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Phytochemical studies and hypoglycemic effects of *chenopodium album* extracts on alloxan induced hyperglycemic the albino rabbits.

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Abstract

Objectives: *Chenopodium album* (*Bathua*) is commonly occurring small herb and has found to have different biological activities. Folk medicine claims its use in the treatment of diabetes in many part of the world.
Method: Different extracts of plant investigated for phytochemical and hypoglycemic potentials, were obtained by successive solvent extraction of the whole plant parts using solvents of different polarities in an increasing order of polarity (Petroleum ether, chloroform and methanol). The phytochemical investigations revealed that all the three extracts of the plant have metabolites of different chemical groups notably glycosides, flavonoids, phenols. Chloroform extract and methanol extract also found to have alkaloids in them. The hypoglycemic investigation of *Chenopodium album* was carried out in alloxan (200 mg/kg) induced diabetic rabbits (albino) by treating them with crude extracts of this plant.
Results: Significant antidiabetic activity was evident with the Petroleum ether and Chloroform extracts at a dose of 100 µg/kg. The methanol extract did not show any hypoglycemic activity at this dose. Active extracts were tested with a double dose of 200 µg/kg and both extracts showed significant reduction in blood glucose level of the alloxan induced diabetic rabbits. The antidiabetic effect of *Chenopodium album* extracts was might be due to presence of glycosides, flavonoids and phenolic constituents.
Isolation of active constituents from these extracts can lead to new drug molecule discovery.

Keywords: Antidiabetic, Bathu, *Chenopodium album*, Hypoglycemic.

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Introduction

Diabetes mellitus is a chronic disease that has a high worldwide prevalence. It is a morbid condition and about 2.8% of the world's population is currently suffering from this disease. Recently studies conducted to estimate the prevalence of Diabetes Mellitus have shown that the number of diabetic patients has increased globally reaching up to 6.6% in 2010. This Figure 1 represents approximately 285 million people of the total population. This Figure 1 will rise to 7.8% in 2030. Reports show that the lower socio-economic groups of the developing countries will more adversely affected group in the future. Presently diabetes has become the most concerned public health challenge. Overall cost for the treatment and prevention of the disease will also increase, because of this high prevalence. The everyday increasing incidence of diabetes mellitus demands the discovery of more modern medicines using herbal source. The growing interests in the use of plants require extensive investigation to find out the hidden uses of medicinal plants. Although much work has been done to dig out the hypoglycemic potential of medicinal plants, WHO recommends more work and investigations in this regard. *Chenopodium album* belonging to the family Chenopodiaceae. This family has vast medicinal value [1]. Its antioxidant property enables its use for the treatment of different diseases as it causes a significant decrease in the production of free radicals. Some other plants of this family also possess hypoglycemic activity such as Chenopodium ambriosioides. *Chenopodium album* has distribution in natural habitat throughout Pakistan. It is a wild plant, which can grow at an altitude of up to 4700 m. It is also cultivated in all parts of India particularly Shimla, Kulu valley and in Rajhistan. Coquillqt (1951) stated that it is among those 5 plants that are widely distributed and grow well throughout the world.

Materials

Plant materials

The plant *Chenopodium album* (*bathua*) was collected in the month of May from Talla Adda near Raiwind Lahore. The plant was authenticated by Dr. Sultan Herbarium GC University Lahore and a voucher specimen number was issued against the plant GC.Herb.Bot.2262.

Chemicals and solvents

Alloxan monohydrate, n-hexane, Chloroform, Methanol and different chemicals reagents were used in the study. All the

other chemicals and solvents used in this project were of B.D.H grade as mentioned or otherwise stated.

Methods

Extraction of the plant material

The plant material was extract by using the maceration process. Plant material about 15-20 kg was air dried in shade for about 15 days at room temperature. The dried plant material was broken into small pieces and then was subject to grinding using a commercial grinder. Plant powder about 1kg, was weighed and taken into a five liter round bottom flask. Successive Extraction of the plant material carried out using three solvents of different polarities successively. The plant material macerated with Petroleum ether, Chloroform and Methanol for 8 days respectively in each of the solvent [2]. These three successive solvent extracts were concentrated by using Rotary Vacuum Evaporator. The extracts further dried in oven at 38ºC for complete drying and stored in refrigerator.

