ORIGINAL ARTICLE

Antidiabetic activity and modulation of antioxidant status by fractions of *Argemone mexicana* in alloxan induced diabetic rats

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Background: *Argemone mexicana* L. (Papaveraceae) commonly known as prickly poppy is an indigenous herb used as a medicinal plant in several countries. **Aim:** The investigation was carried out to study the effects of chloroform and aqueous soluble fractions from hydroalcoholic extract of *Argemone mexicana* in normoglycemic and alloxan induced diabetic rats. It was also intended to establish correlation between the marker antioxidant enzymes and diabetes. **Materials and Methods:** Hyperglycemia was induced in rats by alloxan monohydrate (150mg/kg body weight i.p.). After alloxan induction diabetic rats received chloroform and aqueous fractions orally at 150 mg/kg body weight daily for 21days. The parameters studied were blood glucose, creatinine and urea, serum lipid profile, serum enzymes [serum glutamate pyruvate transaminases and serum glutamate oxaloacetate transaminases, lipid peroxidation, antioxidant enzymes (catalase (CAT), superoxide dismutase and reduced glutathione. The results of test drug were compared with standard hypoglycaemic drug-glibenclamide (5 mg/kg). **Statistical analysis:** All data were expressed as means ± SEM. Dunnet's t-test and one-way ANOVA test was used to compare the mean values of test groups and control. **Results:** . Experimental findings showed that the chloroform and aqueous soluble fractions significantly ($P < 0.01$)normalized blood glucose levels, serum biochemical parameters; decreased LPO and recovered glutathione-S-tranferase (GST) and CAT as compared with those of alloxan controls.. **Conclusion:** From this study it may be concluded that the potential anti-diabetic action of chloroform and aqueous fractions of *A. Mexicana* is plausibly due to its modulation of endogenous antioxidant status

Key words: Anti-diabetic activity, *Argemone mexicana*, alloxan monohydrate

INTRODUCTION

India has one of the oldest, richest and diverse cultural traditions associated with the use of plants and herbs for human, livestock and plant health. Many of the ingredients of Indian cooking, which have been handed down from ages contain medicinal properties. A vastethnobotanical knowledge exists in India from ancient times. However, very few plants used by locals for medicine are subjected to scientific investigation. The need for the conservation of medicinal plants and traditional knowledge, particularly in developing countries like India, taking into account the socio-cultural and economic conditions is urgent.[1] Diabetes is a deadly disease that affects an estimated 135 million people worldwide and the numbers are increasing in rural and poor populations throughout the world. Diabetes mellitus is a non-communicable disease considered to be one of the five leading causes of death worldwide.[2] In the indigenous Indian system of medicine a sizable number of plants was mentioned for the cure of diabetes and some of them have been experimentally evaluated and the active principles isolated.[3] However, search for new anti-diabetic drugs continues. Oxidative stress is the imbalance between the generation of reactive oxygen species (ROS) and the body defence mechanisms. Environmental pollutants, toxic habits (drugs, smoking and/or alcohol), inadequate nutrition, excess solar radiation, large exposure to toxic substances (fertilizers and pesticides), drug metabolism (side effects) and a high physical or psychical stress are the most common exogenous factors originating ROS in the human body.[4] Oxidative stress has also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurologic disorders, as well as in the process of aging.[5]

Argemone mexicana L. (Papaveraceae) commonly known as prickly poppy is an indigenous herb used as a medicinal plant in several countries. In Mexico

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the seeds are considered as an antidote to snake venom. In India the smoke of the seeds are used to relieve toothache. The fresh yellow-milky seed extract contains protein-dissolving substances, effective in the treatment of warts, cold sores, cutaneous infections, skin diseases, itches and also dropsy and jaundice.[6] The plant contains alkaloids as berberine, protopine, sarguinarine, optisine, chelerytherine, etc., Medicinal plants being the effective source of both traditional and modern medicines are genuinely useful for primary health care. Over the years, World Health Organization (WHO) advocated traditional medicines as safe remedies for ailments of both microbial and non-microbial origins.[7] In the USA, some plant-based compounds as well as herbal remedies are used along with other medications. In some cases, patients used these treatments instead of conventional medications, and severe complications including increased hospitalizations, ketoacidosis and acute hyperglycaemia occurred.[8] The present study has been designed to determine the role of chloroform and water soluble fractions of aerial parts of *A.mexicana* for potential anti-diabetic and antioxidant activity, if any, against alloxan-induced hyperglycaemic rats.

