using glucose oxidase-peroxidase reactive strips and a glucometer. In oral glucose tolerance test, glucose (2gm/kg, p.o.) was administered to nondiabetic control, glibenclamide (10 mg/kg, p.o.) and extract treated rats. The serum glucose levels were analyzed at 0, 30, 60 and 120 min after drug administration. The effect of the extract on body weight of the diabetic rats was also observed. The serum insulin level and lipid profile were estimated. The extract showed significant reduction ($p<0.05$) of fasting blood glucose level in alloxan induced type- II diabetic rats on 10th and 15th days. In oral glucose tolerance test, the extract increased the glucose tolerance. It also showed an increase in the body weight of diabetic rats. Serum insulin levels were also increased in the treated group as compared to the control. The treated groups showed a decrease in total cholesterol and LDL levels and an increase in HDL levels.

INTRODUCTION: Diabetes mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein and lipid metabolism and complications like retinopathy, microangiopathy and nephropathy [1]. Currently available synthetic antidiabetic agents produce serious side effects like hematological coma [2] and hepato-renal disturbances [3]. Moreover they are not safe for use during pregnancy [4].

Hence, search for more safer and effective hypoglycemic agents has continued to be an important area of research. Following the recommendations made by the WHO on the beneficial uses of medicinal plants for the treatment of diabetes mellitus [5], investigations of hypoglycemic agents from medicinal plants have also become more important. Several investigations have been conducted and resultantly many plants have shown a positive activity [6]. Though the active principles of have been isolated from some plants, some still remain to be identified.

Artocarpus heterophyllus (Moraceae), commonly known as Jack fruit is found all over India. The roots are used as diuretic and diaphoretic [7]. Traditionally, the pulp and seeds are used as tonic, roots in diarrhea and fever, woods as sedative in convulsions, leaves as antisyphillic and vermifuge, leaf ash is applied to ulcers and wounds (Khan *et al*., 2003) [8, 9]. The infusion of the unripe fruit has been used for the management of diabetes by the tribal around islands Madagascar, Comros and Mascarenes [10] as a cure for diabetes. Though there is no scientific evidence to support the antidiabetic effect of *Artocarpus heterophyllus* (AH), tribals continue to use it in the management of diabetes. The objective of this study was to ascertain the scientific basis for the use of this plant in the management of diabetes using alloxan induced type-II diabetic rats.

MATERIALS AND METHODS:

Collection of plant material: The fruits of AH were collected from the local market in Madhya Pradesh and identified at Department of Botany, Government Agriculture College, Indore.

Preparation of AH Aqueous Fruit Extract: The aqueous extract was prepared by cold maceration of 150 g of the shade dried fruits powder in 500ml of drinking water for 7 days. The extract was filtered, concentrated, dried *in vacuo* (yield 65 g) and the residue stored in a refrigerator at 2-8^{o}C for use in subsequent experiments [11].

Animals: Healthy adult male Wistar Albino rats between 2-3 months of age and weighing 250-280g were used for the study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25 ± 5^{o}C, 35-60% humidity), the animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Study: Animals were starved overnight and divided into five groups (n=6). They were fed orally with the aqueous extract of AH in increasing dose levels of 100, 500, 1000, 3000 and 5000 mg/kg body weight. The animals were observed continuously for 2h for the following [12]:

- Behavioral profile: alertness, restlessness, irritability, and fearfulness.
- Neurological profile: spontaneous activity, reactivity, touches response, pain response and gait.
- Autonomic profile: defecation and urination.

Number of deaths, if any, were recorded after 24 and 72h.

Induction of Non-Insulin Dependent Diabetes Mellitus (NIDDM): NIDDM was induced [13] by a single intraperitoneal injection of 150 mg/kg alloxan monohydrate (Lobachemie) [14]. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. Rats found with permanent NIDDM were only used for the study [15].

Collection of Blood and determination of Serum Glucose: Blood was withdrawn from the retro orbital sinus under ether inhalation and glucose levels were estimated using a glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek, Roche Diagnostics, USA).