Phytochemical analysis

The phytochemical analysis of different extracts carried for the detection of secondary metabolites groups present in the extracts. Chlorophyll removed from the extracts for better observation of results. For this lead acetate solution was added to extracts and shaken well. The extract formed precipitates with lead acetate solution. The precipitated solution was filtered and the filtrate was used for phytochemical analysis after drying. Phytochemical analysis was performed by following standard methods.

For animal study was conducted after taking written approval from animal ethics committee of the college of pharmacy, university of the Punjab Lahore Pakistan. Healthy albino rabbits of 1.5-2 kg average weight were selected and divided into 5 groups each having 5 rabbits (n=5). The rabbits were got acclimatize for one week in the animal house of the college and kept on standard fodder diet and water ad libitum.

Blood sampling

Blood samples were drawn from the marginal vein after shaving and treating with xylene using sterile needle. The blood glucose levels were measured by using glucometer (Lever check glucometer, model TD-4225) as prescribed and used by alarcon.

Induction of diabetes

Alloxan Monohydrate was used to induce diabetes in animals at a dose of 200 mg/kg. Freezing point depression method was used to make isotonic solution. After intraperitoneal administration, the rabbits were monitored for about 8 hours. The rabbits were given free access to 25% Dextrose saline solution and after 2 hours of administration of Alloxan they were given simple syrup orally to treat initial hypoglycemia induced by Alloxan. Rabbits showing persistent high sugar levels (>200) were then selected for the study.

Treatment groups

The diabetic rabbits were placed in five groups, namely Diabetic control (untreated), Positive control (treated with Glibenclamide at dose of 1 mg/kg), Petroleum ether extract group, Chloroform extract group, Methanol extract group. The rabbits were treated with plant extracts (100 µg/kg body weight) for fourteen days and blood samples were withdrawn 24 hours after the administration of the extract [3]. Finally the hypoglycemic effect was studied with 200 µg/kg doses of the active extracts for 7days.

Statistical analysis

Statistical analysis of the hypoglycemic studies were performed. Means and standard errors of means were calculated. The significant reduction in blood glucose levels was estimated by One way ANOVA following Dunnet's test, values lower than the diabetic control were consider as significant with $p<0.05$.

Results

Phytochemical analysis of all the three extracts of *Chenopodium album* revealed that the petroleum ether extract contains glycosides, saponins, flavonoids, fats and fixed oils, terpenoids and phenolic compounds.

The chloroform extract contains alkaloids, glycosides, saponins, flavonoids, fats and fixed oils, terpenoids and phenolic compounds. Methanolic extract of *Chenopodium album* contains alkaloids, glycosides, tannins, saponins, flavonoids, lignans, fats and fixed oils, terpenoids and phenolic compounds (Table 1).

Tests	Petroleum Ether Extract	Chloroform Extract	Methanolic Extract
Alkaloids	–	+ + +	+ + +
Glycosides	+ + +	+ + +	+ + +
Sugars	—	—	–
Proteins	–	–	-
Tannins	-	-	++
Saponins	+ + +	+ + +	+ + +

Flavanoids	+ + +	+ + +	+ + +
Lignans	–	–	+ + +
Terpenoids	+ + +	+ + +	+ + +
Fats and Fixed Oils	+ + +	+ + +	+ + +
Phenolic compounds	+ + +	+ + +	+ + +

Table 1. *Phytochemical analysis of Chenopodium album.*

The hypoglycemic studies were carried out on albino rabbits with all the three extracts of *Chenopodium album*. These studies revealed that blood glucose level was significantly reduced with petroleum ether and chloroform extracts (Table 2 and Figure 1)

