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Protective effect of coadministration of *Cassia auriculata* and pioglitazone in diabetic rats

Navjot Grover and Pallavi Bafna*

Department of Pharmacology, Rayat Institute of Pharmacy, Railmajra, Dist. Nawanshahar, Punjab(INDIA)

ABSTRACT

The present study was designed to evaluate the effect of coadministration of Cassia auriculata and pioglitazone in alloxan induced diabetic rats. Different groups of diabetic animals were treated with pioglitazone (10 mg/kg, p.o.; 100%), C. auriculata (450 mg/kg, p.o.; 100%); and 50:50 and 25:75 combinations of both pioglitazone and C. auriculata, respectively for a period of 28 days. Biochemical parameters like SGPT, SGOT, alkaline phosphatase, total bilirubin, BUN, serum creatinine and LDH were estimated weekly upto 28 days of treatment. The results proved a prominent positive effect of 25:75 combination of pioglitazone and C. auriculata; suggesting that a reduction of 75% of the conventional dose of pioglitazone, supplemented/ combined with 75% dose of C. auriculata (25% reduction in conventional dose), produced protective effects which were comparable to that of 100% pioglitazone (10 mg/kg) with restoration of levels of renal, cardiac and hepatic parameters. The study concluded that coadministration of pioglitazone and C.auriculata may prove to be more beneficial in diabetes than pioglitazone alone; but the clinical appropriateness of the combination has still to be confirmed.

Keywords: Alloxan, diabetes, renal, cardiac, hepatic.

INTRODUCTION

Diabetes mellitus is one of the oldest diseases known to mankind. All the renowned classic texts of Ayurveda like Charaka Samhita (1000 B.C.) and subsequent works refer to this disease under the term *Madhumeha* or *Ikshumeha* (literally meaning sugar in the urine). It is a chronic progressive disease caused by inherited or acquired deficiency of insulin production or resistance to action of the produced insulin [1]. According to a report published by the WHO Expert Committee on Diabetes Mellitus in 1980, the abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin.

The primary organs affected in diabetes are liver and kidney. Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes [2]. Some of the changes associated with diabetic liver are decreased glycolysis, impeded glycogenesis and increased gluconeogenesis. The disease is also associated with marked increase in parameters such as cardiovascular risk factors comprising of hypertriglyceridemia, hypercholesterolemia and low level of high- density lipoprotein- cholesterol [3].

The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. The disease may present with characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss. In its most

severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Before the diagnosis is made, hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time, with or without the presence of symptoms. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. The metabolic consequences of prolonged hyperglycemia and dyslipidemia, including accelerated atherosclerosis, chronic kidney disease, and blindness, pose an enormous burden on patients with diabetes mellitus and on the public health system. Improvements in our understanding of the pathogenesis, complications and prevention methods of diabetes are critical to meet this progressing challenge. Poor control of diabetes accelerates the progression of complications. Thus, to prevent complications, good control of diabetes is essential and the management of diabetes should therefore aim to improve glycaemic control beyond that required to control its symptoms [4]. The available treatments for diabetes are insulin analogues and oral hypoglycemic agents. Amongst the oral hypoglycemics, the sulfonylureas and biguanides have been available the longest and are the traditional initial treatment choice for type 2 diabetes. Novel classes of rapidly acting insulin secretagogues, the meglitinides and D-phenylalanine derivatives, are alternatives to the short-acting sulfonylurea, tolbutamide. The thiazolidinediones are very effective agents that reduce insulin resistance. Alpha-glucosidase inhibitors have a relatively weak antidiabetic effect and significant adverse effects, and they are used primarily as adjunctive therapy in individuals who cannot achieve their glycemic goals with other medications.

Pioglitazone hydrochloride, belonging to the thiazolidinedione class of antidiabetics, has been studied for its effect on different tissues. It has been proved for its ability to activate AMP- activated protein kinase in rat liver and adipose tissue [5]. The study done to elucidate the mechanisms via which pioglitazone improves insulin resistance in patients with type 2 diabetes mellitus is associated with improvements in hepatic and peripheral tissue sensitivity to insulin [6].

A multitude of herbs spices and other plant materials have been described for the treatment of diabetes throughout the world [7, 8]. India has about 45,000 plant species and many of them have medicinal properties; out of which a large number of herbal drugs have been stated to possess anti-diabetic activity in the Ayurvedic system of medicine of India [9]. Various plants traditionally reported for the treatment of diabetes mellitus are *Trigonella foenum graecum* (Fenugreek), *Vaccinium myrtillus (European bilberry)*, *Taraxacum officinale* (Dandelion), *Gymnema sylvester* (Gymnema), *Glycyrrhiza glabra* (Licorice), *Syzygium cumini* (Jambul), *Opuntia streptacantha* (Prickly pear), *Panax ginseng/ P. quinquefolium* (Ginseng), *Lupinus albus* (White lupin) and *Globularia alypum* (Globularia).