Oral Glucose Tolerance Test (OGTT): The oral glucose tolerance test [16] was performed in overnight fasted (18 h) normal rats. Rats divided into four groups (n=6) were administered drinking water or AH aqueous extract 250 and 500 mg/kg respectively. Glucose (2g/kg) was fed 30 min after the administration of extracts. Glibenclamide (10 mg/Kg) was used as the standard drug. Blood was withdrawn from the retro orbital sinus under ether inhalation at 30, 60 and 120 minutes of glucose administration and glucose levels were estimated using a glucose oxidase- peroxidase reactive strips and a glucometer (Accu-chek, Roche Diagnostics, USA).

Experimental design for Antidiabetic Study: Overnight fasted diabetic rats were divided for the antidiabetic study in the following manner: Group I: diabetic control rats administered gum acacia daily for 15 days; Group II: diabetic rats administered AH aqueous extract (250 mg/kg); Group III: diabetic rats administered AH aqueous extract (500 mg/kg); Group IV: diabetic rats administered standard drug glibenclamide (2.5 mg/kg) for 15 days. The effects of administration of AH extracts in normal and diabetic rats were observed by measuring fasting plasma glucose levels, serum insulin level, serum lipid profile, and initial and final changes in body weight. Fasting plasma glucose was estimated on days 0, 10, and 15 of extract administration. All other biochemical parameters were determined on day 15 after blood withdrawal from the retro orbital sinus under ether inhalation. Serum Insulin levels were estimated in a commercial diagnostic laboratory, using a radio immunoassay kit issued by the Board of Radiation and Isotope Research, Bhaba Atomic Research Centre (BARC), Mumbai, India. Serum lipid profiles were measured by an autoanalyzer (Star 21).

Statistical Analysis: Data were statistically evaluated using one-way ANOVA, followed by post hoc Sheffe's test using 7.5 version of SPSS computer software. The values were considered significant when $P < 0.05$ [17].

RESULTS AND DISCUSSION: Acute toxicity studies revealed the non-toxic nature of the fruit extracts of AH. There were no lethality or toxic reactions found at any of the doses selected until the end of the study period. All the animals were alive and active during the observation period. In OGTT, the aqueous extract, from 60 min onwards showed significant reduction in plasma glucose levels (**Table 1**). On repeated administration of the extract for 15 days, a significant decrease in the fasting blood sugar level was observed in the diabetic rats as compared to the control (**Table 2**). Diabetic rats showed a decrease in body weight during the experimental period which was significantly controlled by the extract (**Table 3**). Diabetic rats showed a decrease in the serum insulin levels which were enhanced upon treatment with the extract. A decrease in total cholesterol, LDL (low density lipids) and levels, and an increase in the HDL (high density lipids) cholesterol levels were observed in the treated groups (**Table 4**).

TABLE 1: EFFECT OF *ARTOCARPUS HETEROPHYLLUS* (AH) ON ORAL GLUCOSE TOLERANCE TEST IN NORMAL RATS

Group	Treatment	Plasma glucose concentration (mg/dl)		
		30 min	60 min	120 min
1	Normal (2g/kg)	132.00±2.12	128.83±1.87	125.67±1.49
2	AH (250 mg/kg)	122.50± 2.48 [a]	96.17±1.64 [a]	92.00±1.41 [a]
3	AH (500 mg/kg)	125.83±1.70 [a]	93.50±2.31 [a]	85.83±1.26 [a]
4	Glibenclamide (10 mg/kg)	135.17±1.64	91.67±1.23 [a]	81.50±2.16

Values are mean ± SD, n = 6 in each group. [a] p< 0.05 when compared with normal

TABLE 2: EFFECT OF AH ON FASTING BLOOD SUGAR LEVEL IN TYPE II DIABETIC RATS

Group	Treatment	Fasting blood sugar level (mg/dl)		
		0 day	10^{th} day	15^{th} day
1	Normal (2g/kg)	231.00 ±3.20	235.50 ± 3.43	244.83 ± 1.51
2	AH (250 mg/kg)	224.17 ±3.43	176.00± 1.98[a]	146.50±3.37[a]
3	AH (500 mg/kg)	226.50 ±1.78	162.83± 1.40[a]	137.33±3.71[a]
4	Glibenclamide (10 mg/kg)	220.67 ±2.09	153.50± 1.65[a]	125.50±1.48[a]

