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EVALUATION OF ANTI-DIABETIC ACTIVITY OF *CASSIA ABSUS* IN ALLOXAN-INDUCED DIABETIC RATS MODEL

RASHID A[1], SALEEM U[2]*, NAEEM S[1] AND RASHID A[2]

1: Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture Faisalabad

2: Faculty of Pharmaceutical Sciences, Government College University Faisalabad.

***Corresponding author: Dr. Uzma Saleem: Uzma95@gmail.com**

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ABSTRACT

Diabetes mellitus is a metabolic disorder in which biochemical changes of lipid and glucose metabolism occur and these changes lead to chronic hyperglycemia. Though, antidiabetic drugs are being used but due to side effects, scientists now focus their research to find lead compounds from natural sources. The current study was designed to explore anti-diabetic activity of *Cassia absus*in alloxan induced diabetic rats.Animals were divided into five groups (n=6). Alloxan monohydrate (120 mg/kg) was administered intraperitoneally to induce diabetes. Blood samples were collected at day 0 and 28 by cardiac puncture for measuring serum glucose, insulin, glycosylatedhaemoglobin levels, liver function markers, lipid profile, oxidative stress markers and renal function markers. Results revealed that aqueous and ethylacetate extracts restored the biochemical parameters that were altered by alloxan but aqueous extract exhibited more pronounced effects as compared to ethyl acetate extract. Oxidative stress analysis also revealed that *Cassia absus* extract possessed good antioxidant activity. It may be concluded that *Cassia absus* possessed antidiabetic potential along with antioxidant, hepato-protective, renal-protective and lipid lowering effects. It may help in the management of diabetes along with disease complications.

Keywords: *Cassia absus*, Diabetes, Alloxan

INTRODUCTION

Diabetes mellitus is a metabolic condition in which biochemical changes of lipid and glucose metabolism occur and these changes lead to chronic hyperglycemia [1]. Liver play an important role in balance of glucose and lipids. It likewise contributes inoxidation of free fatty acids, digestion and take-up. Triglycerides, phospholipids and cholesterol synthesis are also associated with liver [2]. In diabetes mellitus significant changes happen not only in concentration of lipids but also in the synthesis of lipids. In the liver of diabetic patients glycolysis and glycogenesis are suppressed alongside huge increase in gluconeogenesis because of inhibition in metabolic system of glucose [3].

Currently in spite of large verity of anti-diabetic drugs, various drugs are used to treat diabetes mellitus because hyperglycemia leads towards many complications [4]. Mainly, diabetic complications include limb amputation, coronary heart disease (CHD), cerebrovascular disease, lipid abnormalities, renal failure (RF) and neurological problems [5]. In diabetes, large number of morbidities and mortalities occur due to its complications. Insulin and other antihyperglycemics are the keystone to treat diabetes, especially agents such as sulfonylureas, alpha-glucosidase inhibitors, biguanides and thiazolidinediones. The problem is that these agents have many side effects and these are not able to maintain euglycemia. So, the need of such remedies is increasing that have very less side effects and more benefits along with more patient acceptability [6].

Many medicinal plants have been testified to be beneficial in diabetes globally and are using empirically in antidiabetic and antihyperlipidemic preparations. Plants show its antidiabetic activity primarily due to their capability to bring back the pancreatic tissues' function which may result in enhanced insulin secretion or preventing the glucose absorption by intestine [7]. Above 400 plant species containing antihyperglycemic action have been reported in literature, though, searching new plants for antidiabetic activity is still striking [6]. Mostly the plants containing flavonoids, alkaloids, terpenoids, carotenoids, glycosides etc. are considered as containing antidiabetic effect. However, the testing of plant for antihyperlipidemic, antioxidant and antihyperglycemic activities may offer different pharmacological approaches to treat diabetes [7].