MATERIALS AND METHODS

Plant Material

The plant material used in this study was aerial parts of *A.mexicana*, collected from road side area from Kasrawad, district Khargone, in Madhya Pradesh (MP), India, during spring (mid-March to mid-April2012) and was authenticated by the former taxonomist Dr. S. K. Mahajan Taxonomist, Department Botany, Government P. G. College Khargone, MP. The plant materials were initially rinsed with distilled water and dried on paper towel in laboratory at (37 ± 1)°C for 24 h.

Preparation and Fractionation of Crude Extracts

The coarse powder was submerged in ethyl alcohol and water (50:50) and allowed to stand for continuous hot extraction. After extraction the solvents were allowed to evaporate using rotary evaporator at a temperature 40-45°C. Thus the highly concentrated crude hydroalcoholic extract was obtained, which was then fractionated using petroleum ether, chloroform and water. The dried chloroform and aqueous-soluble fractions were then preserved in the refrigerator for the experimental use.

Drugs and Chemicals

All chemicals and solvents used were of analytical grade, from S.D Fine Chemicals Ltd, Mumbai, India, The standard anti-diabetic drug glibenclamide was the generous gift from Cipla Ltd, Pithampur, Indore. Total cholesterol (TC) and triglyceride (TG) wet reagent diagnostic kits were the products of Crescent Diagnostic. Alloxan monohydrate was purchased from Sigma-Aldrich.

Anti-diabetic Activity

Animals

Male albino Wistar rats, weighing 150-200 g and Swiss albino mice weighing 20-25 g were used. Prior to the experiments, the selected animals were housed in acrylic cages in standard environmental conditions (20-25°C) fed with standard rodent diet for 1 week in order to adapt to the laboratory conditions and water *adlibitum.* They were fasted overnight (12 h) before experiments, but were allowed free access to water. Six animals were used for each group of study. All the experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Ethical Committee (IAEC No. 1171/C/08/CPCSEA).

Preparation of the Test Samples

The test fractions were suspended in 25% Tween 20 in distilled water prior to oral administration to the experimental animals. Glibenclamide (5 mg/kg) was used as the reference control. Animals in the control group received only the 25% Tween 20 (2 ml/kg). All the test samples were administered through oral route.

Acute Oral Toxicity Study

An acute oral toxicity study was performed as per organisation for economic co-operation and development (OECD-423) guidelines.[9] Male Swiss albino mice (20-25 g) selected by random sampling were used for acute toxicity study. The animals were fasted overnight and the fractions were administered orally at doses of 100, 400, 800, 1500and 3200 mg/kg body weight. The animals were closely observed for the first 24 h for any toxic symptoms and for 72 h for any mortality.

Study on normal glycaemic animals

The animals were fasted for 18 h but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h) blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. Plasma was separated following centrifugation the glucose was estimated by GOD/POD method using the glucose estimation kit from Sigma Diagnostics (India) Pvt. Ltd., Baroda, India. The normal rats were then divided into four groups of six animals each. Group I served as solvent control and received only vehicle (2 ml/kg) through oral route, Group II received glibenclamide (5 mg/kg) and served as reference control. Groups III and IV received the chloroform and aqueous fractions at a dose of 150 mg/kg, respectively, through oral route. Blood glucose levels were determined

after 1, 2, 4, 6, 8 and 10 h of administration of single dose of test and control samples.

Induction of diabetes

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg).[10] Two days after alloxan injection rats screened for diabetes and those having glycosuria with blood glucose level of 200-400 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Determination of blood glucose levels

Fasting blood glucose (FBG) concentration was determined using a Glucometer (Accu Chek), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time intervals.[11,12]

Treatment schedule and determination of blood glucose levels

The diabetic animals were divided into four groups each containing six animals, and one group of normal non-diabetic animals. Both the fractions of *A.mexicana* were given at a dose of 150 mg/kg, for 21 days, as a suspension in Tween 20 at a dose of 2 ml/kg to different groups of animals.

