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Research Article

HYPOGLYCAEMIC ACTIVITY OF AERIAL PARTS OF *ARGEMONE MEXICANA* L. IN EXPERIMENTAL RAT MODELS

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ABSTRACT

Experimental Design, Materials and Methods: The aim of the present study was focus on, to the evaluation of hypoglycaemic activity of hydro-alcoholic extract of aerial parts of *Argemone mexicana* L. (HAAM). The study was carried out on normal, glucose loaded, and streptozotocin (STZ) induced hyperglycaemic rats, using standard experimental procedures at dose levels of 200 and 400 mg/kg by, p.o, by taking Metformin (300 mg/kg) as standard drug.

Statistical Analysis: The data are analyzed by one way ANOVA followed by Turkey – Kramer Multiple Comparison test, $p < 0.05$ considered as significant.

Results: In oral glucose loaded rat model (normal rats and hyperglycemic rats) and in single/multiple (14-days) dose rat models (hyperglycemic rats) HAAM at both the dose levels (200 mg/kg and 400 mg/kg, p.o.) reduces the fasting blood glucose level significantly ($p < 0.001$) in a dose dependent manner, when compared to control group of animals. The effect of the extract at higher dose (400 mg/kg) is comparable with the standard drug metformin (300 mg/kg), in most of the tested animal models.

Conclusion: The hydro-alcoholic extract of *Argemone mexicana* stem is endowed with hypoglycemic potential and the test dose of 400 mg/kg for 14-days is most parallel with that of the effect of standard drug metformin.

Keywords: *Argemone mexicana*, Hypoglycaemia, Streptozotocin, Hyperglycemia, Metformin

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by the classical symptom of hyperglycemia, which in turn leads to various acute and chronic complications if left untreated and may cause massive damage to the renal, cardiovascular, retinal and neurologic systems. It occurs as a result of a relative or an absolute lack of insulin, or its action on the target tissue, or both [1, 2]. Despite the great strides made in understanding and management of diabetes, the incidence of diabetes mellitus is on rise all over the world, especially in Asia and Africa, and is likely to rise to up to 300 million or more by the year 2025 [3, 4]. Many synthetic hypoglycemic agents are currently available but they are either too expensive or produce undesirable side effects on chronic use [5]. Traditionally, many indigenous plants have been used successfully for the management of the disease throughout the world; some of them have been evaluated experimentally and their active ingredients have been isolated [6, 7].

However, a number of such potential plants have remained unexplored.

Argemone mexicana (Linn.) belongs to Family *Papaveraceae* and is commonly known as Mexican poppy or Prickly Poppy. The plant is pantropic in distribution and it is a weed in waste places. It is native to America and naturalized throughout India [8]. The whole plant, roots, leaves, stem, flowers are extensively used in traditional system of medicine for various ailments like leprosy, malaria, jaundice, rheumatism, pain, inflammation, skin diseases, fever, piles, warts, dysentery, tumors and worm infestations [9, 10, 11]. The plant is known to possess antimalarial [12], antimicrobial [13], antibacterial [14] and antifungal [15] activities. In Mexico infusion of aerial part of the plant is used as hypoglycemic [16]. Chemical investigations of this plant have revealed the presence of alkaloids [17, 18], amino acids [19], phenolics [20] and fatty acids [21]. The aerial part of the plant contains isoquinoline and Benzylisoquinoline alkaloids [22]. Alkaloids like berberine & tetrahydroberberine, protopine, Benzophenanthridines has been isolated from the plant [23].

The present study has been designed to determine the effectiveness of extracts of aerial parts of *A. mexicana* for potential hypoglycaemic activity, if any, against normoglycemic and streptozotocin induced hyperglycemic rats.

MATERIAL AND METHODS

Plant material

The aerial parts of *A. mexicana* L. were collected in the month of April – May 2009, from the rural area of the dist. Cuttack, Odisha, India, India and authenticated by the Taxonomist of the Botanical Survey of India, Howrah, India.

Preparation of extract

The aerial parts were dried under shade condition and made into coarse powder using a mechanical grinder. The powdered material was initially defatted with petroleum ether followed by 72-hours extraction with 1: 1 mixture of methanol and water using cold maceration process for 72-hours. The extract was filtered and concentrated by rotary evaporator and kept in vacuum desiccators until use. The yield of the extract was 18.68% w/w with respect to dried powder.