Unfortunately many people suffer from the misconception that herbal products are totally safe and do not possess any side-effects because they are derived from natural sources and have been used for many years. Components of medicinal plants can alter the absorption and/or metabolism of conventional drugs leading to reduced efficacy or systemic drug toxicity [10]. Furthermore, drug-herb interaction could lead to treatment failure and increased drug toxicity [11]. The patients suffering from chronic diseases such as diabetes and who use multiple medications, particularly those drugs with a narrow therapeutic range, are at greatest risk of interactions [12]. The present study was thus initiated as a starting point to address the issue of possible interactions between traditional medicines and current prescription drugs used in type II diabetic care.

The plant, *Cassia auriculata* belonging to family Caesalpiniaceae, has been proved to possess various activities like antioxidant and hepatoprotective [13], anti-cancer [14], anti-hyperlipidemic [15], antimutagenic and antifertility [16], anti-inflammatory [17] and anthelmintic [18]. Extracts of leaves [19], flowers [20] and whole plant [21] of *C.auriculata* were also found to possess anti-diabetic activity in rats. This plant has been widely used in various marketed formulations namely, Diakyur, Diasunil, Diabeta plus, Dianex, Diabkil.

There is scarcity of information on effects of coadministration of C. auriculata with oral hypoglycemic agents. It has been only recently studied for the effect of coadministration with glibenclamide where the results confirmed that the combination helped to modify the serum parameters associated with diabetes [22].

However, the effect of co-administration of C. auriculata has never been studied with thiazolidinedione class of anti-diabetic drugs. Thus, the objective of the study was to investigate the effect of co-administration of aqueous extract of *Cassia auriculata* (AECA) and an oral hypoglycemic agent of thiazolidinedione class, namely pioglitazone hydrochloride, in alloxan induced diabetic animals. Our earlier studies [23] reports the effects of the same on serum levels of glucose and antioxidant markers (TBARS, catalase and reduced glutathione) where the combination revealed comparable antidiabetic and antioxidant effects to that of 100% pioglitazone administration. The present paper reports the effect of the combination of C.auriculata and pioglitazone on the serum markers of liver, kidney and cardiac damage to determine its protective potential on various organs.

MATERIALS AND METHODS

Animals

Rats (either sex) weighing around 120-150 gms were housed in polypropylene cages at an ambient temperature of 25 ± 20° C and 55-65% relative humidity. A 12 ± 1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available pellet diet (Ashirwad Labs, India) and had free access to water. Experiments were performed according to the guidelines for care and use of laboratory animals as mentioned by CPCSEA. Prior approval from Institutional Animal Ethics Committee was obtained for conduction of experiments.

Drugs and Chemicals

Alloxan monohydrate was purchased from Explicit Chemicals (Pune). Pioglitazone hydrochloride was procured from Kwality Pharmaceuticals, Amritsar (Punjab). Dry standardized aqueous extract of *Cassia auriculata* Linn., Family: Caesalpiniaceae (AECA) was procured and authenticated from Amsar Pvt. Ltd. (M.P., India). The plant extract was dissolved in distilled water just before use. Diagnostic kits were procured from Avecon Healthcare Pvt. Ltd. (Saha, India), Span Diagnostics Ltd. (Surat, Gujarat, India) and Transasia Bio-Medicals Ltd. (Solan, H.P., India).

Methodology

Alloxan monohydrate (150mg/kg, i.p.) was administered to 24 hrs fasted rats to induce diabetes. Blood samples were collected after 24 hrs of alloxan administration and blood glucose levels were estimated. Animals which showed a blood sugar level (BSL) in the range of 200-350 mg/dl were considered as diabetic. These diabetic rats were divided into 5 groups of 6 animals each. Group I served as normal control which consisted of non-diabetic animals which received the vehicle (normal saline) for a period of 28 days. Group II served as diabetic control which consisted of diabetic animals and received the vehicle for 28 days. Group III diabetic animals received pioglitazone (10 mg/kg, p.o.; 100%) daily whereas Group IV diabetic animals received AECA (450 mg/kg, p.o.; 100%) daily for a period of 28 days. Group V consisted of diabetic animals treated with different combinations of pioglitazone and AECA. This group was further divided into two: Group Va received a 50%:50% combination of pioglitazone (5 mg/kg, p.o.) and AECA (225 mg/kg, p.o.) daily for 28 days; whereas Group Vb received a 25%:75% combination of pioglitazone (2.5 mg/kg, p.o.) and AECA (337.5 mg/kg, p.o.) daily for 28 days. On the 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment, blood was collected and serum was separated. The serum was then used for the estimation of indicators of liver, heart and kidney damage namely, SGPT, SGOT, alkaline phosphatase, total bilirubin, BUN (Blood urea nitrogen), creatinine and LDH (Lactate dehydrogenase) using diagnostic kits.

Statistical Analysis

The data was expressed as Mean ± SEM. In all the tests, the criterion for the statistical significance was set at $p < 0.05$. The data was analyzed using one-way ANOVA followed by Tukey Kramer test.

RESULTS

Effect on SGPT Level

The SGPT level of Group I animals (Vehicle treated Control group) was found to be in the range of 30-42 units/ml during all the four weeks of observation (0^{th} to 28^{th} day). Alloxan administration to animals of Group II (Diabetic control) resulted in a significant increase ($p<0.001$) in SGPT levels (72-84 units/ml) on all the days of observation as compared to vehicle treated control group (Group I).

