Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

ANTIHYPERGLYCEMIC ACTIVITY IN GREWIA ASIATICA, A COMPARATIVE INVESTIGATION

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Received: 12 Feb 2011, Revised and Accepted: 18 May 2011

ABSTRACT

In the present study we report the comparative anti-hyperglycemic effects of crude ethanolic extracts of the fruit, stem bark and leaves of Grewia asiatica and their fractions in alloxan induced hyperglycemic rabbits. The crude extracts and their fractions were administered orally in suspension and capsule form at the dose of 200mg/kg body weight and 100mg /kg body weight respectively. Oral administration of fruit, stem bark and leaves reduced serum glucose level of alloxan induced diabetic rabbits. The results suggest that the fruit, stem bark and leaves of Grewia asiatica have shown significant antihyperglycemic activity.

Keywords: Grewia asiatica, Antihyperglycemic, Fruit, Stem bark, Leaves.

INTRODUCTION

Diabetes mellitus is regarded as a serious, incurable chronic disease that carries a high risk for a considerably short life span, renal failure, disability, complications including blindness, amputation of organs, sexual impotence, early cardiovascular complications etc[1]. Many oral hypoglycemic agents have been effective recommendations in treating type II diabetes. Selection of an appropriate pharmacological agent is based on considerations of the cost, glucose control levels and characteristics of the patient[2] but no one has ever been reported to have recovered totally from diabetes. Combination of two or more oral antihyperglycemic agents is often needed for disease management. There is a lot of scope for alternative therapy, either from herbal formulations or indigenous plants, as add on therapy, in the long-term management of type II diabetes[3]. Grewia asiatica is reputed to have antihyperglycemic activity. Pakrashi and Mukherjee reported the remarkable antidiabetic effect produced in both cats and rabbits made diabetic respectively by Subtotal pancreatectomy and by oral hydrochlorothiazide treatment with prior injection of insulin. No significant toxic or teratogenetic effects were observed in mice[4]. Dogar et al. reported significant reduction in blood glucose, blood cholesterol and triglycerides levels by ground herbal drugs including Grewia asiatica (bark), Gossypium herbacium and Gymnema sylvestre in normal and alloxan induced diabetic rabbits[5]. The investigation of hypoglycemic agents of plant origin, which are used in traditional medicine, is thus of great significance. In this context the present investigation was undertaken to evaluate the anti-hyperglycemic effects in the crude ethanolic extracts of the fruit, stem bark and leaves of Grewia asiatica and their fractions in alloxan induced diabetic rabbits in a comparative fashion.

MATERIALS AND METHODS

Drugs and chemicals

Alloxan monohydrate was purchased from Sigma-Uldrich, USA. Blood glucose determination kit from Randox Laboratories Ltd, UK. The chemicals used were analytical grades prepared by E. Merk, Germany.

Experimental animals

Healthy rabbits of normal strain, specie of both sexes, weighing 1-1.5 Kg were purchased from the local market. They were kept on a balance feed comprising of green fodder, wheat grains etc.; they were given access to water but food was withdrawn 24 hours prior to experiments.

Preparation of plant extract

The fruit (5kg), stem bark (500 gm) and leaves (1 kg) of Grewia asiatica (Tilliaceae) were collected from the Grewia orchid, Dera Ismail Khan, Pakistan. The plant was identified by Mr. Hamidullah, Department of Pharmacognosy, Faculty of Pharmacy, Gomal University, and D.I.Khan. The plant materials were separately extracted with 95% ethanol by maceration process. This procedure was repeated thrice. The crude ethanolic extract was filtered and then evaporated under reduced pressure in the rotary evaporator and then was fractionated with different organic solvents. A concentrated red colored syrupy extract of the fruit crude (110gm) was obtained which afforded aqueous (46gm), methanolic (13gm), n-butanolic (5gm) and ethyl acetate fraction (1gm). Its fractionation with hexane, carbon tetrachloride and chloroform yielded negligible quantities. The dried brown crude ethanolic extract of stem bark (33gm) yielded aqueous (4gm), methanolic (12gm), n-butanolic (918mg), ethyl acetate (5gm), chloroform (930mg) and carbon tetra chloride fraction(1gm). Its fractionation with hexane yielded negligible quantity. The dried green crude ethanolic extract of the leaves (100gm) yielded aqueous fraction (16.50gm), methanolic (21gm), n-butanolic (2gm), ethyl acetate (5gm), chloroform (2gm), carbon tetrachloride (1.55gm) and hexane fraction (6.3gm).