Group I: Normal animals received Tween 20 at a dose of 2 ml/kg as a suspension in distilled water

Group II: Diabetic animals received Tween 20 at a dose of 2 ml/kg as a suspension in distilled water

Group III: Diabetic animals received glibenclamide at a dose of 5 mg/kg

Group IV: Diabetic animals received chloroform fraction at a dose of 150 mg/kg

Group V: Diabetic animals received aqueous fraction at a dose of 150 mg/kg.

At the end of the experimental period, the animals were fasted overnight for 8 handblood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. Serum was separated by centrifugation at 3000 rpm for about 5 min. The clear straw coloured serum was collected and stored at 4°C for the measurement of marker enzyme levels to assess the liver functions. Blood sugar level was evaluated by GOD/POD method using glucose estimation kit from Sigma Diagnostics (India) Pvt. Ltd. Baroda, India.[13]

Measurement of body weight gain, food and water intake

Body weight gain, food and water intakes were monitored was done weekly on overnight fasted animals during the 21 days experimental period.

Estimation of lipid profile

At the end of 21 days of treatment with the test fractions, the animals were sacrificed by decapitation under ether anaesthesia and blood samples were collected from test, standard and solvent-treated groups including normal animal as reference. The serum supernatant was separated by centrifugation and was subjected for the determination of the lipid profile studies such as TC, TG, High-density lipoprotein, Low-density lipoprotein and Very-low-density lipoprotein[14]

Estimation of serum biochemical parameters

Collected blood was used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), TC and serum TGs.[15,16]

Estimation of liver, kidney and pancreas biochemical parameters

After sacrificing the animals on 21st day the liver, kidney and pancreas tissue from various groups of animals were removed carefully followed by washing thoroughly with ice cold saline, 0.5 g of the wet tissue was weighed and homogenized in 0.1M Tris–HCl buffer, pH 7.4 at 4°C in a Remi homogenizer with a Teflon pestle rotated at 600 rpm for 30 min. The homogenate was centrifuged at 2500 rpm for 10 min at 4°C using refrigerated centrifuge. The supernatant was used for the assay of lipid peroxidation (LPO) products and antioxidant enzymes, such as reduced glutathione (GSH),[17,18] glutathione-S-transferase (GST),[19] superoxide dismutase (SOD),[20] catalase (CAT)[21] and total protein level.[22]

Statistical Analysis

All the results were analysed statistically using one-way analysis of variance followed by Dunnet's *t*-test. A *P* value less than 0.05 are considered significant. All the results are expressed as mean ± SEM for six animals in each group.

RESULTS

Acute Oral Toxicity Study

The chloroform and aqueous fractions of hydroalcoholic extract of *A.mexicana* did not show any mortality and toxic manifestations up to the dose of 3200 mg/kg. Further dosing was not performed to estimate the LD_{50} (lethal dose) value. According to the OECD guidelines for the acute toxicity, an LD_{50} dose of 2000 mg/kg and above is categorized as unclassified and hence the drug is found to be safe. Based on the acute toxicity studies, the dose 150 mg/kg of the fractions has been selected as the therapeutic dose.

Effects of Chloroform and Aqueous Fractions on Single Dose Treated Normoglycemic Animals

The results of chloroform and water soluble fractions on blood sugar level of normoglycaemic rats are depicted in Table 1. The test result indicates that, there is a significant reduction ($P<0.05$) in blood glucose level from 2 h ($P<0.05$)

onwards till the end of 10 hand registered 17.88% and 16.79 reduction at the end of 10 h, in animals treated with 150 mg/kg of the chloroform and aqueous fractions. However, the standard drug glibenclamide on the same day reduces the blood glucose by 21.65% with $P<0.001$, when compared with the solvent control group. The results of the normoglycaemic model showed that the test fractions have hypoglycaemic effect.

Effects of Chloroform and Aqueous Fractions on Fasting Blood Glucose Levels

The results illustrated in Table 2 of the study reveals that, chloroform and aqueous fractions reduces the blood glucose level to an extent of 57.24% and 52.63% at 150 mg/kg body weight, respectively at the end of the 21st day of the study, whereas the standard drug glibenclamide registered 61.87% of reduction on the same day of the study. However, the individual data shows a statistical significance ranges between $P<0.05$ and $P<0.001$, throughout the experiment when compared with solvent control and analysis of variance registered $P<0.01$ level of significance.