Table 1: Effect of *Cassia auriculata* and its combination with Pioglitazone on the SGPT level (units/ml) of diabetic rats.

Groups	Normal Control	Diabetic Control (Alloxan)	Alloxan + 100% Pio	Alloxan + 100% AECA	Alloxan + 50% Pio+ 50% AECA	Alloxan + 25% Pio+ 75% AECA
0th Day	31 ± 0.98	77 ± 1.23***	78 ± 1.26^{NS}	76 ± 1.64^{NS}	77 ± 1.13^{NS}	76 ± 1.24^{NS}
7th Day	35 ± 2.40	74 ± 1.60***	71 ± 2.22^{NS}	71 ± 0.95^{NS}	72 ± 1.18^{NS}	67 ± 1.76^{NS}
14th Day	39 ± 1.55	77 ± 1.07***	66 ± 2.16$^{###}$	63 ± 2.61$^{###}$	64 ± 1.34$^{#}$	59 ± 4.10$^{###}$
21st Day	40 ± 2.00	80 ± 0.87***	49 ± 3.78$^{###}$	56 ± 3.03$^{###}$	59 ± 0.76$^{###}$	53 ± 0.67$^{###}$
28th Day	37 ± 1.59	83 ± 0.91***	43 ± 2.55$^{###}$	53 ± 2.99$^{###}$	55 ± 1.54$^{###}$	48 ± 0.98$^{###}$

Values are represented as Mean ±SEM;
Pio: Pioglitazone; AECA: Aqueous Extract of Cassia auriculata

Group I- Vehicle treated Control Group; Group II- Diabetic Control Group; Group III- Pioglitazone (100%; 10 mg/kg) - treated Diabetic Group; Group IV- AECA (100%; 450 mg/kg) - treated Diabetic Group; Group Va- Pioglitazone (50%) + C.auriculata (50%) -treated Diabetic Group; Group Vb- Pioglitazone (25%) + C.auriculata (75%) -treated Diabetic Group.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared to Vehicle treated control group (Group I) on the respective day [Group II was compared with Group I];

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared to Alloxan treated diabetic control group (Group II) on the respective day [Groups III, IV, Va and Vb were compared to Group II].

NS: Non-significant
Treatment of pioglitazone in Group III diabetic animals and AECA in Group IV diabetic animals produced a significant ($p<0.001$) decrease in SGPT levels on the 14th, 21st and 28th day as compared to alloxan treated diabetic animals (Group II) on the respective days [Table 1].

Treatment with 50%:50% and 25%:75% combination of pioglitazone and AECA to animals of Group Va and Vb, respectively showed a significant reduction in the SGPT level on the 14th (Group Va : $p<0.05$; Group Vb: $p<0.001$), 21st (Groups Va and Vb: $p<0.001$) and 28th (Groups Va and Vb: $p<0.001$) day of observation as compared to that of alloxan-induced diabetic animals (Group II) on the respective days [Table 1].

Effect on SGOT Level
The SGOT level of Group I animals (Vehicle treated Control group) was found to be in the range of 30-34 units/ml during the four weeks of observation (0th to 28th day). Animals of Group II (Diabetic control) showed a significant increase ($p<0.001$) in SGOT levels (72-93 units/ml) on all the days of observation as compared to vehicle treated control group (Group I). On the 0th day of observation the SGOT levels in animals of groups III, IV, Va and Vb were similar to the diabetic control group.

Administration of pioglitazone to Group III diabetic animals and AECA to Group IV diabetic animals significantly ($p<0.001$) declined the SGOT levels as compared to alloxan treated diabetic animals (Group II) on all the days of observation [Table 2].
Similarly treatment with 50%:50% and 25%:75% combination of pioglitazone and AECA to animals of Group Va and Vb, respectively, showed a significant reduction ($p<0.001$) in the SGOT level on all the days of observation as compared to the SGOT levels of alloxan-induced diabetic animals (Group II) on the respective days [Table 2].

Table 2: Effect of *Cassia auriculata* and its combination with Pioglitazone on the SGOT level (units/ml) of diabetic rats.

Groups	Normal Control	Diabetic Control (Alloxan)	Alloxan + 100% Pio	Alloxan + 100% AECA	Alloxan + 50% Pio + 50% AECA	Alloxan + 25% Pio + 75% AECA
0th day	33.5 ±0.9	75 ± 1.44***	76 ± 1.24^{NS}	75 ± 1.07^{NS}	77 ± 1.05^{NS}	77 ± 1.13^{NS}
7th day	31 ± 1.14	80 ± 0.79***	70 ± 0.70$^{###}$	70 ± 0.66$^{###}$	72 ± 0.90$^{###}$	71 ± 1.02$^{###}$
14th day	30 ± 1.26	80 ± 3.35***	63 ± 2.40$^{###}$	63 ± 1.84$^{###}$	67 ± 1.33$^{###}$	64 ± 1.49$^{###}$
21st day	30 ± 1.48	85 ± 2.80***	54 ± 2.21$^{###}$	54 ± 2.43$^{###}$	64 ± 1.74$^{###}$	54 ± 1.33$^{###}$
28th day	32 ± 1.53	92 ± 1.14***	44 ± 1.90$^{###}$	49 ± 1.97$^{###}$	60 ± 1.14$^{###}$	49 ± 2.61$^{###}$