Experimental induction of diabetes in rabbits

Alloxan monohydrate was administered intravenously as a 5% aqueous solution in the marginal ear vein at a dose of 150 mg/kg [6]. The rabbits developed diabetes in 7 days after administration of alloxan. Rabbits with blood glucose level above 200 mg/dl were considered as diabetic and employed for the bioassay [7].

Experimental design

Experimental animals were distributed at random into two major groups containing normal rabbits (non-diabetic) and diabetic rabbits. They were further divided into groups, each containing six animals. Amongst the normal rabbits group I, II & III received G.asiatica fruit, stem bark and leaves crude extract 200 mg/kg body weight respectively, group IV received Glucophage (Merck) 500mg/kg body weight and group V served as control receiving 20 ml 2% Gum tragacanth suspension.

In the alloxan induced hyperglycemic rabbits group VI, VII and VII received G. asiatica fruit, stem bark and leaves crude extract (200 mg/kg body weight) respectively. Group IX, X, XI & XII received G. asiatica fruit aqueous methanolic, butanolic and ethyl acetate fraction (100mg/kg body weight) respectively. Group XIII, XIV, XV, XVI, XVII, XVIII received G.asiatica bark aqueous, methanolic, butanolic, ethyl acetate, chloroform and carbon tetrachloride fraction (100mg/kg body weight) respectively. Group XIX, XX, XXI, XXII, XXIII, XXIV, XXV received G.asiatica leaves aqueous, methanolic, butanolic, ethyl acetate, chloroform, carbon tetrachloride, hexane fraction (100mg/kg body weight) respectively. Group XXVI received Glucophage (500 mg/kg body weight). Group XXVII received only 20 ml 2% Gum tragacanth suspension. Group XXVIII served as diabetic control. Blood samples (0.2ml) were collected at 0, 2, 4, 8, 12 and 24 hours after the drug administration[8]. Blood glucose levels were determined by Gluco-oxidase method[9].

Statistical analysis

All the values were expressed as Mean ±S.E.M. Student "t" test was used to check the significance of the data for "t" value. Values of $p<0.05$ were considered as statistically significant.

RESULTS

Excellent results were obtained in normal and alloxan induced diabetic rabbits as compared to standard oral hypoglycaemic agent, Glucophage (Merk) and control.

Normal rabbits treated with crude extracts of the Fruit, Stem bark and Leaf of Grewia asiatica

The normal rabbits after treatment with 200mg/kg body weight of the fruit of Grewia asiatica in Group I showed a non significant decrease in blood glucose level after 2 hours. The reductions were significant at 4 hour and highly significant at 8 hour as compared to zero hour level 97.83±1.89mg/dl. The blood glucose level was statistically significant at 12 hour and non significant at 24 hour as compared to the zero hour level. The mean blood glucose level of the normal rabbits after treatment with 200mg/kg body weight of the stem bark of Grewia asiatica showed a statistically non significant decrease at 2, 4, 8, and 24 hours while the decrease in blood glucose level was statistically significant at 12 hours (G-II). The crude extract of the leaf did not affect the blood glucose level as there was no statistical difference at 2, 4, 8, 12 and 24 hours interval (G-III). Normal rabbits in Group IV after treatment with Glucophage 500 mg/kg showed highly significant decrease in blood glucose level at 2, 4 & 8 hours while at 12 hour blood glucose level reached to 87.16±0.75 mg/dl which was statistically significant. The gum tragacanth did not affect the blood glucose levels (Group-V) as there was no statistical difference at 2, 4, 8, 12 and 24 hours interval. Results are shown in Table1.

Diabetic rabbits treated with crude extracts (200mg/Kg body weight) of the Fruit, Stem bark and Leaf of Grewia asiatica

The blood glucose level of Group VI after treatment with fruit crude at zero hour showed a non significant effect of the drug at 2 hours while at 4 hr the decrease in blood glucose level was significant. The data pertaining to 8, 12 and 24 hours response reveal a highly significant decrease in blood glucose level with respect to the zero hour level. Diabetic rabbits in Group VII showed a highly significant decrease in blood glucose level at 12 and 24 hours.