Cassia absus is known as *Sennainsularis* or *Chamaecrista absus* and it belongs to family of *Caesalpiniaceae* (*Fabaceae, Leguminosae*). It has certain common names like Pig's Senna, Chimed, Chaksu, and Jasmeejaz but traditionally *C. absus* seeds are known as Chaksu [8]. Characteristically, *Cassia absus* is a small and flowering, plant. It is an annual plant having 30–70 cm length, sparingly branched. Therapeutically it has great significance. It has astringent and bitter taste [9]. There are many other therapeutic effects of seeds that are reported in different studies especially hypotensive, laxative, and anti-spasmodic effects. It prevents hemorrhage and it is also useful in many skin, kidney and liver diseases [10].

The main objective of this study was to evaluate the antidiabetic effect of aqueous and ethyl acetate extracts of seeds of *Cassia absus*.

MATERIALS AND METHODS

Collection and identification of plant material

Cassia absus seeds were purchased from the local market of Faisalabad. Identification and authentication was done by the Taxonomist of Department of Botany, University of Agriculture-Faisalabad and then seeds were ground to fine powder.

Preparation of extracts

Water and ethyl acetate were used as solvents. Extracts were prepared by using soxhlet apparatus. Excess of solvent was removed with rotary evaporator at 40 ^{o}C.

Drugs

Tablet glibenclamide (Daonil) was purchased from Sanofi Aventis, Pakistan. Alloxan monohydrate was purchased from AppliChem GmbH, Germany.

Animal husbandry

Adult healthy albino rats (n=30) of either sex weighing approximately 180-200gm were used from the stockpile of animal house of Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture-Faisalabad. Rats were kept in animal room of Institute of Pharmacy, Physiology and Pharmacology under standard conditions *i.e*. twelve hours light and twelve hoursdark cycle and 25±2ºC room temperature. To all experimental animals standard diet and water was given*ad libitum.* After the adaption period of seven days, rats were distributed into 5 groups, each group consisting of six rats.

Induction of Diabetes

Rats were made diabetic by administration of single dose(120mg/kg) of alloxan monohydrate intraperitoneally. After three days of injection, animals were

screened for diabetes. Rats with blood glucose level greater than 300 mg/dL were considered diabetic rats andwere used in thisexperiment. 0 day was the day when the diabetes has been induced.

Experimental Protocol

5 groups (n=6 in every group) of animals were made. Group I served as normal control and II was disease control group. Group III served as standard (given glibenclamide, 10 mg/kg/day, orally)and IV-V groups were treatment groups receiving aqueous extract (900 mg/kg/day, orally) and ethyl acetate extract (900 mg/kg/day, orally) respectively.

Blood Sampling

Blood samples were collected by cardiac puncture on 0 and 28thday of the experiment after overnight fasting. Serum was collectedvia centrifugation of blood at 4000 rpm for 10 minutes and used in biochemical testing i.e. glucose level, insulin level,HbA1c,alanine transaminase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST), total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), urea, creatinine by employing diagnostic kits.

Oxidative Stress Analysis

Total oxidant status in serum was measured by procedure established by Erel [11], Malondialdehyde (MDA) was determined via the method established by Ohkawa*et* [12], total antioxidant concentration was determined by procedure established by Erel [13], superoxide dismutase (SOD) and catalase (CAT) activity was determined by procedure established by Alam *et al* [14].

Statistical Analysis

Results were showed as (mean ±SEM). One way ANOVA subsequent lyposthoc Tukey's test were applied to evaluate data by using SPSS software version 17. P value < 0.05 was set as statistically significant value.

RESULTS

Table 1 is showing the results of serum glucose, insulin and HbA1c level. Aqueous extract showed promising decrease in serum glucose level (120.67 mg/dL), increase in insulin level (13.17 IU/L) and lowered HbA1c value (4.05%) as compared to results of ethyl acetate extract. There was maximum 62.49 % decrease in serum glucose level and 44.42 % increase in serum insulin level in group treated with aqueous extract, calculated from values given in table 1 (Fig. 1). These three parameters were significantly different statistically (P<0.05) with respect to disease control group (Table 1).