Values are represented as Mean ±SEM;
Pio: Pioglitazone; AECA: Aqueous extract of Cassia auriculata

Group I- Vehicle treated Control Group; Group II- Diabetic Control Group; Group III- Pioglitazone (100%; 10 mg/kg) -treated Diabetic Group; Group IV- AECA (100%; 450 mg/kg) -treated Diabetic Group; Group Va- Pioglitazone (50%) + C.auriculata (50%) -treated Diabetic Group; Group Vb- Pioglitazone (25%) + C.auriculata (75%) -treated Diabetic Group

* p<0.05, **p<0.01, ***p<0.001 as compared to Vehicle treated control group (Group I) on the respective day [Group II was compared with Group I]

$^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 as compared to Alloxan treated diabetic control group (Group II) on the respective day [Groups III, IV, Va and Vb were compared to Group II].

NS: Non-significant

Effect on Serum Alkaline Phosphatase Level

The alkaline phosphatase level of Group I animals (Vehicle treated Control group) was found to be in the range of 45-51 IU during the four weeks of observation (0^{th} to 28^{th} day).

Animals of Group II (Diabetic control) showed a significant increase (p<0.001) in serum alkaline phosphatase levels (254-385 IU) on all the days of observation as compared to vehicle treated control group (Group I).

Table 3: Effect of *Cassia auriculata* and its combination with Pioglitazone on the serum alkaline phosphatase level (IU) of diabetic rats.

Groups	Normal Control	Diabetic Control	Alloxan + 100% Pio (Std)	Alloxan + 100% AECA	Alloxan + 50% Pio + 50% AECA	Alloxan + 25% Pio + 75% AECA
0^{th} Day	48 ± 1.29	258 ± 3.74***	250 ± 1.24^{NS}	265 ± 3.77^{NS}	268 ± 4.00^{NS}	261 ± 4.59^{NS}
7^{th} Day	50 ± 1.16	276 ± 1.42***	188 ± 3.15$^{\#\#\#}$	216 ± 7.16$^{\#\#\#}$	212 ± 4.23$^{\#\#\#}$	207 ± 2.20$^{\#\#\#}$
14^{th} Day	48 ± 1.29	318 ± 3.53***	156 ± 2.91$^{\#\#\#}$	176 ± 2.55$^{\#\#\#}$	155 ± 2.35$^{\#\#\#}$	156 ± 1.86$^{\#\#\#}$
21^{st} Day	47 ± 2.03	371 ± 1.75***	111 ± 3.99$^{\#\#\#}$	126 ± 2.30$^{\#\#\#}$	121 ± 2.10$^{\#\#\#}$	111 ± 2.10$^{\#\#\#}$
28^{th} Day	49 ± 1.75	383 ± 1.88***	80 ± 2.99$^{\#\#\#}$	112 ± 2.56$^{\#\#\#}$	102 ± 1.45$^{\#\#\#}$	81 ± 2.16$^{\#\#\#}$

Values are represented as Mean ±SEM;
Pio: Pioglitazone; AECA: Aqueous extract of Cassia auriculata

Group I- Vehicle treated Control Group; Group II- Diabetic Control Group; Group III- Pioglitazone (100%; 10 mg/kg) -treated Diabetic Group; Group IV- AECA (100%; 450 mg/kg) -treated Diabetic Group; Group Va- Pioglitazone (50%) + C.auriculata (50%) -treated Diabetic Group; Group Vb- Pioglitazone (25%) + C.auriculata (75%) -treated Diabetic Group

* p<0.05, **p<0.01, ***p<0.001 as compared to Vehicle treated control group (Group I) on the respective day [Group II was compared with Group I]

$^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 as compared to Alloxan treated diabetic control group (Group II) on the respective day [Groups III, IV, Va and Vb were compared to Group II].

NS: Non-significant

Treatment with 100% pioglitazone (Group III), 100% AECA (Group IV), 50%:50% (Group Va) and 25%:75% (Group Vb) combinations of pioglitazone and AECA to diabetic animals was found to significantly (p<0.001) decrease the serum alkaline phosphatase levels on all the days of observation as compared to the alkaline phosphatase levels in Group II animals on the respective days [Table 3].

Effect on Serum Total Bilirubin Level

The bilirubin level of Group I animals (Vehicle treated Control group) was found to be in the range of 0.91- 3.80 mg/dl during the four weeks of observation (0^{th} to 28^{th} day). Animals of Group II (Diabetic control) showed a significant increase (p<0.001) in serum bilirubin levels on all the days of observation as compared to vehicle treated control group (Group I).

Treatment of pioglitazone in Group III diabetic animals and AECA in Group IV diabetic animals was found to control the rise in serum bilirubin levels as compared to alloxan treated diabetic animals (Group II). A significant

($p<0.05$) decrease in serum bilirubin value was observed on 14th, 21st and 28th day as compared to the bilirubin levels in Group II animals on the respective days [Table 4].