The animals in Group VIII showed a statistically significant decrease at 24 hour as compared to zero hour level. The alloxan diabetic rabbits in Group XXVI treated with Glucophage (500mg/Kg) showed a decrease in blood glucose level at 2 & 8 hour time interval and a highly significant effect was observed at 4 hour while the data pertaining to 12 & 24 hours reveal a non significant change with respect to zero hour level. In Group XXVII the mean ± SEM blood glucose level of diabetic animals treated with 2% gum tragacanth suspension at 2, 4, 8, 12 & 24 hours was statistically insignificant. In Group XXVIII no statistically significant change in blood glucose level was observed at 2, 4, 8, 12 and 24 hours as compared to zero hour level.

Table1: Effect of the crude ethanolic extract of the Fruit, Stem bark and Leaf of Grewia asiatica on glucose levels in Normal rabbits.

Sub		Mean blood glucose level (mg/100ml ± SEM)					
Group	Treatment	0hr	2hr	4hr	8hr	12hr	24hr
I	F crude	97.83±1.89	104.16±2.19[c]	80.5±2.22[c]	60.33 ±1.82 [a]	79.66 ±0.99 [a]	105.16±1.87 [c]
II	S crude	101.5±1.69	106.00± 1.99 [c]	107.00± 1.77 [c]	110.16± 4.06 [c]	88.83± 4.17[a]	121.00 ± 3.84 [c]
III	L crude	79± 1.79	117.5±1.88[c]	86.66±2.01[c]	112± 1.75 [c]	87.5±1.71 [c]	92.16±1.70 [c]
IV	Glucophage	92.16±0.83	92.00±0.96 [a]	72.50±1.31 [a]	79.66±1.76 [a]	87.16±0.75 [b]	92.00±0.57 [c]
V	Control	94.17± 0.60	94.00± 0.58 [c]	94.33± 0.81	93.50± 0.67 [c]	91.67 ±0.50 [c]	92.50± 1.05 [c]

a= Significant decrease in blood glucose level as compared to zero hour level. ($p<0.005$)
b = Significant decrease in blood glucose level as compared to zero hour level. ($p<0.05$)
c= Non significant decrease in blood glucose level as compared to zero hour level. ($p>0.05$)

Table 2: Effect of the crude extracts of Fruit, Stem bark and Leaf of Grewia asiatica on glucose levels in Diabetic rabbits

Group	Mean blood glucose level (mg/100ml ± SEM)						
	Treatment	0hr	2hr	4hr	8hr	12hr	24hr
VI	F crude	386.33± 2.15	419.83±2.40 [c]	377.50 ±2.50[b]	327.66 ±1.58 [a]	271.33 ±1.20 [a]	133.00 ±1.06[a]
VII	S crude	308.33 ±1.54	323.16 ±1.53[c]	306.33 ± 1.89 [c]	305.50 ± 2.10[c]	258.16 ±2.21 [a]	179.83 ±1.40 [a]
VIII	L crude	329.33 ± 1.98	412.33±1.75 [c]	415.66±1.63 [c]	418.83 ±1.80 [c]	341.66±2.05[c]	299.83± 2.45 [a]
XXVI	Standard	335.16±3.04	318.50±2.96 [b]	306.50±1.65[a]	322.66±3.08[b]	327.00±2.50 [c]	340.80±3.63 [c]
XXVII	Placebo	309.00± 1.62	309.66± 1.78 [c]	309.00± 0.89 [c]	309.16± 3.25[c]	312.00 ±2.85 [c]	310.33± 2.50[c]
XXVIII	Control	294.83± 3.25	295.00± 3.05[c]	294.00± 4.18[c]	290.83± 2.99[c]	293.16± 2.49[c]	293.83± 3.08[c]

Table3: Effect of the Fractions of the crude ethanolic extracts of the Fruit, Stem bark and Leaf of Grewia asiatica on glucose levels in Diabetic rabbits

