Research Article

Antidiabetic and Antihyperlipedemic Activity of "ATH-2K13" in Normal and Alloxan Induced Diabetic Rats

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ABSTRACT

Alloxan monohydrate is a chemical which is selectively toxic analogue for glucose. Alloxan monohydrate will destroys the pancreatic β-cells and may causes non-insulin-dependent diabetes mellitus (NIDDM), after administration with a selective dose to the animals. The main aim of the present study is the screening of the anti-diabetic and anti-hyperlipidemic activities of polyherbal formulation named by ATH-2K13.This poly herbal formulation is also subjected to acute toxicity studies for the safety sake. Either sex of Wistar Albino variety of rats which are in the range of 200-300gm was selected for the present study. The blood glucose levels were checked in regular intervals and the standard drug used in the present study is Glibenclamide. After 14 days of study the serum profile of each group of rats would examine and the rats were sacrificed from each group and liver and pancreas are collected for histopathology studies. The readings are compared to that of the control group. Phytochemical analysis of the herbals is done before screening. No toxicity is observed.

Keywords: ATH-2K13, Alloxan monohydrate, Acute toxicity studies, Anti diabetic activity, Anti-hyperlipedimic activity, Glibenclamide, Histopathology studies.

INTRODUCTION

A number of wide variety plant species are present worldwide are known having anti hyperglycaemic[1] activity. There are wide varieties of Ayurveda and allopathic dosage forms are available in the treatment of Diabetes and lipedimia. Even though wide range of availability of drugs in the market still the screening of new antidiabetic sources from herbal source is welcoming, because the herbal formulations are having less side effects and safe too. The present poly herbal formulation ATH-2K13used in the screening of anti-diabetic and anti hyperlipedimic activities consists the herbals called dried rhizomes of turmeric, dried fruits of amla and Honey. The methanolic extracts of these herbals are used to prepare the formulation.

Objective

The objective of our present study is to prove the anti-diabetic and anti-hyperlipedimic activity of the poly herbal formulation by using alloxan induced diabetic rats.

MATERIALS AND METHODS

Procurement and identification of plant material

The dried rhizomes of Curcuma longa, fruits of *Emblica officinalis* and honey were procured at Erragadda market. The dried rhizomes and fruits were brought to the laboratory, cleaned the rhizomes and washed fruits thoroughly in running tap water to clean the adhering sand particles and then rinsed in distilled water, shade dried, coarsely powdered and stored in air tight containers for further use.

Preparation of the extract[2]

After washing the rhizomes of curcuma longa and dried fruits of *Emblica officinalis*, the dried powder submitted to successive extraction by soxhlet apparatus with 100% methanol at 72ºC temperature for 18hrs. All the extract was filtered through membrane filter and then the extract dried in room temperature. Then prepared the suspension of herbals by adding equal quantities of methanolic extract of Amla and turmeric, Honey. In the preparation of this poly herbal formulation tween 80 is used as suspending agent. After preparation of poly herbal formulation by adding methanolic extracts of dried fruits of amla and rhizomes of turmeric in 1:1ratio and honey is used as vehicle the two doses were prepared from the formulation called 100mg/kg body weight and 200mg/kg body weight.

Drugs and chemicals

Alloxan monohydrate was procured from LOBA CHEMIE Laboratory Reagents and Fine Chemicals, Mumbai. Glibenclamide (Batch no: G080851) was a gifted sample from TABLETS INDIA LTD, Chennai. Standard Glucose estimation kits were procured from ROBONIK (INDIA) PVT.LTD, Mumbai. Enzymatic kits for the estimation of lipid profile were obtained from CHEMA DIAGNOSTICA (INDIA).

Animals

Healthy adult albino rats of Wister strain of either sex between the age of 2-3 months and weighing 200-300 grams were used for the present study. The animals were housed individually in polypropylene cages, maintained under standard conditions (12 hours light and12 hours

dark cycle, 24±5°C and 40-60% humidity).[3] They were fed with standard rat pellet diet (National Institute for Nutrition, Hyderabad) and provided water ad libitum. All the animals are collected from central animal house SICRA LABS PVT.LTD, KUKATPALLY, HYDERABAD and all experiments were conducted according to the ethical norms approved by CPCSEA, Ethical committee IAEC reg. no. (769/2011/CPCSEA).