Treatment with 50%:50% and 25%:75% combinations of pioglitazone and AECA to animals of Group Va and Vb, respectively, showed a significant reduction ($p<0.001$) in the serum bilirubin level on 14th, 21st and 28th days of observation as compared to the bilirubin levels of alloxan-induced diabetic animals (Group II) on the respective days [Table 4].

Table 4: Effect of *Cassia auriculata* and its combination with Pioglitazone on the serum total bilirubin level (mg/dl) of diabetic rats.

Groups	Normal Control	Diabetic Control	Alloxan + 100% Pio (Std)	Alloxan + 100% AECA	Alloxan + 50% Pio + 50% AECA	Alloxan + 25% Pio + 75% AECA
0th Day	0.91 ± 0.22	3.25 ± 0.11*	3.50 ± 0.95^{NS}	3.76 ± 0.10^{NS}	3.80 ± 0.17^{NS}	3.69 ± 0.11^{NS}
7th Day	1.19 ± 0.23	3.44 ± 0.09***	2.73 ± 0.06$^{\#}$	3.41 ± 0.11^{NS}	3.15 ± 0.20^{NS}	3.01 ± 0.11^{NS}
14th Day	1.18 ± 0.24	3.58 ± 0.08***	2.07 ± 0.13$^{\#\#\#}$	2.52 ± 0.10$^{\#\#\#}$	2.14 ± 0.22$^{\#\#\#}$	2.17 ± 0.15$^{\#\#\#}$
21st Day	1.12 ± 0.22	3.57 ± 0.18***	1.50 ± 0.16$^{\#\#\#}$	1.54 ± 0.13$^{\#\#\#}$	1.47 ± 0.16$^{\#\#\#}$	1.58 ± 0.15$^{\#\#\#}$
28th Day	1.24 ± 0.19	4.03 ± 0.05***	0.61 ± 0.02$^{\#\#\#}$	0.80 ± 0.02$^{\#\#\#}$	0.84 ± 0.04$^{\#\#\#}$	0.61 ± 0.02$^{\#\#\#}$

Values are represented as Mean ±SEM;
Pio: Pioglitazone; AECA: Aqueous extract of Cassia auriculata

Group I- Vehicle treated Control Group; Group II- Diabetic Control Group; Group III- Pioglitazone (100%; 10 mg/kg) -treated Diabetic Group; Group IV- AECA (100%; 450 mg/kg) -treated Diabetic Group; Group Va- Pioglitazone (50%) + C.auriculata (50%) -treated Diabetic Group; Group Vb- Pioglitazone (25%) + C.auriculata (75%) -treated Diabetic Group

* $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared to Vehicle treated control group (Group I) on the respective day [Group II was compared with Group I]

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared to Alloxan treated diabetic control group (Group II) on the respective day [Groups III, IV, Va and Vb were compared to Group II].

NS: Non-significant

Effect on Serum BUN Level

The BUN level of Group I animals (Vehicle treated Control group) was found to be in the range of 13-16 mg/dl during the four weeks of observation (0th to 28th day). Animals of Group II (Diabetic control) showed a significant increase ($p<0.001$) in serum BUN levels (50-60 mg/dl) on all the days of observation as compared to the vehicle treated control group (Group I) [Table 5].

Table 5: Effect of *Cassia auriculata* and its combination with Pioglitazone on the serum BUN level (mg/dl) of diabetic rats.

Groups	Normal Control	Diabetic Control (Alloxan)	Alloxan + 100% Pio	Alloxan + 100% AECA	Alloxan + 50% Pio + 50% AECA	Alloxan + 25% Pio + 75% AECA
0th Day	14.8 ± 0.75	54.02 ± 2.52***	55.3 ± 1.6^{NS}	58.4 ± 2.99^{NS}	53.1 ± 1.65^{NS}	56.3 ± 2.82^{NS}
7th Day	14.3 ± 0.83	58.50 ± 1.00***	33.8 ± 0.57$^{\#\#\#}$	51.4 ± 3.10$^{\#}$	45.8 ± 0.66$^{\#\#\#}$	40 ± 0.81$^{\#\#\#}$
14th Day	15.3 ± 0.92	55.20 ± 2.02***	28.2 ± 0.28$^{\#\#\#}$	33.3 ± 0.89$^{\#\#\#}$	40.5 ± 0.57$^{\#\#\#}$	26.2 ± 0.39$^{\#\#\#}$
21st Day	14.1 ± 0.95	53.07 ± 2.28***	23.74 ± 0.31$^{\#\#\#}$	28.4 ± 1.57$^{\#\#\#}$	33.2 ± 0.45$^{\#\#\#}$	17.4 ± 0.41$^{\#\#\#}$
28th Day	14.4 ± 0.72	54.9 ± 2.50***	13.15 ± 0.21$^{\#\#\#}$	24.5 ± 0.75$^{\#\#\#}$	21.9 ± 0.44$^{\#\#\#}$	14.05 ± 0.44$^{\#\#\#}$

Values are represented as Mean ±SEM;
Pio: Pioglitazone; AECA: Aqueous extract of Cassia auriculata

Group I- Vehicle treated Control Group; Group II- Diabetic Control Group; Group III- Pioglitazone (100%; 10 mg/kg) -treated Diabetic Group; Group IV- AECA (100%; 450 mg/kg) -treated Diabetic Group; Group Va- Pioglitazone (50%) + C.auriculata (50%) -treated Diabetic Group; Group Vb- Pioglitazone (25%) + C.auriculata (75%) -treated Diabetic Group

* $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared to Vehicle treated control group (Group I) on the respective day [Group II was compared with Group I]

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared to Alloxan treated diabetic control group (Group II) on the respective day [Groups III, IV, Va and Vb were compared to Group II].