Effect on Body Weight, Food and Water Intake

Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 21 days [Table 3] Alloxan caused body weight reduction, which was significantly reversed in both test fractions after 7 days of treatment. Tables 4 and 5 illustrate that diabetic control rats showed significantly higher intake of food and water when compared with normal control groups ($P<0.001$). The food and water intake significantly ($P<0.05$) decreased in diabetic rats treated with both test fractions and glibenclamide.

Effects of Chloroform and Aqueous Fractions on Serum Lipid Profile

Table 6 illustrates the levels of serum lipid profile such as TC, total TGs, HDL, LDL and VLDL on 21st day of the study. The diabetic rats showed significantly ($P<0.001$) increase level of all tested lipid profiles except HDL, which showed a significantly decreased value ($P<0.05$). Both the test fractions showed significant ($P<0.05$ to $P<0.001$) reduction in total

Table 1: Effect of aqueous and chloroform fractions of *Argemone mexicana* on blood glucose single dose treated on normoglycaemic rats in oral route

Groups and treatment	Blood glucose level (mg/dl)							
	0 h	1 h	2 h	4 h	6 h	8 h	10 h	% decreases at 10th h
Solvent control (tween+water)	96.5±4.80	95.66±4.66	94.5±4.77	99.16±4.04	98.83±4.15	99.83±5.60	101.16±4.90	
Glibenclamide (5 mg/kg)	91.43±1.31	81.22±2.63	67.53±2.34*	58.12±2.61**	54.72±2.44**	73.83±1.42**	71.63±2.81**	21.65
Chloroform fraction (150 mg/kg)	91.18±0.93	87.19±0.78	85.71±2.61	84.23±1.37	82.83±2.38*	80.11±1.21**	74.87±2.73**	17.88
Aq. fraction(150 mg/kg)	92.34±1.23	89.23±2.32	88.01±1.21*	87.76±1.42**	85.21±1.89**	83.65±1.67***	76.83±2.07**	16.79

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *$P<0.05$,** $P<0.01$, respectively, in comparison to group-I)

Table 2: Effect of aqueous and chloroform fractions of *Argemone mexicana* on blood glucose level in alloxan induced diabetic rats

Groups	Blood glucose level (mg/dl)					
	0th day	5th day	10th day	15th day	21st day	% decrease at 21 day (%)
Solvent control (tween+water)	82.40±1.63	83.04±2.30	82.20±1.96	81.00±1.30	81.00±1.39	-
Diabetic control	264.0±6.48	292.22±7.73	308.00±6.11	314.20±3.32	325.20±3.51	-
Glibenclamide (5 mg/kg)	250.23±7.50	203.24±7.43**	149.00±6.56**	129.02±7.22**	95.33±5.44**	(61.87)
Chloroform fraction (150 mg/kg)	281.11±3.60	230.10±9.21*	205.33±7.24*	188.00±5.68**	120.20±6.65**	(57.24)
Aq. fraction (150 mg/kg)	291.33±4.40	242.33±5.36*	212.00±4.35**	188.66±4.40**	138.00±4.97**	(52.63)

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *$P<0.05$, **$P<0.01$, respectively, in comparison to diabetic control group)

Table 3: Effect of aqueous and chloroform fractions of *Argemone mexicana* on body weight on treated alloxan induced diabetic rats

Groups	Body weight(g)		
	Initial	Final	% Increase/decrease in body weight
Solvent control (tween+water)	159.16±6.77	161.83±9.58	-
Diabetic control	152.60±2.49	122.80±2.22	-
Glibenclamide (5 mg/kg)	160.00±6.47	168.10±1.81**	4.81
Aq. fraction (150 mg/kg)	158.83±8.39	162.00±1.18**	1.95
Chloroform fraction (150 mg/kg)	152.83±8.34	159.20±1.2**	4.00

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *$P<0.05$, **$P<0.01$, respectively, in comparison to diabetic control group)

TGs, LDL and VLDL, however, a decrease in the levels of TC was also recorded, when compared with the diabetic control group, while the HDL levels were approaching almost normal values when compared with the without-treatment normal-control group.