Animals

Healthy Wistar albino rats (150 – 250 gm body weight) supplied by Central Animal House of School Of Pharmaceutical Sciences, SOA University, Bhubaneswar, India were used in the experiments. Animal care and handling was done as per the guidelines set by the Indian National Science Academy New Delhi, India. The animals were housed in polypropylene cages inside a well-ventilated room. They were maintained under standard laboratory conditions of temperature 24-28°C, relative humidity 60-70% and 12 hour light/dark cycle. They were fed a standard commercial pellet diet and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week before commencement of experiment. The study was approved by the Institutional Animal Ethics Committee and the

experiments were performed based on animal ethics guidelines of the University Animals Ethics Committee.

Preparation of Interventions

HAAM (at dose levels of 200 and 400 mg/kg) was suspended in distilled water using 25% Tween 20 as suspending agent. The standard drug metformin (300mg/kg) was also prepared in a similar manner. The test and standard drugs were administered by oral route based on dose and corresponding weight of the animals.

Induction of Hyperglycaemia

Hyperglycaemia was induced by intraperitoneally administered multiple low doses of Streptozotocin (STZ) 40 mg/kg/day for five consecutive days. A freshly prepared solution of STZ (40 mg/kg) in ice- cold citrate buffer (0.01 M, pH 4.5) was injected intraperitoneally to the overnight fasted rats [24]. After 12days of STZ administration, the fasting blood glucose levels were measured and the rats showing fasting blood glucose level >250 mg/dL were considered to be diabetic and were used in the study.

Blood Glucose Level and Biochemical Parameters Measurement

Drop of blood was collected from the tip of the tail vein of each rat and glucose level was estimated by using a Glucose Oxidase – Peroxidase reactive strip and Glucometer (One Touch Horizon, Lifescan, Johnson and Johnson Company). On terminating the dosing, the rats were fasted for 12 h, sacrificed by decapitation blood samples were collected by standard method for estimation of serum urea, creatinine, triglycerides and cholesterol by using commercially available diagnostic kit.

EXPERIMENTAL DESIGN

Acute Toxicity Study

Healthy Wistar albino rats of either sex starved overnight, were divided into five groups (n=4). Group I-IV animals were orally fed HAEAM in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg. b.w., while group V (untreated) served as control. The animals were observed continuously for the first 2 h for any gross change in behavioral, neurologic and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again at 24 h , 48 h and 72 h for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity were selected for the experiment [25].

Oral Glucose Tolerance Test

Normal rats

Fasted rats were divided into four groups of six animals each. Group I served as control and solvent. Group II and III received HAAM extract at an oral dose of 200 and 400 mg/kg respectively. Group IV received the standard drug Metformin 300 mg/kg orally. All the groups were loaded with glucose (2 g/kg/p.o) 30 minutes after drug administration [26]. Blood samples were collected from the tail vein just prior to drug administration and at 30, 60, 120 & 180 minutes after glucose loading. Serum glucose levels were measured immediately.

STZ-Induced hyperglycemic rats

The hypoglycemic effect of extract was also measured through oral glucose tolerance test (OGTT) in streptozotocin-induced diabetic rats. The apportioning of animals was similar to OGTT in Normal Rats. The rats were fasted for 18 h and the test performed by oral administration of glucose (5 g/kg) to diabetic rats [27]. Solvent, the extracts (200 and 400 mg/kg) and metformin (300 mg/kg) were given 1 h prior to the glucose challenge and blood samples collected at 0, 30, 60, 120 and 180 minutes following the challenge.

Single Oral Dose Hypoglycaemic Study [25, 27, 28]

Normoglycaemic rats

The effect of single dose administration of HAAM extract on fasting blood glucose was studied in normal rats. Animals were divided into four groups of six rats each. Each group received single dose of either solvent, HAAM extract (200 mg and 400 mg/ kg) or Metformin 300 mg/kg. The blood was collected at 0, 1, 2, 4, 6 and 12 hrs after drug administration, to estimate blood sugar.

STZ-Induced hyperglycaemic rats

The effect of single dose administration of HAAM extract on fasting blood glucose was studied in STZ-induced diabetic rats. Animals were divided into four groups of six rats each. Each group received single dose of either solvent, HAAM extract (200 mg and 400 mg/ kg) or Metformin 300 mg/kg. The blood was collected at 0, 1, 2, 4, 6, and 12 hrs after drug administration, to estimate blood sugar.

Multiple Oral Doses Hypoglycaemic Study [27, 28]

Normoglycaemic rats

The normal healthy rats were divided into four groups of six animals each and received the intervention for 9 days. Each group received either solvent, HAAM extract (200 mg and 400 mg/ kg) or Metformin 300 mg/kg everyday 30 minutes before food. Fasting blood sample was collected on day 0, 1, 3, 6 and 9 for estimating blood sugar. On day 10 blood sample has been collected for estimation of Biochemical parameters as well. During the 10 days of observation of the rats were observed for any changes in the body weight relative to day 0, i.e. before the start of the treatment.