NS: Non-significant

Treatment of pioglitazone in Group III diabetic animals significantly ($p<0.001$) decreased the serum BUN levels on all the days of observation as compared to Group II animals. Also, treatment with AECA (Group IV) significantly reduced the serum BUN value on the 7th ($p<0.05$), 14th ($p<0.001$), 21st ($p<0.001$) and 28th ($p<0.001$) day as compared to the BUN levels of diabetic control animals (Group II) on the respective days [Table 5].

Treatment with 50%:50% and 25%:75% combination of pioglitazone and AECA to Groups Va and Vb, respectively showed a significant reduction ($p<0.001$) in the serum BUN level on all the days of observation as compared to the BUN levels of alloxan-induced diabetic animals (Group II) on the respective days [Table 5].

Effect on Serum Creatinine Level

The creatinine level of Group I animals (Vehicle treated Control group) was found to be in the range of 0.71- 0.80 mg/dl during the four weeks of observation (0th to 28th day). Animals of Group II (Diabetic control) showed a significant increase ($p<0.001$) in serum creatinine levels (1.3-1.6 mg/dl) on all the days of observation as compared to vehicle treated control group (Group I).

Treatment of pioglitazone in Group III diabetic animals significantly decreased the serum creatinine levels on the 7th ($p<0.05$), 14th ($p<0.001$), 21st ($p<0.001$) and 28th ($p<0.001$) day; whereas AECA treatment to Group IV diabetic animals produced significant effects only on the 14th ($p<0.05$), 21st ($p<0.05$) and 28th ($p<0.001$) day as compared to the creatinine levels in Group II animals on the respective days [Table 6].

Treatment with 50%:50% combination of pioglitazone and AECA to animals of Group Va significantly decreased the serum creatinine levels on the 14th ($p<0.05$), 21st ($p<0.05$) and 28th ($p<0.01$) day; whereas treatment with 25%:75% combination to Group Vb diabetic animals produced significant effects on all the days of observation, namely 7th ($p<0.05$), 14th ($p<0.05$), 21st ($p<0.05$) and 28th ($p<0.001$) day as compared to the creatinine levels in Group II animals on the respective days [Table 6].

Table 6: Effect of *Cassia auriculata* and its combination with Pioglitazone on the serum creatinine level (mg/dl) of diabetic rats.

Groups	Normal Control	Diabetic Control (Alloxan)	Alloxan + 100% Pio	Alloxan + 100% AECA	Alloxan + 50%Pio + 50% AECA	Alloxan + 25% Pio + 75% AECA
0th Day	0.85 ± 0.04^{NS}	$1.4 \pm 0.06^{***}$	1.7 ± 0.06^{NS}	1.6 ± 0.05^{NS}	1.7 ± 0.01^{NS}	1.5 ± 0.01^{NS}
7th Day	0.74 ± 0.03^{NS}	$1.5 \pm 0.05^{***}$	$1.39 \pm 0.02^{\#}$	1.52 ± 0.02^{NS}	1.48 ± 0.01^{NS}	$1.36 \pm 0.01^{\#}$
14th Day	0.77 ± 0.03^{NS}	$1.6 \pm 0.03^{***}$	$1.10 \pm 0.04^{\#\#\#}$	$1.37 \pm 0.01^{\#}$	$1.27 \pm 0.02^{\#}$	$1.24 \pm 0.02^{\#}$
21st Day	0.77 ± 0.02^{NS}	$1.5 \pm 0.06^{***}$	$0.96 \pm 0.03^{\#\#\#}$	$1.21 \pm 0.02^{\#}$	$1.27 \pm 0.02^{\#}$	$1.24 \pm 0.02^{\#}$
28th Day	0.76 ± 0.04^{NS}	$1.6 \pm 0.02^{***}$	$0.82 \pm 0.02^{\#\#\#}$	$0.94 \pm 0.03^{\#\#\#}$	$1.19 \pm 0.03^{\#\#}$	$0.65 \pm 0.01^{\#\#\#}$

Values are represented as Mean ±SEM;
Pio: Pioglitazone; AECA: Aqueous extract of Cassia auriculata

Group I- Vehicle treated Control Group; Group II- Diabetic Control Group; Group III- Pioglitazone (100%; 10 mg/kg) -treated Diabetic Group; Group IV- AECA (100%; 450 mg/kg) -treated Diabetic Group; Group Va- Pioglitazone (50%) + C.auriculata (50%) -treated Diabetic Group; Group Vb- Pioglitazone (25%) + C.auriculata (75%) -treated Diabetic Group

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared to Vehicle treated control group (Group I) on the respective day [Group II was compared with Group I]

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared to Alloxan treated diabetic control group (Group II) on the respective day [Groups III, IV, Va and Vb were compared to Group II].