Effects of Chloroform and Aqueous Fractions on Serum Biochemical Parameters

Table 7 illustrate that biochemical parameters like SGOT, SGPT and total creatinine in the diabetic control group were significantly (P<0.001) elevated as compared with the normal control group. Treatment with both test fractions significantly (P<0.05 to P<0.001) brought their levels towards normal values.

Effects of Chloroform and Aqueous Fractions on Liver, Kidney and Pancreas Biochemical Parameters

The levels of SOD, CAT, glutathione-S-transferase (GST), GSH, LPO level and total protein level [Tables 8-13] were significantly (P<0.05) reduced in alloxan-induced rats. The level of SOD, CAT, GSH, GT, total protein and LPO level was significantly (P<0.05 to P<0.001) elevated, approaching normal levels upon administration of both test fractions as compared with diabetic control group.

Table 4: Effect of aqueous and chloroform fractions of *Argemone mexicana* on food intake on treated alloxan induced diabetic rats

Groups and treatments	Food intake habit (g/rat/week)			
	Initial	1st week	2nd week	3rd week
Normal control (tween+water)	27.12±0.087	29.23±0.92	27.22±1.01	28.21±0.096
Diabetic control	26.66±0.76	34.33±1.02	38.44±1.1	41.50±0.96
Glibenclamide (5 mg/kg)	27.5±0.76	21.167±0.87**	18.11±.97	13.667±0.88**
Chloroform fraction (150 mg/kg)	25.667±0.88	20.33±1.23**	17.99±1.17	18.167±0.98**
Aq. fraction (150 mg/kg body wt)	25.83±0.70	21.667±0.71**	19.886±.089	19.667±1.02**

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *P<0.05, **P<0.01, respectively, in comparison to diabetic control group)

Table 5: Effect of aqueous and chloroform fractions of *Argemone mexicana* on water intake on treated alloxan induced diabetic rats

Groups and treatments	Water intake habit (ml/week/rat)			
	Initial	1st week	2nd week	3rd week
Normal control (tween+water)	78.99±0.85	76.43±0.84	79.63±0.89	77.45±0.92
Diabetic Control	76.83±0.60	81.85±0.82	89.33±1.17	100.17±1.66
Glibenclamide (5 mg/kg)	79.33±0.95	65.21±0.98	59.16±1.10**	39.83±1.30**
Chloroform fraction (150 mg/kg)	76.167±1.43	72.23±0.97	58.83±1.89**	43.167±2.44**
Aq. fraction (150 mg/kg)	75.83±1.25	70.03±0.79	62.16±0.87**	53.33±0.99**

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *P<0.05, **P<0.01, respectively, in comparison to diabetic control group)

Table 6: Effect of aqueous and chloroform fraction of *Argemone mexicana* on some serum lipid profile on treated alloxan induced diabetic rats

Groups	TG (mg/dl)	TC (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Solvent control (tween+water)	90.22±2.08	43.10±2.64	9.46±3.10	11.53±0.30	22.00±0.57
Diabetic control	231.33±1.85	77.19±1.20	36.19±3.60	30.87±0.58	8.33±0.88
Glibenclamide (5 mg/kg)	135.02±3.71**	51.55±6.50**	14.42±3.74**	18.73±1.60**	19.05±0.57**
Chloroform fraction (150 mg/kg)	141.45±4.40**	57.03±2.08*	22.73±1.61*	20.93±0.92*	16.33±0.33**
Aq. fraction (150 mg/kg)	145±6.11**	61.20±0.750*	25.08±2.39*	22.75±1.38*	14.33±0.88*

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *P<0.05, **P<0.01, respectively, in comparison to diabetic control group). TG-Triglyceride; TC-Total cholesterol; LDL-Low-density lipoprotein; VLDL-Very Low-density lipoprotein; HDL-High-density lipoprotein

Table 7: Effect of aqueous and chloroform fraction of *Argemone mexicana* on some serum biochemical parameters on treated alloxan induced diabetic rats