Phytochemical Screening

A preliminary phytochemical screening of methanol extracts was carried by using standard procedures. The results are shown in table 1 and 2.

Acute Oral Toxicity Studies

Acute oral toxicity studies[4] of the extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted and received from Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India.

Albino wistar rats (n=30) were fasted for 16 to 18hrs and were divided into four groups of 6 animals each and the treatment protocol was described below. Before the drug administration, blood samples were collected by spinning the tail vein for the estimation of glucose levels.

Treatment protocol

All the rats were randomized into five groups comprising of six animals in each group as given below.

Group I: Normal rats received 100mg of ATH-2K13

Group II: Normal rats received 200mg/kg ATH-2K13

Group III: Normal rats received 500mg/kg ATH-2K13

Group IV: Normal rats received 750mg/kg ATH-2K13

Group V: Normal rats received 1000mg/kg ATH-2K13

ATH-2K13of various doses were administered orally using an intra-gastric tube and monitored for 48hrs.After the administration of single dose of ATH-2K13 no mortality rates are observed.

Oral Glucose Tolerance Test

Albino wistar rats (n=30) were fasted for 16 to 18hrs and were divided into four groups of 6 animals each and the treatment protocol was described below. Before the drug administration, blood samples were collected by spinning the tail vein for the estimation of glucose levels.

Treatment protocol

All the rats were randomized into five groups comprising of six animals in each group as given below.

Group I: Normal rats received (10% acacia mucilage)

Group II: Normal rats received glucose

Group III: Normal rats received 5mg/kg Glibenclamide.

Group IV: Normal rats received 100mg/kg ATH-2K13

Group V: Normal rats received 200mg/kg ATH-2K13

ATH-2K13(100mg/kg, 200mg/kg) and Glibenclamide (5mg/kg)[5] were administered orally using an intra-gastric tube.After30mins of the administration of standard and poly herbal formulation ATH-2K13 orally 1% glucose is administered. Immediately blood glucose levels were monitored at 0hr, 1hr, 2hr and 4hr and6 hrs. By using "Glucocheck"[6] blood glucose testing instrument after the administration of single dose of 1%glucose. The Percentage reduction in serum glucose was calculated with respect to the initial level.

Anti-diabetic activity Treatment protocol

All the rats were randomized into five groups comprising of six animals in each group as given below.

Group I: normal rats received (10% acacia mucilage)

Group II: Diabetic control received 150mg/kg 0f Alloxan-monohydrate.

Group III: Normal rats received 5mg/kg Glibenclamide.

Group IV: Normal rats received 100mg/kg ATH-2K13.

Group V: Normal rats received 200mg/kg ATH-2K13.

Induction of experimental diabetes

Albino rats weighing about 200-300gm were fasted overnight (14-16 hours) and their weight and fasting blood glucose level was recorded. Rats were then made diabetic by a single intraperitonial injection of alloxan monohydrate (150 mg/kg body weight).[7] One hour after administration of alloxan, the animals were fed with standard laboratory diet and water ad libitum.[8] Alloxan was first weighed individually for each animal according to their body weight & then solubilized with 3ml normal saline just prior to injection in order to avoid the decomposition of alloxan. Food and water were presented to the animals 60 minutes after drug administration. 48hr after alloxan injection, plasma blood glucose level of each animal was determined and animals with a fasting blood glucose range above 300 mg/dl were included in the study. The blood samples were collected from the tail vein of the rats at regulation intervals for checking the blood glucose levels by using "Glucocheck[9] "glucometer to check the blood glucose levels. After the administration of single dose of ATH-2K13 or Glibenclamide (for acute study) as well as on the 1st, 3rd, 5th, 7th, 14th day respectively (for prolonged effect).