STZ-Induced hyperglycemic rats

The STZ-Induced diabetic rats were divided into four groups of six animals each and received the intervention for 14 consecutive days. Each group received either solvent, HAAM extract (200 mg and 400 mg/ kg) or Metformin 300 mg/kg everyday 30 minutes before food. Fasting blood sample was collected on day 0, 1, 3, 6, 9 and 14 for estimating blood sugar. On day 15 blood sample has been collected for estimation of Biochemical parameters as well. During the 15 days of observation of the rats were observed for any changes in the body weight (Day 7 and Day 14) relative to day 0, i.e. before the start of the treatment. Food and water intake was monitored daily for each rat during 15 days of experimental period (Day 0, Week 1 and Week 2).

STATISTICAL ANALYSIS

Results are expressed as Mean ± SE. The data was analyzed by one way ANOVA followed by Turkey – Kramer Multiple Comparison Test. Confidence Interval has been considered as 95% and $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of Single Dose Administration of HAAM

Effect of a single dose of HAAM on blood glucose levels of normoglycaemic rats

The results for the normoglycaemic rats are cited in Table-1, revealed that the test extract at the tested dose levels progressively decreases the blood glucose level with maximum fall (14.12% and 8.47% at 400 and 200 mg/kg dose level respectively) at the end of 12 h of the experiment with statistical significance of $p < 0.001$ & $p < 0.05$ with 400 mg/kg & 200 mg/kg dose respectively. However the standard drug metformin at the end of 12th h showed a significant ($p<0.001$) decrease of blood sugar level, measured about 25% fall, when compared with solvent control group. Since there is a remarkable % fall of blood sugar level in both the dose levels of the test extract, hence it can be suggested that, the test extract is having hypoglycemic property, and also shows a dose proportionate effect. So, accordingly the further studies have been undertaken.

Table 1: Effect of single dose haam on blood glucose in normoglycaemic rats

Treatment ↓	Fasting Blood Glucose (mg/dL)						% Change From 0Hr
	0hr	1 hr	2 hrs	4 hrs	6 hrs	12 hrs	
Normal Control	96.5±2.09	96.66±1.56	96.50±1.57	97.83±1.42	96.6±2.09	98.33±1.52	---
HAAM 200mg/kg	96.5±1.73	96.6±2.09	94.667±1.76	92.66±1.67	90.16±1.99	88.33±2.16*	8.47
HAAM 400mg/kg	96.83±3.72	95.167±4.52	91.50±4.39	84.83±3.11*	83.66±3.35*	83.16±2.02***	14.12
Metformin 300mg/kg	96.16±3.57	90.5±3.16	78.50±2.49***	75.66±3.59***	72.66±3.30***	71.83±2.73***	25.30

Values are expressed as Mean ± SEM; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test;

*p<0.05, **p<0.01, ***p<0.00 *vs.* Control Group

Effect of a single dose of HAAM on blood glucose levels of STZ Induced hyperglycaemic rats

The results of the study are depicted in Table-2, showed that the test extract at both the tested dose levels exhibited a change in plasma glucose level significantly (p < 0.001) at 2 h post-dosing and did so throughout the duration of the testing afterwards, registering 28.70 and 31.42% fall of blood sugar at the end of 12 h corresponding to 200 and 400 mg/kg dose levels. The standard drug metformin at the same time showed a progressive fall of blood sugar level till the end of the experiment and to a significant extent (p<0.001) and with a quantitative fall of 43.05% of blood sugar at 12 h. The observed effect of the test in hyperglycaemic rats is greater than that observed in normoglycaemic rats. The difference in % decrease of blood sugar in both the dose levels at the end of the 12 h, is approximately three, and is not insignificant, which suggests that the extract may have antidiabetic activity. The antidiabetic activity of the plant extract may be due to the pancreatic or extra pancreatic effect of the extract.