NS: Non-significant

Effect on Serum LDH Level

The LDH level of Group I animals (Vehicle treated Control group) was found to be in the range of 309-389 IU/L during the four weeks of observation (0^{th} to 28^{th} day). Animals of Group II (Diabetic control) showed a significant increase ($p<0.001$) in serum LDH levels (533-748 IU/L) on all the days of observation as compared to vehicle treated control group (Group I).

Table 7: Effect of *Cassia auriculata* and its combination with Pioglitazone on the serum LDH level (IU/L) of diabetic rats.

Groups	Normal Control	Diabetic Control	Alloxan + 100% Pio (Std)	Alloxan + 100% AECA	Alloxan + 50% Pio + 50% AECA	Alloxan + 25% Pio + 75% AECA
0^{th} Day	313 ± 4.4	551 ± 17.8***	575 ± 27.4^{NS}	573 ± 23.69^{NS}	627 ± 13.63^{NS}	613 ± 9.62^{NS}
7^{th} Day	330 ± 4.2	611 ± 5.5***	556 ± 6.48$^{\#}$	528 ± 25.4$^{\#\#\#}$	566 ± 11.6^{NS}	546 ± 5.05$^{\#}$
14^{th} Day	331 ± 8.03	668 ± 19.4***	449.5 ± 6.09$^{\#\#\#}$	499 ± 20.5$^{\#\#\#}$	521 ± 12.8$^{\#\#\#}$	519 ± 6.7$^{\#\#\#}$
21^{st} Day	377 ± 11.9	737 ± 7.6***	407 ± 4.2$^{\#\#\#}$	466 ± 13.7$^{\#\#\#}$	446 ± 8.7$^{\#\#\#}$	447 ± 9.1$^{\#\#\#}$
28^{th} Day	348 ± 8.9	746 ± 2.4***	342 ± 12.5$^{\#\#\#}$	440 ± 18.7$^{\#\#\#}$	383 ± 15.06$^{\#\#\#}$	334 ± 13.8$^{\#\#\#}$

Values are represented as Mean ± SEM;
Pio: Pioglitazone; AECA: Aqueous extract of Cassia auriculata

Group I- Vehicle treated Control Group; Group II- Diabetic Control Group; Group III- Pioglitazone (100%; 10 mg/kg) -treated Diabetic Group; Group IV- AECA (100%; 450 mg/kg) -treated Diabetic Group; Group Va- Pioglitazone (50%) + C.auriculata (50%) -treated Diabetic Group; Group Vb- Pioglitazone (25%) + C.auriculata (75%) -treated Diabetic Group

* $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared to Vehicle treated control group (Group I) on the respective day [Group II was compared with Group I]

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared to Alloxan treated diabetic control group (Group II) on the respective day [Groups III, IV, Va and Vb were compared to Group II].

NS: Non-significant

Treatment of pioglitazone in Group III diabetic animals significantly decreased the serum LDH levels on 7^{th} ($p<0.05$), 14^{th} ($p<0.001$), 21^{st} ($p<0.001$) and 28^{th} ($p<0.001$) day. Also treatment with AECA of Group IV diabetic animals significantly ($p<0.001$) decreased serum LDH values on all the days as compared to the LDH levels of Group II animals on the respective days [Table 7].

Treatment with 50%:50% and 25%:75% combinations of pioglitazone and AECA to animals of Group Va and Vb, respectively, showed a significant reduction ($p<0.001$) in the serum LDH level on the 14^{th}, 21^{st} and 28^{th} day of observation as compared to the LDH levels of alloxan-induced diabetic animals (Group II) on the respective days [Table 7].

DISCUSSION

Diabetes is one of the most common metabolic disorders and 1.3% of the population suffers from this disease throughout the world. In 2010, 285 million people, corresponding to 6.4% of the world's adult population, suffered from diabetes. The number is expected to grow to 438 million by 2030, corresponding to 7.8% of the adult population. The pathological changes associated with diabetes namely, thickening of the capillary basement membrane and narrowing of the vessel lumina, causing inadequate perfusion of critical regions of certain organs, contribute to some of the major complications of diabetes, including premature atherosclerosis, intercapillary glomerulosclerosis, retinopathy, neuropathy, nephropathy, and ulceration and gangrene of the extremities. Prolonged hyperglycemia results in the formation of advanced glycation end products [24]. These macromolecules are thought to induce many of the vascular abnormalities that result in the complications of diabetes [25].

The present paper aims to study the effect of coadministration of C. auriculata (family Ceasalpiniaceae), a plant traditionally used for treating diabetes and pioglitazone hydrochloride, an oral hypoglycemic drug of thiazolidinedione class, for their effect against various biochemical parameters in alloxan induced diabetes.