Groups	SGOT (IU/dl)	SGPT (IU/dl)	Creatinine (mg/dl)
Solvent control (tween+water)	169.66±17.14	123.17±1.91	1.64±0.04
Diabetic control	297.46±62.17	208.3±37. 83	1.85±0.01
Glibenclamide (5 mg/kg)	180.33±12.21**	121.43±9.59**	1.69±0.009**
Chloroform fraction (150 mg/kg)	183.60±12.01*	112.58±6.41**	1.74±0.02**
Aq. fraction (150 mg/kg)	189.93±14.03*	120.4±5.98**	1.71±0.01**

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *P<0.05, **P<0.01, respectively, in comparison to diabetic control group). SGPT –Serum glutamate pyruvate transaminases; SGOT –Serum glutamate oxaloacetate transaminases

Table 8: Effect of aqueous and chloroform fraction of *Argemone mexicana* on superoxide dismutase (U/ml) level in alloxan induced diabetic rats

Group	Liver	Kidney	Pancreas
Solvent control (tween+water)	306.00±3.94	145.19±1.97	136.17±1.99
Diabetic control	114.99±3.146	78.25±2.38	60.65±1.815
Glibenclamide (5 mg/kg)	289.31±2.11**	139.53±1.67**	129.83±1.62**
Chloroform fraction (150 mg/kg)	224.16±7.15**	134.29±4.13**	116.92±0.64**
Aq. fraction (150 mg/kg)	160.21±8.63**	105.54±1.68*	87.43±0.367**

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *P<0.05, **P<0.01, respectively, in comparison to diabetic control group)

Table 9: Effect of aqueous and chloroform fraction of *Argemone mexicana* on catalase (U/ml) level in alloxan induced diabetic rats

Group	Liver	Kidney	Pancreas
Normal control (tween+water)	52.83±0.23	56.79±0.22	31.78±0.32
Diabetic control	22.57±1.21	26.60±0.31	15.34±0.29
Glibenclamide (5 mg/kg)	49.78±0.89**	52.86±0.42**	29.31±0.43**
Chloroform fraction (150 mg/kg)	45.83±0.30**	49.08±0.50**	26.26±0.09**
Aq. fraction (150 mg/kg)	30.95±0.62**	39.25±0.29**	22.36±0.46**

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *P<0.05, **P<0.01, respectively, in comparison to diabetic control group)

Table 10: Effect of aqueous and chloroform fraction of *Argemone mexicana* on reduced glutathione (μM/ml) level in alloxan induced diabetic rats

Group	Liver	Kidney	Pancreas
Normal control	638.93±8.21	486.83±18.75	424.25±19.25
Normal diabetic control	260.55±21.52	214.40±6.38	79.19±5.98
Glibenclamide (5 mg/kg)	560.45±9.21**	453.76±5.97**	395.257.43**
Chloroform fraction (150 mg/kg)	522.61±11.66**	436.76±13.76**	348.83±8.31**
Aq. fraction (150 mg/kg)	409.20±8.03**	301.78±18.88**	160.14±17.14**

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *P<0.05, **P<0.01, respectively, in comparison to diabetic control group)

Table 11: Effect of aqueous and chloroform fraction of *Argemone mexicana* onglutathione-S-transferase (μM/ml) level in alloxan induced diabetic rats

Group	Liver	Kidney	Pancreas
Normal control	63.21±2.74	53.01±2.64	34.05±2.59
Normal diabetic control	36.06±2.38	22.71±2.67	14.83±1.89
Glibenclamide (5 mg/kg)	57.38±1.07**	48.06±1.46**	29.27±1.95**
Chloroform fraction (150 mg/kg)	55.20±0.51**	43.58±1.65**	24.13±1.45**
Aq. fraction (150 mg/kg)	46.95±0.56**	31.59±1.95*	20.11±0.85*

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *P<0.05, **P<0.01 respectively, in comparison to diabetic control group)

Table 12: Effect of aqueous and chloroform fraction of *Argemone mexicana* on total protein level (mg/ml) in alloxan induced diabetic rats

Groups	Liver	Kidney	Pancreas
Normal control	60.27±4.78	50.35±1.43	33.85±1.13
Normal diabetic control	33.05±0.94	16.21±1.64	10.46±0.35
Glibenclamide (5 mg/kg)	54.85±1.34	41.75±1.06	27.55±0.79
Chloroform fraction (150 mg/kg)	51.37±1.66**	36.41±2.84**	21.97±0.36**
Aq. fraction (150 mg/kg)	42.21±0.73*	21.63±0.68*	17.94±0.45**

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *P<0.05, **P<0.01 respectively, in comparison to diabetic control group)

DISCUSSION

Diabetes mellitus affects glucose and lipid metabolism, the increased blood sugar levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from β-cells manifested in the decreased serum insulin levels.