Table 2: Effect of single dose haam on blood glucose in stz induced hyperglycaemic rats

Treatment ↓	Fasting Blood Glucose (mg/dL)						% Change From 0Hr
	0hr	1hr	2hr	4hr	6hr	12hr	
Diabetic Control	361.83±2.60	359.66±5.93	350.83±3.4	345.33±2.60	333.33 ± 2.72	328.66 ± 2.69	---
HAAM 200mg/kg	361.16±3.40	352.16±5.53	308.66±3.75***	265.50±4.45***	260.83±4.51***	257.50±4.83 ***	28.70
HAAM 400mg/kg	365.50±4.85	341.83±4.99	303.66±4.44***	276.00±5.87***	264.50±2.89***	250.66±4.24***	31.42
Metformin 300mg/kg	369.66±4.74	290.83±4.82***	259.83±3.04 ***	236.83±2.97***	214.16±4.11***	210.50±3.74***	43.05

Values are expressed as Mean ± SEM. (n = 6) One Way ANOVA followed by Turkey – Kramer Multiple Comparison test

*p<0.05, **p<0.01, ***p<0.00 *vs.* Control Group

Effect of HAAM on Oral Glucose Tolerance Test

In Normal Rats

Effects of the extract on glucose loaded normal rats are shown in Table - 3. The maximum blood glucose level is observed at 30 minutes after glucose loading in all the groups. It is observed that the treatment with the test extract at both the dose levels reduced blood sugar level significantly (p<0.001) at 30, 60, 120 and 180 minutes when compared to solvent control rats, while the standard drug metformin showed decrease in blood glucose levels in a similar manner. The glycaemia showed a maximum rate in the solvent control group. The estimated difference between the values after 30 minutes of glucose loading, suggests that the test extract has oral glucose tolerance effect on normoglycaemic rats or it resists the intestinal absorption of glucose when pretreated. At the end of the experiment (180 minutes), the percentage reduction from the highest value observed is 24. 37%, 28.35% & 29.82% in case of pretreatment with HAAM 200 mg/kg, HAAM 400 mg/kg & metformin 300 mg/kg respectively.

Table 3: Oral glucose tolerance test in normal rats

Treatment↓	Fasting Blood Glucose (mg/dL)						% change from Highest value
	-30 Mnts	Base Line	30 Mnts	60 Mnts	120 Mnts	180 Mnts	
Normal Control	94.83±1.58	96.67±2.12	178.83±1.74	175.17±2.43	171.83±3.32	167.33 ± 3.28	---
HAAM 200 mg/kg	97.67±2.278	96.17±2.09	131.33±3.06***	125.17±2.14***	110.67±3.20***	99.33±2.04***	24.37
HAAM 400 mg/kg	98.83±1.82	96.67±2.04	128.17±4.09***	123.50±4.04***	103.83±3.65***	91.833±3.53***	28.35
Metformin 300mg/kg	102.33±1.73	96.33±2.19	115.17±2.10***	95.50±4.47***	83.50±3.48***	80.83±2.73***	29.82

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test;

*p<0.05, **p<0.01, ***p<0.00 *vs.* Control Group

In STZ induced Hyperglycaemic Rats

The oral glucose tolerance effect of the extract in STZ induced hyperglycemic rats is depicted in Table 4. After 30 minutes of bolus oral administration of glucose (5 g/kg) the blood glucose concentration reaches the maximum level in all the groups and then starts falling down. The trend of change in blood glucose concentration shows similar trend like exposure of normal rats to oral glucose administration. At dose level of 400 mg/kg the blood glucose level drops significantly (p < 0.01) after 30 minutes of oral glucose administration and becomes highly significant (p < 0.001) starting from the 2nd hour.

In case of 200 mg/kg dose the significant decrease starts from the 2nd hour only (p < 0.001) and does so till end of the experiment with no significant difference from 400 mg/kg dose at the end. Metformin reduces blood glucose level starting from 30 minutes of glucose administration (p < 0.001) and does so at each testing interval.

Table 4: Oral glucose tolerance test in stz induced hyperglycaemic rats

Treatment↓	Fasting Blood Glucose (mg/dL)						% change from Highest value
	-1Hrs	Base Line	30 Mnts	60 Mnts	120 Mnts	180 Mnts	
Diabetic Control	308.67±16.78	306.5±16.80	414.83±21.09	409.17±20.84	398.17±18.95	369.67±19.58	---
HAAM 200 mg/kg	306.17±15.29	292.17±12.86	372.83±15.22	362.50±15.31	314.67±12.86***	268.00±9.49***	28.12
HAAM 400 mg/kg	303.17±10.95	278.33±8.22	339.67±9.67**	314.00±9.56**	272.83±8.11***	236.83±5.64***	30.28
Metformin 300mg/kg	304.5±7.85	234.67±7.69	283.5±9.91***	238.00±10.795***	200.33±8.62***	180.5±6.57***	36.33

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test;