Alloxan monohydrate is one of the most commonly used inducer of experimental diabetes. It has been shown that the inhibition of mechanism of oxidative phosphorylation in the beta cells is the primary cause of diabetogenic action of alloxan [26]. Alloxan acts by interfering with some essential enzyme/ enzymes of beta cells; which are also present in liver and kidneys in a specific concentration. Therefore, these organs, namely liver and kidneys are also affected by suitable concentrations of alloxan. In the present study, alloxan at the dose of 150 mg/kg body weight (i.p.) was found to induce diabetes (glucose levels greater than 150 mg/dl) in 24 hour fasted rats.

Earlier studies have reported a significant antihyperglycemic activity of the aqueous extract of flowers of *Cassia auriculata* in alloxan-induced diabetic rats [21]. Our earlier study [23] also proved the glucose lowering or antidiabetic effect of C.auriculata (100%) as a whole plant. The study also revealed a significant anti-diabetic effect of co-administration of 25%:75% and 50%:50% of pioglitazone and C. auriculata, which was found to be comparable to that produced by the administration of 100% pioglitazone.

ALT i.e. Alanine Transaminase or SGPT is a cytosolic enzyme primarily present in the liver, and AST i.e. Aspartate Transaminase or SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscles and kidney. SGOT and SGPT are both sensitive markers of hepatocellular injury. When the liver cell is injured or dies, these proteins can leak through the liver cell membrane into the circulation and serum levels will rise. Diabetic rats have been found to be associated with an increase in the activities of SGOT and SGPT. It may be indicative of severe liver and cardiac damage. The higher levels of SGOT and SGPT may give rise to a high concentration of glucose. In other words, the gluconeogenic action of SGOT and SGPT plays the role of providing new supplies of glucose from other sources such as amino acids [27]. The present study revealed an increase in both SGPT and SGOT in diabetic animals which indicated damage to vital organs like liver and heart. Treatment with the drugs alone and their combinations helped to restore the SGOT and SGPT levels to normal.

Alkaline phosphatase is a hydrolase enzyme, which is responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids. Serum alkaline phosphatase is produced by many tissues, especially bone, liver, intestine and placenta and is excreted in the bile. In the absence of bone disease and pregnancy, an elevated serum alkaline phosphatase levels generally reflect hepatobiliary disease. The mechanism of elevated ALP levels may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells. Previous studies suggested an increase in the serum alkaline phosphatase levels on administration of alloxan in the experimental animals [28]. Similarly in this study a significant elevation in alkaline phosphatase levels was observed in the diabetic rats, which in all the treatment groups was restored to normal.

Estimation of bilirubin, metabolic product of the breakdown of heme is one of the better liver function tests. Normally, 0.25 mg/dl of conjugated bilirubin is present in the blood of an adult. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in hemolysis and defects of hepatic uptake and conjugation of bilirubin treatment such as Gilbert's disease. It is secreted by the liver and stored in gall bladder [29]. In the present study, alloxan induced diabetic animals showed a marked increase in the level of bilirubin; whereas the treatment groups showed a marked reduction in the levels of total bilirubin.

BUN (blood urea nitrogen) level indicates the functioning of the kidney. If the kidneys are not able to remove urea from the blood normally, the BUN level is found to increase. Alloxan induced diabetic animals in the present study showed an increase in the level of BUN in serum; which was significantly reduced in all the treatment groups.

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). In chemical terms, creatinine is a spontaneously formed cyclic derivative of creatine. Creatinine is filtered out of the blood by kidneys and there is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance. In the present study, alloxan-induced diabetic animals showed an increase in the level of creatinine, which was indicative of damage to kidneys. Treatment with 100% pioglitazone, 100% C.auriculata and 25%:75% and 50%:50% combination of pioglitazone and C.auriculata were found to reduce the levels of creatinine significantly.

LDH (Lactate Dehydrogenase) is an enzyme found in almost all organs and tissues in the body. When cells are actively growing or when their membranes are damaged, enzymes leak into the circulation. Elevated levels, therefore, indicate growing/ healing tissues or cellular damage. The increased levels in diabetic control group in the

present study indicated the presence of cellular damage caused by administration of alloxan; which was restored to normal in all the treatment groups.

Overall, the 25%:75% combination of pioglitazone and C.auriculata was found to produce significant effect on diabetes and all the serum indicators of renal, cardiac and hepatic damage as compared to the 50%:50% combination of drugs and also to that of 100% C.auriculata. Also, the effects caused due to treatment with the 25%:75% combination was found to be similar to that of 100% pioglitazone, a standard conventional hypoglycemic drug for diabetes.

CONCLUSION

The present study revealed a prominent positive effect of 25%:75% combination of pioglitazone and *Cassia auriculata* suggesting that a reduction of 75% of the conventional dose of pioglitazone (ie. administration of only 25% dose of pioglitazone) combined with 75% dose of C. auriculata (25% reduction in dose), produced anti-diabetic effects comparable to that of 100% pioglitazone (10 mg/kg) along with lesser liver damage (indicated by decreased levels of SGPT, SGOT, alkaline phosphatase and total bilirubin), heart damage (decreased level of LDH and SGOT) and kidney damage (indicated by decreased levels of serum creatinine and BUN). This study thus suggests the use of combination of pioglitazone and C. auriculata for the effective treatment of diabetes. Clinical feasibility of the beneficial effect of this combination in diabetic patients needs to be confirmed.

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