Table 2 demonstrates the serum total cholesterol, LDL, triglycerides and HDL levels. Results showed that alloxan induced diabetes significantly raised the serum cholesterol, triglycerides, LDL level and lowered the HDL level at day 0. The aqueous and ethyl acetate extract lowered the level of cholesterol, triglycerides and LDL significantly (P <0.05) in comparison to disease control group at 28th day after their oral administration. Serum HDL level was significantly raised (P <0.05) in treated diabetic groups.

Liver function markers are presented in Table 3. The data elaborate the values of ALT, AST and ALP at 0 and 28th day. The results were identical to that of Table 1 as aqueous extract showed promising decrease in ALT, AST and ALP levels on day 28th. The renal function markers, urea and creatinine was also decrease more in aqueous extract treated group as compared to ethyl acetate extract group (Table 4).

Alloxan induced oxidative stress in β-cells which induced diabetes. Table 5 showedthe elevated levels of MDA and total oxidative stress (TOS) in serum of diabetic rats and decreased level of SOD, total antioxidant capacity (TAC) and catalase in diabetic rats. In treated groups serum SOD, TAC and catalase levels were significantly increased while serum MDA and TOS levels were significantly reduced in comparison to disease control group.

Table 1: Effect of aqueous and ethyl acetate extracts of *C. absus* seeds on fasting serum Glucose, Insulin and HbA1c level in different groups

Parameters	Days	Normal Control	Disease Control	Standard	Aqueous extract	Ethyl acetate extract
Glucose (mg/dl)	0	91.17±1.66	335.17±9.91	320.17±2.72	321.67±4.46	321.50±4.16
	28	90.83±1.99*	339.167±10.1	94.33±2.52*	120.67±2.99*	174.50±5.81*
Insulin (IU/L)	0	16.45±0.36	7.350 ± 0.232	7.20 ± 0.35	7.32 ± 0.28	7.20 ± 0.25
	28	16.45±0.36*	7.27 ± 0.19	15.08±0.32*	13.17± 0.6*	12.50 ± 0.76*
HbA1c (%)	0	3.36 ±0.01	7.94 ± 0.09	7.92 ± 0.08	7.96 ± 0.11	7.956± 0.09
	28	3.36 ± 0.01*	7.95 ± 0.08	3.43 ± 0.04*	4.05 ± 0.13*	5.05 ± 0.09*

Values are demonstrated as Mean + SEM, n=6; * p<0.05 when the values of treated groups are compared with values of Disease control group

Table 2: Effect of aqueous and ethyl acetate extract of *C. absus* seeds on serum lipid profile in different groups

Parameters	Days	Normal Control	Disease Control	Standard	Aqueous extract	Ethyl acetate extract
TC (mg/dl)	0	70.67±6.34	101.33±4.47	101.50±4.54	102.50±3.41	102.67±4.40
	28	71.67±6.45*	102.67±4.72	73.67±5.26*	77.17±4.00*	80.67±4.83*
TG (mg/dl)	0	89.17±3.83	149.33±11.84	136.00±12.45	149.00±10.07	150.17±7.82
	28	88.83±3.96*	144.67±11.09	94.33±7.36*	97.83±3.05*	104.17±8.81*
LDL (mg/dl)	0	13.33±0.95	33.17±1.70	32.00±1.86	32.67±1.78	34.17±1.74
	28	13.67±1.08*	34.17±1.92	16.50±1.38*	19.50±1.43*	24.33±1.54*
HDL (mg/dl)	0	22.50±1.12	15.33±2.20	14.67±0.33	13.67±0.61	14.17±0.79
	28	22.50±1.09*	13.17±1.14	21.67±0.67*	19.83±0.83*	18.33±0.92*

TC: total cholesterol, TG: triglycerides, LDL: low density lipoproteins, HDL: high density lipoproteins, Values are demonstrated as Mean + SEM, n=6; * p<0.05 when the values of treated groups are compared with values of Disease control group

Table 3: Effect of aqueous and ethyl acetate extracts of *C. absus* seeds on liver function markers in different groups