*p<0.05, **p<0.01, ***p<0.00 *vs.* Control Group

The estimation of glycaemia showed a maximum rate in the solvent treated group (414.83 mg/dL). The group of animals pretreated with the test extract at 200mg/kg and 400 mg/kg dose levels showed the maximum level being 372.83 mg/dL & 339.67 mg/dL respectively at 30minutes after pretreatment. A fall of blood sugar level to 268 and 236.83 mg/dL is observed in case of HAAM 200 mg/kg (28.12 %) & HAAM 400 mg/kg (30.28 %) treated groups respectively at 180 minutes of the study with respect to the blood sugar measured at 30 minutes. Similarly at the same time the standard drug metformin showed a reduced blood sugar level of 180.5 mg/dL (36.33 %). However treatment with increasing doses of the plant extract from 200 to 400 mg/kg induce significant reduction in hyperglycemia during the glucose tolerance test in diabetic rats sooner. The results of oral glucose tolerance tests suggest that the pretreatment with the extract causes, inhibition of glucose intake-induced hyperglycemia when compared with the solvent control group.

A single administration of HAAM at tested dose levels remarkably decrease blood sugar level in normal rats, as well as in diabetic rats. Concurrently, in the case of glucose fed hyperglycaemic rat, the HAAM improved the condition probably by enhancing glucose uptake or inhibiting intestinal absorption of glucose. These results from all the single dose administration experiment indicate that, single dose administration of 400 mg/kg of extract is more potent than 200 mg/kg dose, though the difference is not significant. It significantly reduced the sugar level in both streptozotocin and glucose-induced hyperglycemia in rats with approximately equal strength, but did not diminish to the levels of normal control and metformin treated groups.

The outcome of single oral administration of *A. mexicana* is similar to the findings of Abdel-barry JA, *et al* [29] and Ojewole JAO [30], who reported respectively that hydro-alcoholic root of *Clausena anisata* (Willd) and ethanolic and aqueous leaf of *Trigonella foenumgraecum* extracts have hypoglycemic effect both in normoglycaemic and in diabetic rat. Concurrently, in the case of glucose fed hyperglycaemic rat, the *A. Mexicana* extract improved the condition probably by enhancing glucose uptake or inhibiting intestinal absorption of glucose. This finding is in agreement with the finding of Pari and Saravanan [31]; Luo Q, *et al.* [32] who reported that *cogen db* (mixture derived from *Azardirachta indica, Phyllanthus emblia, Terminalia bellerica, Terminalia chebula, Tribulus terrestris, Trigonella foenum-graecum*, *Curcuma longa, Syzigium cumini, Rotula aquatica*) and *Lycium barbarum* respectively improved glucose tolerance in diabetic rats.

Even at a higher dose of 400 mg/kg the extract did not improve glucose tolerance or reduce blood glucose level significantly in comparison to dose of 200 mg/kg. This may be due to antagonism of different substances present within the extract. Therefore, at low doses, the concentrations of this antagonistic substance(s) are low, thus, offering no hindrance to the hypoglycemic causative substance(s) [33]. Prince PSM, *et al.* [34] and Kameswara RB, *et al.* [35] while working in *Tinospora cardifolia* root and *Pterocarpus santalinus* bark on blood glucose levels observed a similar dual action.

Table 5: Effect of multi dose haam on blood glucose in normoglycaemic rats

Treatment ↓	Fasting Blood Glucose (mg/dL)					% Change From Day - 0
	Day 0	Day 1	Day 3	Day 6	Day 9	
Normal Control	94.5±1.544	97.83±1.87	97.00±0.73	95.833±1.58	95.833±1.96	---
HAAM 200mg/kg	97.5±1.86	93.16±1.92	88.33±2.84*	86.33±2.62 *	83.667±2.28**	14.19
HAAM 400mg/kg	100.83±1.62	92.33±1.28	88.167±1.96*	83.83±2.34 **	71.33 ± 2.99***	29.26
Metformin 300mg/kg	96.5±3.75	90.16±2.47	81.00±2.39***	73.83±1.97***	64.00±1.97***	33.68

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test;

*p<0.05, **p<0.01, ***p<0.00 *vs.* Control Group

Effect of Repeated Dose Administration of HAAM

Effect of Repeated oral administration of HAAM on blood glucose levels of normal rats

In normal rats, HAAM was capable of reducing the sugar level at the end of day 9 by 14 % & 29 % with dose levels of 200 mg/kg (p < 0.01) and 400 mg/kg (p < 0.001) respectively (Table - 5). The significant decrease (p < 0.05) in blood glucose level with both the doses start on 3rd day, whereas at end of the experiment period the extract at dose level 400 mg/kg shows better effect (p < 0.001) than dose level of 200 mg/kg (p < 0.01). After repeated administration for 9days the blood glucose level comes down significantly in comparison to normal group and the effect with dose 400 mg/kg is comparable with effect of metformin. The study result suggests that, the extract exhibits a dose proportionate hypoglycaemic effect.