Table 13: Effect of aqueous and chloroform fraction of *Argemone mexicana* on lipid peroxidation level (nanomole/mgprotein) in alloxan induced diabetic rats

Groups	Liver
Normal control	36.41±3.21
Normal diabetic control	185.39±7.05
Glibenclamide (5 mg/kg)	57.73±1.65
Chloroform fraction (150 mg/kg)	80.31±0.74**
Aq. fraction (150 mg/kg)	130.61±3.17*

Values are expressed in mean±SEM of six animals. One way ANOVA followed by Dunnet's *t*-test (*t*-value denotes statistical significance at *P<0.05, **P<0.01 respectively, in comparison to diabetic control group)

The fundamental mechanism underlying hyperglycaemia in diabetes mellitus involves the overproduction of glucose by excessive hepatic glycogenolysis and gluconeogenesis, decreased hepatic glycogenesis and, or decreased utilization of glucose by the tissue.[23]

In our earlier work, we have reported hypoglycaemic and anti-diabetic activity of ethanolic and aqueous extracts of *A.mexicana* whole plants in normoglycaemic and alloxan-induced hyperglycaemic rats by single-dose and multi-dose treatment.[24] In continuation of our earlier work, the present study aims to find out the potent anti-hyperglycaemic fraction from hydroalcoholic extract of *A.mexicana* towards a mechanistic hypoglycaemic and antioxidant potential upon 21 days of study.

The results of the study revealed that chloroform fraction in comparison with water-soluble fraction at doses of 150 mg/kg significantly normalized elevated blood glucose level, body weight and restored serum and liver biochemical parameters towards normal values.

The results of the investigations revealed that treatment with chloroform and water soluble fractions produced hypoglycaemia in normoglycaemic (euglycaemic) rats. This suggests that single dose of test fractions have hypoglycaemic effect and it is in comparison with the standard hypoglycaemic agent glibenclamide. In concordance with this hypoglycaemia was observed up to 10th hour after the administration of the test fractions in glucose-loaded normal rats.

Alloxan a beta-cytotoxin induces "chemical diabetes" by pancreatic cell damage mediated through generation of cytotoxic oxygen-free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation.[25] This damages a large number of β-cells, resulting in decrease in endogenous insulin release which leads to decreased utilization of glucose by the tissue.[26] The results depicted in this study revealed that the sub-acute anti-diabetic, hypoglycaemic effects of chloroform fraction were similar to those of glibenclamide. The possible mechanism, by which the plant extract mediates its anti-diabetic action, is potentiation of pancreatic secretion of insulin from existing residual β-cell of islets and due to enhanced utilization of blood glucose by peripheral tissues as well.

Hyperglycaemia was observed after 2 days of alloxan induction. Treatment with chloroform and aqueous fractions of *A. mexicana* in alloxan-induced diabetic rats started reducing fasting blood glucose levels 5 days and made them normoglycaemic after 21 days. The anti-hyperglycaemic effect of chloroform and aqueous fractions of *A. mexicana* at a dose of 150 mg/kg was found to be comparable with the effect exerted by the reference drug glibenclamide at a dose of 5 mg/kg.