Parameters	Days	Normal Control	Disease Control	Standard	Aqueous extract	Ethyl acetate extract
ALT (IU/L)	0	37.17±3.26	62.50±4.75	63.17±3.05	62.17±3.35	63.83±3.51
	28	37.67±3.28*	62.67±4.75	39.33±3.11*	47.17±5.82	57.17±4.10
AST (IU/L)	0	79.83±3.59	122.50±7.82	125.83±3.26	123.50±3.17	125.33±2.52
	28	79.83±4.09*	122.17±7.94	88.67±4.76*	95.67±5.33*	105.50±7.86
ALP (IU/L)	0	221.34±3.25	280.61±4.14	281.51±3.24	280.39±2.64	283.78±2.16
	28	220.33±4.40*	282.50±3.25	230.67±3.85*	232.50±2.82*	251.00±2.65*

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase Values are demonstrated as Mean + SEM, n=6; * p<0.05 when the values of treated groups are compared with values of Disease control group

Table 4: Effect of aqueous and ethyl acetate extracts of *C. absus* seeds on serum Urea and Creatinine in different groups

Parameters	Days	Normal Control	Disease Control	Standard	Aqueous extract	Ethyl acetate extract
Urea (mg/dl)	0	15.33±0.76	30.33±2.68	27.83±1.67	29.00±2.95	30.33±3.04
	28	15.33±0.76*	30.33±2.67	25.17±2.52*	22.00±2.92*	27.00±3.04*
Creatinine (mg/dl)	0	0.58±0.03	1.10±0.06	1.13±0.09	1.05±0.03	1.12±0.07
	28	0.58±0.03*	1.12±0.09	0.63±0.04*	0.70±0.04*	0.82±0.03*

Values are demonstrated as Mean + SEM, n=6; * p<0.05 when the values of treated groups are compared with values of Disease control group

Table 5: Antioxidant activity of aqueous and ethyl acetate extracts of *C. absus* seeds in diabetic rats

Parameters	Days	Normal Control	Disease Control	Standard	Aqueous extract	Ethyl acetate extract
TAC(µmol/L)	0	1.49±0.04	0.86±0.03	0.87±0.04	0.85±0.03	0.86±0.04
	28	1.49±0.04*	0.87±0.03	1.29±0.05*	1.35±0.06*	1.33±0.04*
TOS(µmol/L)	0	4.82±0.24	6.72±0.22	6.61±0.44	6.91±0.67	6.71±0.48
	28	4.82±0.24*	6.91±0.68	5.23±0.38*	4.96±0.44*	5.06±0.48*
Catalase(KU/l)	0	52.93±1.16	41.85±1.84	41.48±1.39	40.53±1.19	41.62±1.42
	28	52.93±1.16*	40.58±1.60	48.00±1.15*	50.00±1.03*	46.33±1.23*
MDA(µmol/ml)	0	10.27±1.10	36.83±0.62	37.54±0.77	37.55±0.85	36.90±0.85
	28	10.31±1.07*	36.83±0.62	21.42±2.06*	16.73±2.48*	24.63±2.32*
SOD(U/mg of protein)	0	6.28±0.031	0.49±0.06	0.60±0.09	0.489±0.03	0.58±0.09
	28	6.28±0.031*	0.49±0.09	4.02±0.04*	5.20±0.04*	3.86±0.03*

TAC: total antioxidant capacity, TOS: total oxidant status, MDA: malondialdehyde , SOD: superoxide dismutase , Values are demonstrated as Mean + SEM, n=6; * p<0.05 when the values of treated groups are compared with values of Disease control group