Effect of Repeated oral administration of HAAM on blood glucose levels of STZ induced Hyperglycaemic rats

The results of the study are depicted in Table-6. During the 14 days of treatment with HAAM, the diabetic rats had an improvement in the normalization of the blood glucose levels.

The decrease in happens significantly (p < 0.001) on 1st day in both the dose levels. Irrespective of the sampling day, both the dose levels of 200 mg/kg (4.93 % - 15.44 %) and 400 mg/kg (3.52% - 28.33%) showed a better antidiabetic action (p < 0.001), when compared with the control group. After two weeks of administration the extract at dose 200 mg/kg decreases the glucose concentration by 39.26% while it becomes 51.90 % with dose 400 mg/kg. Metformin had shown better action than the extract on all sampling day, except day 14, on which there is no significance difference between

Metformin and extract at dose level of 400 mg/kg. After two weeks of treatment, the blood glucose level returned to level comparable with metformin treated group with 400 mg/kg dose indicating that the better antidiabetic effect of the plant extract is achieved through repeated and not single administration.

These results from repeated dose study reveal that with repeated administration, the efficacy of the extract increases and shows dose proportionality with increasing doses. At higher dose and repeated administration the efficacy of the extract is comparable with that of the standard drug. The dose proportionality with increasing doses may be due to increased absorption. The of hypoglycemic effect of the extract is similar to that of metformin and underlying mechanism may be similar to that of metformin i.e., decreasing hepatic glucose production, decreasing intestinal absorption of glucose or/and improving insulin sensitivity by increasing peripheral glucose uptake and utilization.

Table 6: Effect of multi dose haam on blood glucose in stz induced hyperglycaemic rats

Treatment↓	Fasting Blood Glucose (mg/dL)						% Change From Day - 0
	Day 0	Day 1	Day 3	Day 6	Day 9	Day 14	
Diabetic Control	361.33±2.11	372.16±2.81	389.83±2.94	401.16±3.54	398.50±2.57	401.66±3.30	---
HAAM 200mg/kg	354.5±4.86	312.50±1.99***	298.66±2.86***	267.88±5.522***	254.66±4.51***	215.33±3.81***	39.258
HAAM 400mg/kg	355.83±4.05	307.66±2.68***	296.83±3.40***	264.33±5.57***	238.83±3.92***	171.16±3.75***	51.898
Metformin 300mg/kg	370.16±5.79	264.83±4.41***	246.66±4.37***	240.00±4.68***	202.66±2.26***	165.66±1.86***	55.246

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test;

*p<0.05, **p<0.01, ***p<0.00 *vs.* Control Group

Effect of repeated oral administration of HAAM on Physical parameters (Body Weight, Food Intake, Water Intake)

The effects of HAAM on the body weight changes of normal rat and diabetic rats are shown in Table – 7 and 8 respectively. During 9days study in normal rats, it has been observed that the body weight of rats has decreased at both the dose levels of 200 mg/kg (1.69%) and 400 mg/kg (9.23%) relative to day 0, but not significantly. The group treated with metformin has shown a significant (15.4 %, $p < 0.01$) decrease in body weight relative to day 0 and when compared with normal group. During the two weeks of observation of body weight in case of diabetic rats there was weight loss in all the groups, relative to day 0, i.e. before the start of the treatment. The untreated diabetic rats lost 34.23 % of their body weight. The loss was 25.728 % and 26.14 % for 200 mg/kg and 400 mg/kg respectively, lower as compared to control. The diabetic rats treated with metformin also showed a bodyweight reduction of 30.69 %, which is not significantly lesser than control diabetic rats. Though the weight loss in treated groups are not significantly lesser than the control group, but the percentage weight loss is lesser than that of control group. The result suggests that the extract inhibits weight loss in case of diabetic rats.