Alloxan-induced diabetes is characterized by severe loss in body weight. This may be due to increased muscle wasting and due to loss of tissue proteins. In this study a significant weight loss was observed in the alloxan induced diabetic control rats. The chloroform and aqueous fractions-treated rats showed significant recovery in body weight gain when compared with diabetic control rats. This may be due to controlling muscle wasting and improvement in insulin secretion as well as glycaemic control by the chloroform and aqueous fractions. The other parameters like food intake and water intake affected due to hyperglycaemia was significantly reversed to normal by drug treatment. Elevation of serum biomarker enzymes such as SGOT, SGPT was observed in diabetic rats indicating impaired liver function, which is obviously due to hepatocellular necrosis. The decreased total protein content in alloxan-induced animals also substantiated the hepatic damage by alloxan. Diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activities.[27] The 21-day treatment with both fractions restored all the above mentioned hepatic biochemical parameters towards the normal levels. Hypercholesterolemia and hypertriglyceridemia have been induced in alloxan-induced diabetic rats.[28] It is well known that in uncontrolled diabetes mellitus, there is an increase in TC in blood which may contribute to coronary artery diseases.[29] In the present study, the elevated serum TC and TG levels in diabetic rats were brought down by both fractions treatment.

Alloxan induced diabetic rats showed significantly increased serum lipid profiles except HDL, when compared with normal rats. The glibenclamide, chloroform and aqueous fractions of *A. mexicana* treated rats showed a significant decrease in the content of lipid profiles, when compared with diabetic induced rats. Similarly HDL level decreased in alloxan induced diabetic rats when compared with normal rats. On administration of chloroform and aqueous fractions of *A. mexicana* and glibenclamide to the diabetic rats, HDL level was found to be restored to normal. The level of serum lipid profiles are usually raised in diabetic rats in the present study and such elevation represents a risk factor for

coronary heart diseases.[30] Lowering the serum lipid level through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease.[31] Glutathione plays an important role in the endogenous non-enzymatic antioxidant system. It primarily acts as a reducing agent and detoxifies hydrogen peroxide in the presence of the enzyme glutathione peroxidase.[32] The depleted GSH may be due to reduction in GSH synthesis or degradation of GSH by oxidative stress in alloxan-induced hyperglycaemic animals.[33] Chloroform and aqueous fractions treatment significantly elevated the liver, kidney and pancreas GSH levels towards normal in diabetic rats. The results showed that the anti-hyperglycaemic activity of test fractions was accompanied by enhancement in non-enzymatic antioxidant protection. Glutathione-S-tranferase (GST) compress the multigene family of proteins with destings cell locations. Earlier researcher reported that the decrease of GST activity was found in hepatic tissue under various pathological conditions. In the present study the diabetes induction might have been caused oxidative damage in liver, kidney and pancreas by elevating peroxidation of membrane lipid. It is evidenced that the diabetes induced alterations in the antioxidant system by inhibiting key enzyme like GST activity. However, on treating with both test fractions the rate of decreases GST activity was minimized, which suggest that the diabetes-induces alterations in the antioxidation system could have been prevented to some extent.

Enzymatic antioxidant such as SOD and CAT are considered primary enzymes since they are involved in the direct elimination of ROS.[34] SOD is one of the most important enzymes and scavenges O^{2-} anion (which is the first product of O_2 radicals) to form H_2O_2 in the enzymatic antioxidant defence system and hence abolishes the toxic effects due to this radical or other free radicals derived from secondary reactions.[35] The O^{2-} anion is reported to inactivate CAT and GSH-Px.[36] CAT has been recognized as a major determinant of hepatic and cardiac antioxidant status[37] and is known to be involved in detoxification of H_2O_2 concentrations.[38] In diabetes, the alloxan-generated ROS causes non-enzymatic glycosylation and oxidation resulting in the inactivation and inhibition of antioxidant enzymes such as SOD and CAT. In the present study, it was observed that long-term treatment with the test fractions had reverse the activities of these enzymatic antioxidants (SOD, CAT) in liver, kidney and pancreas by significantly increasing the activity of such enzymes.

CONCLUSION

In the present study, the administration of chloroform and aqueous fractions of hydroalcoholic extract of *A.mexicana* to alloxan induced hyperglycaemic rat's demonstrated prominent reduction in blood sugar level, normalization of serum biochemical profile including lipid contents, as compared with alloxan control rats. Also with both test fractions treatment resulted in significant modulation of endogenous non-enzymatic (GSH) and enzymatic (CAT) antioxidant and detoxification status. Therefore, it can be concluded that chloroform and aqueous fractions of hydroalcoholic extract of *A.mexicana* is remarkably effective against alloxan-induced diabetes in Wistar rats plausibly by virtue of its augmenting the endogenous antioxidant mechanisms.

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