Values are mean ± SD, n = 6 in each group. [a] p< 0.05 when compared with diabetic control

TABLE 3: EFFECT OF AH ON BODY WEIGHT IN TYPE II DIABETIC RATS

Group	Treatment	Fasting blood sugar level (mg/dl)		
		0 day	10^{th} day	15^{th} day
1	Normal (2g/kg)	215.12±1.42	206.14±2.11	200.25±1.64
2	AH (250 mg/kg)	212.22±1.21	204.32±0.51	208.41±1.12
3	AH (500 mg/kg)	204.10±2.14 [a]	200.43±0.18	206.35±0.44
4	Glibenclamide (10 mg/kg)	205.12±1.31 [a]	201.32±1.45	210.35±0.65[a]

Values are mean ± SD, n = 6 in each group. [a] p< 0.05 when compared with diabetic control

TABLE 4: EFFECT OF AH ON CHOLESTEROL AND SERUM INSULIN LEVELS IN TYPE II DIABETIC RATS

Group	Treatment	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	High density lipoprotein (mg/dl)	Insulin level (µU/ml)
I	Control	149.67 ± 1.45	125.33 ± 1.54	17.50 ± 1.48	4.35 ± 0.45
II	AH (250 mg/Kg)	121.83 ± 2.50 [a]	101.17± 1.25 [a]	25.67 ± 2.32	8.26 ± 0.33
III	AH (500 mg/Kg)	110.67 ± 2.46 [a]	94.00 ± 1.59 [a]	31.33 ± 1.94 [a]	10.64 ± 0.28 [a]
IV	Glibenclamide (0.25mg/kg)	92.00 ± 1.53 [a]	85.83 ±1.08 [a]	44.83 ± 2.12 [a]	11.70 ± 0.12 [a]

Values are mean ± SD, n = 6 in each group. [a] p< 0.05 when compared with diabetic control

The present work evaluated the antidiabetic effect of the aqueous fruit extract of *Artocarpus heterophyllus* in alloxan induced type- II diabetic rats. Alloxan induces diabetes by pancreatic cell damage (massive reduction of β cells of pancreas) which is mediated through generation of cytotoxic oxygen free radical. The primary target of these radicals is DNA of pancreatic cells causing DNA fragmentation [18]. Over-production and decreased utilization of glucose by the tissues form the fundamental basis of hyperglycemia in diabetes mellitus [19]. When AH aqueous fruit extract was administered to glucose loaded normal rats, reduction in fasting blood sugar level was observed after 60 minutes and it reached the maximum at 2 h.

Administration of extract to diabetic rats showed a significant decrease in the levels of blood glucose and an increase in the serum insulin levels. Hence, the possible mechanism by which AH brings about its hypoglycemic action in diabetic rats may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form [20]. Induction of diabetes with alloxan is associated with the characteristic loss of body weight, which is due to increased muscle wasting and due to loss of tissue proteins [21].

Diabetic rats treated with the extract showed an increase in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis [22]. A marked increase in total cholesterol and decrease in HDL cholesterol have been observed in untreated diabetic rats. Under normal conditions, the enzyme lipoprotein lipase hydrolyses triglycerides. Diabetes mellitus results in failure to activate this enzyme thereby causing hyper-triglyceridemia. Under normal circumstances insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides [23]. Insulin deficiency results in failure to activate the enzymes thereby causing hyper-triglyceridemia [24]. The significant control of the levels of serum lipids in the aqueous extract treated diabetic rats may be directly attributed to improvements in insulin levels upon AH therapy.

CONCLUSION: The aqueous extract of the fruit of *Artocarpus heterophyllus* has antidiabetic activity as it lowers serum glucose levels in diabetic rats and significantly increases glucose tolerance. It also increases the body weight of diabetic rats. Hence, chronic studies of *Artocarpus heterophyllus* and its isolated compounds are necessary to elucidate the exact mechanism of action so as to develop it as a potent antidiabetic drug.

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