Table 7: Effect of multi dose am extract on body weight in normoglycemic model

Treatment ↓	Body Weight (gm)		Change in Weight	Percentage Change
	Day 0	Day 9		
Normal Control	198.33 ± 6.902	210.66 ± 7.182	12.33 ± 2.894	6.22
HAAM 200mg/kg	206.00± 5.404	202.5 ± 5.284	-3.5 ± 2.729	-1.69
HAAM 400mg/kg	205.83 ± 4.861	186.83 ± 4.847	-19.00 ± 4.091	-9.23
Metformin 300mg/kg	210.00 ± 4.082	177.66 ± 7.168	-32.34 ± 4.169 **	-15.4

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test;

*p<0.05, **p<0.01, ***p<0.00 *vs.* Control Group

Table 8: Effect of multi dose haam on body weight in stz induced hyperglycemic rats

Treatment↓	Body Weight (gm)			Change in Weight Day - 0 & Day - 7	Change in Weight Day - 0 & Day - 14	% Change Between Day -0 & Day - 7	% Change Between Day - 0 & Day - 14
	Day 0	Day 7	Day 14				
Diabetic Control	203.50±7.33	159.33±6.31	133.83±5.81	-44.16±7.18	-69.66±6.33	-21.7	-34.23
HAAM 200mg/kg	200.17±5.58	157.33±7.60	148.67±6.20	-42.83±3.40	-51.5±2.66	-21.396	-25.728
HAAM 400mg/kg	204.67±7.11	157.50±7.72	151.17± 7.09	-47.16±8.37	-53.5±8.43	-23.041	-26.139
Metformin 300mg/kg	202.00±5.47	160.33±8.54	140.00±4.20	-41.66±8.37	-62.00±11.95	-20.623	-30.693

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test;

*p<0.05, **p<0.01, ***p<0.00 *vs.* Control Group

Table – 9 & 10 shows the outcome of exposure of the extract on food and water intakes of diabetic rat. The extract at both the dose levels, considerably reduced food and water intakes of diabetic rats. The food intake of diabetic control rats is 41.50 gm/rat/week during the second week, where as the food intake for the rats treated with HAAM 200 mg/kg, HAAM 400 mg/ Kg and metformin 300 mg/kg is 17.67 gm/rat/week, 14.17 gm/rat/week and 13.67 gm/rat/week respectively. Similarly the water intake habit for the rats treated

with HAAM 200 mg/kg, HAAM 400 mg/ Kg and metformin 300 mg/kg is 53.33 ml/rat/week, 43.17 ml/rat/week and 39.83 ml/rat/week respectively. The water intake habit of diabetic control rats is 100.17 ml/rat/week. This result of extract treated group is comparable to that of metformin treated group. During 2nd week HAAM shows significant decrease in food intake and water intake in comparison to the control group.

It is well known that streptozotocin-provoked hyperglycemia is accompanied by symptoms like loss of weight, polydipsia and polyphagia [36]. Induction of diabetes with streptozotocin is also associated with a characteristic loss of body weight, which is probably due to muscle wasting [37]. In our study, the extract did not significantly prevent loss in body weight but diminished food intake and the water consumption when compared with diabetic control rats. The similar pattern has been observed in the rats treated with standard drug metformin. The improvements of these parameters could be attributed to the hypoglycemic properties of the plant.

Table 9: Effect of multi dose haam on food intake of stz induced diabetic rats

Treatment↓	Food Intake Habit (gm/rat/week)		
	Day 0	Week 1	Week 2
Diabetic Control	26.66±0.76	38.33±1.02	41.50±0.96
HAAM 200mg/kg	25.83±0.70	20.667±0.71***	17.667±1.02***
HAAM 400 mg/kg	25.667±0.88	19.33±1.23***	14.167±0.98***
Metformin 300mg/kg	27.5±0.76	19.167±0.87***	13.667±0.88***

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.00 *vs*. Control Group

Table 10: Effect of multi dose haam on water intake of stz induced diabetic rats

Treatment↓	Water Intake Habit (ml/rat/week)		
	Day 0	Week 1	Week 2
Diabetic Control	76.83±0.60	89.33±1.17	100.17±1.66
HAAM 200mg/kg	75.83±1.25	62.16±0.87***	53.33±0.99***
HAAM 400 mg/kg	76.167±1.43	58.83±1.89***	43.167±2.44***
Metformin 300mg/kg	79.33± 0.95	59.16±1.10***	39.83±1.30***

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.00 *vs*. Control Group

Effect of repeated oral administration of HAAM on biochemical parameters

Studies on some of the biochemical parameters of normal rats (Table 11) showed that at the end of treatment (9 days), serum lipid levels (cholesterol and triglycerides) were appreciably reduced ($p < 0.001$) only when treated with 400 mg/kg of extract. The study on normal rat also reveals that the extract doesn't have any effect on serum Urea or Creatinine which shows the extract doesn't have any effect on renal function of normal rat. In normal rat metformin has shown significant ($p < 0.05$) increase in creatinine level, which is sometimes expected in case of metformin therapy due to metformin accumulation and lactic acidosis. The decrease in body weight observed in normal rat treated with extract may be due to the decrease in body cholesterol level expressed by the extracts in normal rats.