	Mean blood glucose level (mg/100ml ± SEM)						
Group	Treatment	0hr	2hr	4hr	8hr	12hr	24hr
IX	FA	318.80± 0.86	308.20±1.60[b]	277.40±1.86 [a]	234.40±1.34[a]	175.80 ±1.34 [a]	123.60 ±1.12[a]
XIII	SA	436.16 ±1.35	374.00 ±1.80[a]	383.00 ± 1.69[a]	308.66 ± 1.58[a]	352.66 ±1.77[a]	240.66±1.45[a]
XIX	LA	460.66 ± 1.48	393.33±1.45[a]	360.50±1.71[a]	348.50±1.48[a]	240.16±1.54[a]	116.00±1.46[a]
X	FM	365.33± 1.54	402.00±1.15[c]	369.50 ±2.70[c]	353.66 ±1.49 [c]	363.83 ±2.11[c]	292.83 ±2.82 [a]
XIV	SM	405.50 ±1.30	353.83 ±1.40 [a]	407.33 ± 1.78[c]	427.83 ±2.08 [c]	367.00 ±1.86[a]	244.33±1.85[a]
XX	LM	431.16 ± 1.92	490.33± 2.28[c]	422.33±2.47[c]	412.66 ±1.38[b]	426.50±2.52[c]	245.33± 1.54 [a]
XI	FB	416.33± 1.94	314.83±1.35[a]	292.66 ±3.08[a]	262.66 ±2.60[a]	175.30 ±1.68[a]	130.33 ±1.54[a]
XV	SB	404.16 ±1.30	344.16 ±1.57 [a]	380.16 ± 1.35[a]	372.00 ± 2.15[a]	193.33 ±1.58[a]	92.33 ± 1.76[a]
XXI	LB	412.50 ± 3.54	233.16±3.77[a]	426.33±2.23[c]	359.00 ±2.64[a]	256.83±2.50[a]	212.50± 2.64[a]
XII	FEA	366.33± 1.60	277.33±2.36[a]	294.50 ±1.33[a]	378.33 ±2.05[c]	429.00 ±2.17[c]	321.33 ±2.36[a]
XVI	SEA	424.66 ±1.68	374.66±1.62 [a]	340.66 ±2.33[a]	460.16 ±1.95[c]	467.66±2.07[c]	327.16 ±1.90[a]
XXII	LEA	478.00 ± 1.93	349.40±1.76[a]	444.40±2.09[a]	368.80 ±2.00[a]	388.60±2.02[a]	204.40± 1.28[a]
XVII	SC	402.00±1.41	391.00±1.41[b]	299.50 ±1.46[a]	296.50 ±1.30[a]	329.33 ±1.68[a]	169.50 ±1.87[a]
XXIII	LC	469.50 ±2.01	481.60 ±1.90[c]	378.00 ±2.40[a]	297.50 ±1.38[a]	419.83 ±1.64[a]	165.16 ±1.35[a]
XVIII	SCT	385.00±0.84	341.60±1.48[a]	285.80 ±1.38[a]	287.00 ±1.43[a]	182.80 ±1.47 [a]	233.00 ±1.81[a]
XXIV	LCT	340.83 ±1.51	324.83 ±2.00[a]	432.33 ±1.94[c]	355.50 ±1.38[c]	343.66 ±1.62 [c]	111.66 ±1.92 [a]
XXV	LH	363.33 ± 1.47	322.33±1.56[a]	328.50±1.33[a]	337.50 ±1.89[a]	127.50±1.85[a]	387.5± 1.62 [c]

Diabetic rabbits treated with Aqueous fractions of the Fruit, Stem bark and Leaf of Grewia asiatica (100mg/Kg body weight)

Administration of the aqueous fraction of the fruit showed a lowering of the mean blood glucose level of diabetic animals (G-IX). The reductions were significant at 2 hours and highly significant at 4, 8, 12 and 24 hours as compared to the zero hour blood glucose level. The aqueous fraction of the stem bark caused a highly significant decrease in blood glucose levels (G-XIII) at 2, 4, 8, 12 and 24 hours as compared to zero hour level.