Collection of blood samples and estimation of Biochemical parameters

The treatment of grouped animals with the standard /extracts of ATH 2K13 was started from 7th day of alloxan administration and continued for the next 15 days. On the 15th day, blood samples (Approximately 0.5ml) were collected from overnight fasted rats by puncturing the retro orbital sinus, under mild ether anaesthesia for biochemical estimations.[8] Blood samples were not

allowed to clot for 30 min and serum was separated by centrifugation at 3000rpm.

Antihyperlipidemic studies

The animals were divided into five groups of five rats each. The first group was given standard pellet diet, water and orally administered with 10% acacia. The second group was given a single dose of Alloxan monohydrate (150mg/kg) administered through I.P.route. After 48 hours of injection; this group received a daily dose of normal saline for 14days. The third group administered with 5mg/kg body weight Glibenclamide. Fourth and fifth group was administered a daily dose of ATH2K13 100mg/kg and 200 mg/kg suspended in tween 80 for 14 days, after inducing diabetes mellitus.

Collection of blood

On the 15thday, blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed and the liver and pancreas are collected and the stains used were Haematoxylin and eosin.[10]

Determination of serum lipid profile

Serum cholesterol and triglycerides were estimated on initial and final days of experiment of each model by CHOD – POD method and enzymatic colorimetric method (GPO which is highly influenced by level of fasting). HDL cholesterol was determined by using standard enzymatic kits obtained from ROBONIK (INDIA) PVT.LTD, Mumbai. While the LDL-C was derived from cholesterol and triglyceride values.

Observations

After the intraperitonial injection of Alloxan monohydrate, general conditions like polyuria, polydipsia was observed. Among all the study group of animals, one animal showed writhing's like abdominal constrictions and trunk twisting. An important observation in case of that animal was that the colour of the urine turned to pink after 2hr of the intraperitonial injection of freshly prepared Alloxan, which increased in intensity as the time progressed. The following images represent the colour change of urine after the Alloxan injection. Due to these observations that animal was excluded from the study.

Statistical analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and analysed for ONE WAY ANOVA and post hoc Dennett's t-test using computerized Graph Pad Prism In Stat version 5.0, Graph Pad software. Differences between groups were considered significant at $P<0.001$ and very significant at $P < 0.0001$ levels.

Table 1: Phytochemicals constituents of curcuma longa

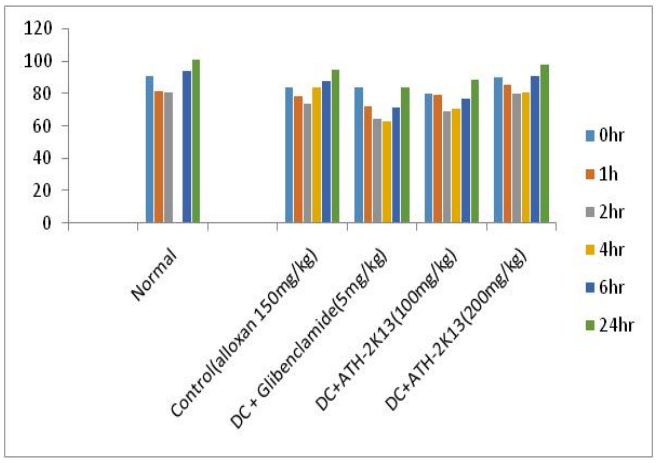
Phytoconstituents	Curcuma longa
Alkaloids	+
Carbohydrates	+
Glycosides	+
Tannins	+
Proteins and amino acids	+
Saponins	+
Steroids	+
Flavonoids	+
Phenols	+

Table 2: Phytochemicals constituents of *Emblica officinalis*

Phyto constituents	Emblica officinalis
Alkaloids	+
Carbohydrates	+
Glycosides	+
Tannins	+
Proteins and amino acids	+
Saponins	-
Flavonoids	+
Phenols	+

Table 3: Acute Oral Toxicity Studies

Dose	No of Rats/No of Moratlity			
	6hr	12hr	24hr	48hr
100mg/kg	6/0	6/0	6/0	6/0
200mg/kg	6/0	6/0	6/0	6/0
500mg/kg	6/0	6/0	6/0	6/0
750mg/kg	6/0	6/0	6/0	6/0
1000mg/kg	6/0	6/0	6/0	6/0



Graph 1: Effect of ATH-2K13 on blood glucose level (mg/dl) in Alloxan induced diabetic rats on day 1 of the treatment (ACUTE STUDY).