In case of diabetic rats (Table - 12) serum lipid levels were significantly reduced at both the dose levels at end of treatment (14 days). Significant decrease in serum creatinine level in diabetic rats can be observed only in the group treated with HAAM 400 mg/kg ($p < 0.001$), where as 200 mg/kg dose and standard drug has no effect. Serum urea has not been controlled by any of the interventions.

Table 11: Effect of multi dose haam on biochemical parameters of normoglycaemic rats

Treatment ↓	Urea (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Normal Rats	33.948±1.29	0.61±0.03	80.890±2.47	61.488±1.59
HAAM 200mg/kg	35.162± 1.42	0.637 ± 0.02	78.678±2.07	56.022 ± 1.70
HAAM 400mg/kg	37.898 ± 1.99	0.6783±0.02	62.910±1.77***	37.363±1.39***
Metformin 300mg/kg	38.683 ± 1.01	0.7133±0.02565*	59.015±1.61***	34.680 ± 0.95***

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test;

*p<0.05, **p<0.01, ***p<0.00 *vs*. Control Group

Table 12: Effect of multi dose haam on biochemical parameters of stz induced diabetic raTS

Treatment ↓	Urea (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Normal Rats	44.610±4.52	0.94 ± 0.03	142.95 ± 6.62	93.70 ±4.33
HAAM 200mg/kg	43.278 ± 2.40	0.828 ± 0.03	121.42 ± 1.67**	77.378 ± 1.59**
HAAM 400mg/kg	39.483 ± 1.46	0.78 ± 0.03**	88.483 ± 1.02***	64.438 ± 1.61***
Metformin 300mg/kg	45.718 ± 1.19	0.823 ± 0.03	69.115 ± 1.60***	38.295 ± 1.33***

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test;

*p<0.05, **p<0.01, ***p<0.00 *vs*. Control Group

The results indicated that the administration of *A. Mexicana* extract for two weeks prevented increases of serum cholesterol and triglycerides. These findings also correlated with those of Dhandapani S, *et al* [38]; Luo Q, *et al.* [32] who respectively reported that *Lycium barbarum* and *Cuminum cyminum* reduced cholesterol and triglycerides in experimentally-induced diabetic rats. The comparable effects of the extract with metformin suggest the possibility of a similar mode of action.

The results of all the repeated dose study reveals that the effect of the extract on blood glucose, serum lipid profile, change in physical parameters confirms the hypoglycemic effect of the plant. In this study, we also observed a better decrease in blood glucose level in normal as well as diabetic rats at a higher dose level. The disturbed serum lipid profiles of diabetic rats are better normalized with a higher dose as well. All these results suggest that, HAAM is more effective at a higher dose.

The latex and aerial part of the plant is known to contain an isoquinoline alkaloid berberine [22, 23]. Berberine has expressed diverse pharmacological properties in various preclinical and clinical experiments [39]. Different animal experimental results showed that berberine has significant hypoglycemic effect, modulating lipids metabolic effects and free radical scavenging activity in diabetes induced by alloxan, streptozotocin. Berberine expresses hypoglycemic and hypolipidemic action in a dose proportionality manner [40, 41, 42]. Berberine has been shown to regulate glucose and lipid metabolism in a pilot study for efficacy and safety in the treatment of type 2 diabetes mellitus patients [43]. In study A, 36 adults with newly diagnosed type 2 diabetes mellitus were randomly assigned to treatment with berberine or metformin (0.5 g 3 times a day) in a 3-month trial. The hypoglycemic effect of berberine was similar to that of metformin. Significant decreases in hemoglobinA1c were observed [44]. In our experiment it has been observed that, the hypoglycemic and hypolipidemic effect of the extract is similar to that of berberine. So, the effect observed in our experiments may be attributed to presence of Berberine or Berberine like substances in the extract. In certain experiments (Glucose Tolerance Test, Single Dose treated Diabetic rat) it has been observed that even at higher dose (400 mg/kg) the extract doesn't show significantly different result from the lower dose (200 mg/kg), which may be due to antagonistic effect of some other substances that may be present in the extract.

CONCLUSION

A. Mexicana hydro-alcoholic extract can lower the blood glucose level in normoglycaemic and diabetic rats; prevent increase in serum cholesterol and triglycerides of normal and diabetic rats. The effect is more significant at a higher dose. More studies is required to understand the exact active principle responsible for such effect and for understanding the mode of action as well as dose proportionality effect of the extract.

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