The diabetic rabbits after treatment with aqueous fraction of leaf indicated a highly significant decrease in blood glucose level at 2, 4, 8, 12 and 24 hours time interval (G-XIX).

Diabetic rabbits treated with Methanolic fractions of the Fruit, Stem bark and Leaf of Grewia asiatica

Diabetic rabbits treated with G. asiatica fruit methanolic fraction (G-X) and leaf fraction (G-XX) showed highly significant decrease in blood glucose levels at 24 hour. Diabetic rabbits treated with G.asiatica bark methanolic fraction showed a highly significant reduction in the mean blood glucose level at 2 hour with respect to zero hour level (G-XIV). Further recordings of the blood glucose levels at 4 & 8 hours were non significant. The reduction was highly significant at 12 hour and at 24 hour.

Diabetic rabbits treated with Butanolic fractions of the Fruit, Stem bark and Leaf of Grewia asiatica

Diabetic rabbits treated with G. asiatica fruit butanolic fraction (G-XI) showed a blood glucose lowering pattern with reductions highly significant at 8, 12 and 24 hours as depicted in Table: 3. The mean blood glucose level of the animals in the diabetic group XV receiving stem bark fraction showed highly significant reductions at 2, 4, 8, 12 and 24 hours. Diabetic rabbits treated with G. asiatica leaves butanolic fraction (G-XXI) showed a highly significant reduction in blood glucose level after 2 hours. The reduction observed at 4 hour was non significant while those recorded at 8, 12 & 24 hours were highly significant.

Diabetic rabbits treated with Ethyl acetate fractions of the Fruit, Stem bark and Leaf of Grewia asiatica

Group XII receiving ethyl acetate fraction of fruit showed a highly significant decrease in blood glucose level at 2 and 4 hour but non significant at 8 and 12 hrs as compared to zero hour level. The mean blood glucose level at 24 hour was highly significant. Diabetic rabbits (G-XVI) treated with G. asiatica stem bark ethyl acetate fraction showed a highly significant decrease in blood glucose level at 2 & 4 hours and nonsignificant at 8 and 12 hours. The mean blood glucose level recorded at 24 hour was significant as compared to the zero hour reading. Diabetic rabbits (G-XXII) treated with leaves ethyl acetate fraction showed significant lowering at 24 hours as compared to the zero hour.

Diabetic rabbits treated with Chloroform fractions of the Stem bark and Leaf of Grewia asiatica

The lowering in blood glucose level of the diabetic group XVII was significant at 2 hours. The reduction was comparatively highly significant at 4, 8, 12 and 24 hours. Diabetic rabbits (G-XXIII) treated with G. asiatica leaves chloroform fraction showed a non significant reduction at 2 hr as compared to zero hour level. The reduction in the blood glucose level was highly significant at 4, 8, 12 & 24 hours.

Diabetic rabbits treated with carbon tetrachloride fractions of the Stem bark and Leaf of Grewia asiatica and Hexane fraction of Leaf.

The mean blood glucose level of the diabetic group XVIII at zero hour was 385±0.84 which was lowered at further intervals. The lowering was highly significant at 2, 4, 8, 12 & 24 hours The reduction after administration of leaf fraction (G-XXIV) was highly significant at 2 & 24 hours and non significant after 4, 8 and 12 hours with respect to the zero hour value. The reduction in the mean glucose level receiving hexane fraction of leaves showed highly significant reductions at 2, 4, 8 and 12 hours and non significant response at 24 hour as compared to the zero hour level (G-XXV).

DISCUSSION

Results show that administration of 2% Gum tragacanth suspension in normal (Group V) and alloxan diabetic group (Group XXVII) have no significant change in blood glucose concentration confirming that 2% gum tragacanth aqueous suspension produced no significant change in blood glucose level of normal and diabetic rabbits at any time interval [10] as shown in Table 1 and 2.