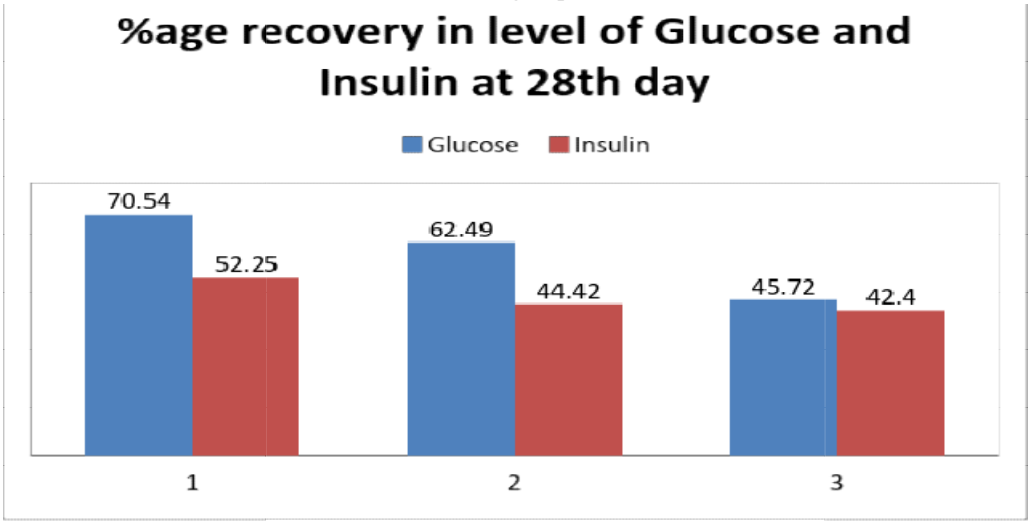

Fig 1: %age recovery in level of Glucose and Insulin at 28th day of study in treated groups. 1: treated with glibenclamide. 2: treated with aqueous extract of *C. absus*. 3: treated with ethyl acetate extract of *C. absus*.

DISCUSSION

Diabetes mellitus is a widespread metabolic disorder. It's prevalence is almost 6% of the population. It describes to a metabolic condition of chronic hyperglycemia that results from inadequacy in insulin secretion or action or both [3].

Alloxan is most widely used in experimental animal models to induce type I diabetes. It is toxic to β-cells of pancreas as it cumulates in these cells being a glucose analogue. Furthermoreits toxicity to β-cells is due to the production of ROS. Quick β-cells destructionis primarily due to the massiverise in cytosolic calcium ion level [15].

Asalloxan causes the destruction of pancreatic β-cells, the serum glucose level increases and serum insulin level reduces [7]. Mean ± SEM values of fasting serum glucose was more than300mg/dl in rats of 2nd, 3rd. 4th and 5th group because of alloxan administration, that was significantly higherthan normal group (91.167±1.662 mg/dl). At 28th day this level was significantly decreased in treated groups. Glibenclamide (10mg/kg/day) brought the values towards normal. Aqueous and ethyl acetate extract's (900mg/kg/day) oral administration significantly lowered the values of glucose as compared to disease control group. However, aqueous extract exhibited better results than ethyl acetate extract. In a previous study, effect of *Cassia auriculata* was checked on alloxan induced diabetic rats. Alloxan at rate of 150mg/kg i.p was administered. Rats with blood glucose level of 300-400mg/dl were considered as diabetic. Aqueous and ethanolic extract of *C. auriculata* lowered the glucose level towards normal [2]. Same effect was shown by the aqueous and ethyl aceate extract of *C. absus*.

At 0 day in diabetic rat's serum insulin level was significantly decreased. At 28th day significant elevation in serum insulin level was seen in treated groups than disease control group. Reduction inserum glucose level and rise in serum insulin level may be due to the regeneration of β-cells or enhanced insulin secretion and action. In a previous study, effect of *Cassia auriculata* was checked on alloxan induced diabetic rats. Alloxan at rate of 150mg/kg i.p was administered. Alloxan lowered the serum insulin level significantly. Aqueous and ethanolic extract of *C. auriculata* at dose 0.25 and 0.5g/kg for 30 days increased the insulin level [2]. HbA1c is diagnostic marker in diabetes which helps to identify the glycation of protein, long term blood glucose level and correlation of diabetes related

problems. In diabetes glycosylated Hb is formed when excess glucose in blood reacts with Hb. The rate of haemoglobin glycation is directly proportional to the blood glucose concentration [7]. In current study, HbA1c level of diabetic rats was significantly rise ($p < 0.05$). At 28th day of study, in treated groups HbA1 level was significantly reduced as compared to diabetic rats.