Table 4: Effect of ATH-2K13 on blood glucose level (mg/dl) in alloxan induced diabetic rats on day 1 of the treatment (ACUTE STUDY)

Treatment	Fasting Blood Glucose Level (mg/dl)					
	0hr	1h	2hr	4hr	6hr	24hr
Normal	91±3.95	82±4.45	80.7±4.63	85.8±3.9	94.2±4.09	101.5±3.75
Control (alloxan 150mg/kg)	84.5±3.81	79±3.70	73.8±3.65	84±3.84	88.2±3.93	95.4±3.33
DC + Glibenclamide (5mg/kg)	84.5±3.81	72.3±3.71	65±3.57	63±3.2***	71.8±3.72***	84.5±2.4***
DC+ATH-2K13 (100mg/kg)	80±3.82	79.5±3.78	69.3±3.60	70.6±3.52***	77.5±3.75***	89.2±2.8***
DC+ATH-2K13 (200mg/kg)	90.5±4.12	85.7±3.86	80±3.80	81.2±3.77***	91.5±4.03****	98.5±3.21**

Values are expressed as Mean ± SEM; *P<0.001; **P<0.001; ***P<0.0001.

Table 5: Effect ATH-2K13 on blood glucose level (mg/dl) in alloxan induced diabetic rats

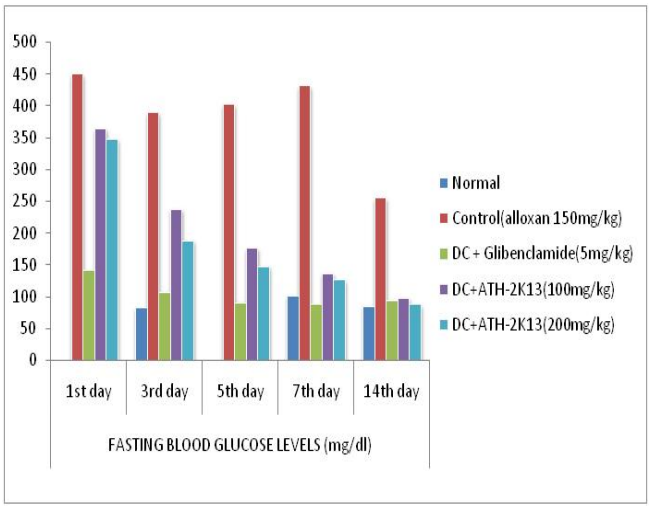
Treatment	Fasting Blood Glucose Levels (mg/dl)				
	1st day	3rd day	5th day	7th day	14th day
Normal	102.32±2.9	82.78±1.41	100±1.94	102.52±0.93	85.14±3.68
Control (alloxan 150mg/kg)	451.1±5.55	390.54±5.50	404.12±8.2	432.7±11.46	255.96±11.8
DC+Glibenclamide (5mg/kg)	141.8±13.76***	107.7±4.9***	91.8±5.14**	88.4±2.5***	95.02±4.3***
DC+ATH-2K13 (100mg/kg)	365.72±13.4***	238.62±9.20***	176.62±9.9***	137.1±3.6***	98.14±4.42***
DC+ATH-2K13 (200mg/kg)	348.82±14.7***	189.2±3.75***	148.38±5.2***	126.8±3.63***	88.22±5.50***

Values are expressed as Mean ± SEM; **P<0.001; ***P<0.0001

Table 6: Effect of ATH-2K13 on serum lipid profile in alloxan induced diabetic rats after 14 days treatment