The mode of action of Grewia asiatica has not been studied so far. But the comparative studies of the fruit, stem bark and leaves of G. asiatica show that the crude extract of the fruit and stem bark reduced the blood glucose level in normal and diabetic rabbits whereas the crude ethanolic extract of the leaves showed no effect in normal animals but reduced the blood glucose level in diabetic rabbits only. In normal rabbits the crude ethanolic extract of the fruit produced a significant reduction in blood glucose level at 8 and 12 hours. In case of the stem bark the reduction was significant at 12 hours. In contrast to oral antidiabetic agents the exogenous administration of Insulin is well known to produce hypoglycemia in normal and alloxan diabetic subjects[11, 12]. It is therefore conceivable that hypoglycaemic principle in the fruit and stem bark of Grewia asiatica exert a direct effect on the diabetic rabbits probably by a mechanism similar to insulin. These drugs do not seem to act directly by stimulating the release of insulin as the alloxan treatment causes permanent destruction of β–cells[13]. In fact it may be supposed that in the normal rabbits these substances act not only by direct insulin like action but also by stimulating the release of insulin. It must however be accepted that the possibility of some other factors producing this alteration cannot be excluded at present. It may be hypothesized that the fruit and stem bark of Grewia asiatica contains more than one active principles which are potent in normal as well as in diabetic rabbits. This view is similar to that reported by others [14, 15] who reported that Momordica charantia possesses more than one hypoglycaemic principle i.e. functioning in normal and diabetic rabbits both. In literature the antidiabetic activity of the seed extract of Strychnos nuxvomica[16] and the fruit extract of Abelmoschus esculentus[17] has been linked to the hypoglycaemic and free radical scavenging activity. The hypoglycemic effect of Nyctanthes arbortristis Linn has been attributed directly to its regenerative and radical scavenging activity on the pancreas of alloxan induced diabetic rats[18]. The fruit of Grewia asiatica has also been reported to possess a strong radical scavenging activity[19]; therefore the antihyperglycemic activity of the fruit of Grewia asiatica may also be hypothesized to be attributed to its antioxidant and radical scavenging activity.

The crude ethanolic extract of the leaves produced no change in the blood glucose level of normal rabbits but significantly reduced the blood glucose level in diabetic rabbits. Gluconil does not produce hypoglycemia in normal animals, because the peripheral utilization of glucose is compensated by hepatic synthesis of glucose[20]. It seems that the leaves have an action similar to Gluconil and it can be said with certainty that there are some active principles that are functioning in diabetic rabbits

Further hypoglycaemic studies with the fractions of the crude extract of the fruit and stem bark and leaves indicate that the aqueous fractions produced a highly significant decrease in blood glucose level from 2 to 24 hours; the fruit exhibiting a much greater and better blood glucose lowering pattern as compared to the stem bark and leaves. The methanolic fraction of fruit showed significant reduction in blood glucose level at 24 hours.

Reduction in blood glucose level was observed, in case of stem bark at 2 hours and from 12- 24 hours and that in case of leaves at 8 and 24 hours. The butanolic fraction of the fruit and stem bark lowered the blood glucose level from 2 hours to 24 hours where as the butanolic fraction of the leaves showed prompt reduction in blood glucose level at 2 hours. Further reductions were significant from 8 to 24 hours as shown in Table 3 .The ethyl acetate fraction of the fruit and stem bark showed significant reduction at 2,4 and 24 hours whereas that of the leaves showed significant reduction from 2-24 hours. The chloroform fraction of the stem bark and leaves showed

significant reduction in blood glucose level from 2-24 hours and from 4-8 hours and at 24 hours respectively. The carbon tetrachloride fraction of the stem bark showed significant lowering from 2-12 hours and at 24 hours; whereas that of the leaves showed highly significant lowering in blood glucose level from 2-24 hours. The hexane fraction showed significant reduction from 8-12 hours as shown in Table 3. The statistically significant decline in the blood glucose level observed at later hours suggests that extracts either take time to reach the target tissues in the body or they get metabolized and the metabolites are active [21].

The presence of antihyperglycemic activity in the fruit, stem bark and leaves of Grewia asiatica provides a hopeful sign for the presence of some new hypoglycemic agents. Further research has to be carried out to find out the active compound(s) responsible for the antihyperglycemic activity such as the isolation and structural elucidation of the active constituents responsible for this activity.

Abbreviation; F-Fruit, S-Stem bark, L-Leaf, A-Aqueous, M-Methanolic, B-Butanolic, EA- Ethyl acetate, C-Chloroform, Ct-Carbon tetrachloride, H-Hexane.

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