In current study, alloxan induced diabetes significantly raised the serum cholesterol, triglycerides, LDL level and lowered the HDL level at day 0. Glibenclamide, aqueous and ethyl acetate extract lowered the level of cholesterol, triglycerides and LDL significantly in comparison to disease control group at 28th day after their oral administration. Serum HDL level was significantly raised in treated groups. In a previous study effect of *Adansonia digitata* fruit pulp was examined on lipid profile of diabetic rats. Alloxn at dose of 150mg/kg intraperitoneally was given which induced diabetes and increased the serum total cholesterol, LDL and triglycerides level and decreased the HDL level. Methanolic extract of fruit pulp showed significant results in 28 days [16]. Similar results have been observed in the present study at dose rate of 900 mg/kg per day of aqueous and ethyl acetate extract.

The elevated lipid's concentration in serum may be due to disturbancein lipase activity in case of alloxan induced diabetes. Lipase covert triglycerides into glycerol and free fatty acids.Insulin inhibits this enzyme in adipose tissues. In alloxan induced diabetes deficiency of insulin occurs due to destruction of β-cells so free fatty acids level increase in plasma. Catabolism of these free fatty acids into acetyl CoA occurs in the liver. The excess acetyl CoA is transformed into cholesterol, ketone bodies and triglycerides. Increased cholesterol, phospholipids and triglycerides are squared into blood as lipoproteins which may cause atherosclerosis and coronary heart diseases [16].

In diabetes, disruption in liver cells leads towards liver dysfunction. So the liver enzymes such as ALT, AST and ALP ooze out from cytosol and their serum levelrise [7]. In diabetes elevated glycosylated proteins and glucose level in tissues cause renal damage so serum Creatinine and urea level increases [17]. In present study, serum ALT, AST, ALP, urea and Creatininelevel at 0 day in diabetic rats of group2, 3, 4 and 5 was significantly higher than that of normal control group. At 28th day in treated groups this level was significantly lowered. Aqueous

extract exhibited marked effect than ethyl acetate extract.

In a previous study effect of *Melastomam alabathricum* was examined on liver function markers of diabetic rats. Alloxn at dose of 150mg/kg intraperitoneally was given which induced diabetes and increased the serum ALP, SGOT and SGPT level. Ethanolic leaf extract of this plantshowed significant reduction in these parameters in 14 days [7]. In a study diabetes in rats was induced by alloxan 120mg/kg intraperitoneally which raised the serum urea and Creatinine level along with glucose. Aqueous, chloroform and ethanolic extract of *Citrullus colosynthis* roots extract (200mg/kg/day) at 14th day significantly lowered these parameters. But aqueous extract showed better results as compared to other extracts [17]. Similar results have been observed in the present study at dose rate of 900 mg/kg per day ofaqueous and ethyl acetate extract.

In diabetes produced by alloxan due to oxidative stress, super oxide dismutase (SOD) boosts up β-cells tolerance. The deficient antioxidant defense to oppose damage arbitrated by ROS may be due to reduction in SOD actions. Studies revealed the lessen activities of SOD and catalase in diabetes which boosted oxygen and H_2O_2 production [18]. In current study, rats of treated groups showed higher TAC, Catalase and SOD serum level at 28th day as compared to 0 day, so compete against the damage produced by ROS and inhibit the free radicals accumulation in β-cells. Level of TOS and Malondialdehyde (MDA) was elevated at 0 day. At 28th day it was decreased in treated groups. SOD and catalase adjusted the alloxan induced toxicity.

CONCLUSION

From the current study it can be concluded that the ethyl acetate and aqueousextract of *C. absus* seeds contain antidiabetic potential in diabetic rats produced by alloxan as they dropped the serum glucose level, glycosylated haemoglobin, increased serum insulin level and returned the liver function markers, serum lipid profile, and renalparameters to normal. Oxidative stress markers revealed that both extracts also have the antioxidant potential against alloxan induced oxidative stress. The aqueous extract exhibited better resultsthan ethyl acetate extract.

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