Treatment	TC (mg/dl)	HDL-C	LDL-C	TG (mg/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Normal	108.6±2.3	46.61±2.00	36.24±0.84	75.30±1.30	96.54±1.25	30.04±0.5	110.4±0.24
Control (alloxan 150mg/kg)	147.6±1.5	26.65±0.9	66.04±1.42	175.9±0.92	288.0±0.7	93.91±0.45	286.8±0.33
DC + Glibenclamide (5mg/kg)	127.6±.98***	57.83±1.62**	48.35±1.8***	120.6±1.9***	174.7±1.65***	43.04±0.9***	124.7±1.33***
DC+ATH-2K13(100mg/kg)	155.9±1.73***	44.10±0.4	51.4±1.08***	139.2±1.4***	205.5±4.15***	62.9±0.91***	158.4±1.84**
DC+ATH-2K13(200mg/kg)	135.6±1.13***	52.97±1.09**	48.32±1.81***	128.9±1.45***	186.8±2.20***	52.4±1.0***	150.6±3.24**

TC= total cholesterol; TG= triglycerides; HDL= high density lipoproteins; LDL= low density lipoproteins; and ALP.
All the values are expressed as Mean ± SEM and are very significant at ***P<0.0001.



Graph 2: Effect ATH-2K13 on blood glucose level (mg/dl) in Alloxan induced diabetic rats

RESULTS AND DISCUSSION

In order to induce the Type-1 Diabetes mellitus there many types of methods are used. But among all the methods to get the better results chemical methods are most widely employed. To induce the type-1 diabetes by chemical method of induction Alloxan monohydrate is most preferably used. In the present study of screening of anti-diabetic and anti-hyperlipidemic activities the poly herbal formulation ATH2K13 is used against the Alloxan monohydrate. To induce the type-1 diabetes mellitus 150g/kg body weight of alloxan monohydrate is used. This dose is given by dissolve in normal saline solution and administered through intraperitonial route. The standard drug used in this study is 5mg/kg body weight Glibenclamide. The test doses are 100mg/kg and 200 mg/kg. The screening continues till 14 days. In regular interval days the blood glucose levels are checked. After 14 days the 15th day the blood is collected from the each group of rats and serum is collected from the blood by

centrifugation technique. The serum profile of Rats Were summarized in Table 5 and Table 6 respectively. The obtained data is biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and analysed for ONE WAY ANOVA and post hoc Dennett's t-test using computerized Graph Pad Prism In Stat version 5.0, Graph Pad software. Differences between groups were considered significant at P<0.001 and very significant at P < 0.0001 levels. Till the completion of screening the body weights of all groups of rats are recorded and tabulated. The untreated group blood glucose level is decreased from 451.1 ± 5.55 to 255.96 ± 11.8. The standard group blood glucose level is decreased from 141.8 ± 13.76 to 95.02 ± 4.3. The test group 1 (100mg/kg) blood glucose level is decreased from 365.72 ± 13.4 to 98.14 ± 4.42. The test group 2 (200mg/kg) blood glucose level is decreased from 348.82 ± 14.7 to 88.22 ± 5.50.The gradual decrease of blood glucose levels are clearly observed. The anti-hyperlipidaemia is clearly observed from the initial day to final day. After observing all the statistics the test drug named ATH2K13 which is poly herbal formulation of methanol extracts is efficient at current doses. So it can be suggested in the treatment of type-1 Diabetes mellitus.

CONCLUSION

ATH-2K13 is a combination of fruits of Amla, dried rhizomes of turmeric and Honey. It is more effective in the treatment of Diabetes mellitus, and hyperlipidaemia. The significant p-value <0.0001 in alloxan induced diabetic rats. The main mechanism of action of poly herbal formulation is to stimulates the b-cells of pancreas there by it reduces hyperglycaemic levels of blood glucose and it also efficiently acts on the lipid profile also. The Histopathological studies also reveal that it also effectively acts on the damaged liver cells and pancreas too. So the present study suggests that the poly herbal formulation ATH-2K13 had potent anti-diabetic and anti-hyperglycaemic activities

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