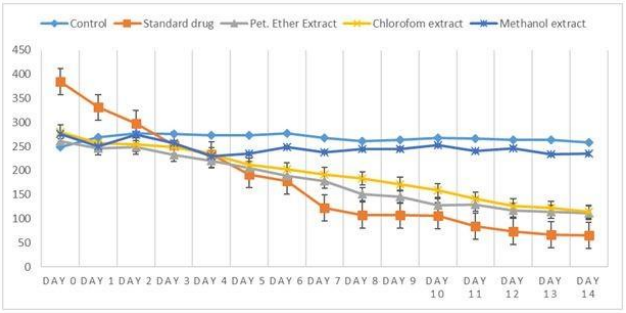

Figure 1. *Graphical representation of the positive control, negative controls and test extracts at 100 µg/kg.*

However the methanol extract did not exhibit hypoglycemic potential. Once the hypoglycemic potential of the Petroleum Ether and the Chloroform extract was established with the initial dose of 100 µg/kg, further studies were conducted by doubling the dose of the two active extracts (200 µg/kg).

These studies were conducted for 7 days and it was observed that there was a remarkable reduction in the blood glucose levels of the rabbits (Table 2 and Figure 2).

Groups	**Mean ± Standard Error of Mean**						
	Base line	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6
Diabetic Control	249.3 ± 27.7	269.3 ± 28.7	277 ± 24.5	276.33 ± 23.77	273.66 ± 18.35	272.66 ± 6.35	277 ± 13.86
Glibenclamide Treated Group	384.6 ± 60.6	331 ± 71.2	297.33 ± 55.66	252.66 ± 60.46	233.33 ± 58.48*	192 ± 29.14*	177.66 ± 30.69*
P.E Treated Group	304 ± 93.6	266.6 ± 97.8	233.33 ± 75.4	204 ± 52.48	180.66 ± 40.1*	153.33 ± 24.3*	118 ± 21.1*
CHCl3 Treated Group	260.3 ± 33.2	253 ± 32.8	224.66 ± 34.6	183.33 ± 14.31	159.66 ± 9.7	135.667 ± 13.8*	109 ± 5.5*

Table 2. *Statistical analysis of different groups treated with Standard and tested drugs at 200 µg/kg.*

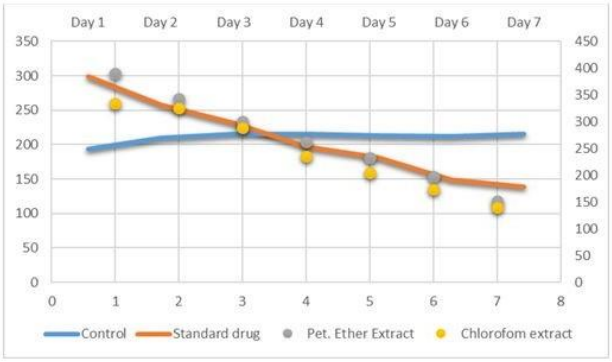

Figure 2. *Graphical representation of the positive control, negative controls and test extracts at 200 µg/kg.*

Discussion

Chenopodium album is very important herb, found frequent in wheat fields and is used traditional herbal system for the treatment of diabetes [4]. Phytochemical results showed that this plant is rich in glycoside, phenolic compounds, lignans and also contain alkaloids. These results were found in accordance with the previous work of different scientists. The different chemical constituents present in *Chenopodium album* are β-sitosterol, Campesterol, Xanthotoxin, Stigmasterol, n-triacontanol, imperatorin, Saponins, apocarotenoids, crytomeridiol, n-transferuloyl-4-O-methyl dopamine, lupeol and 3-hydroxy nonadecyl henicosanoate. An unusual xyloside was also reported to be discovered from the plant. The essential oils isolated from *Chenopodium album* were Limonene, α-terpinyl acetate, α-terpinene and cis-ascaridole. Chenopodium contains pyridine, piperidine and tropane alkaloids. Chenoalbicine was the alkaloid isolated from the plant. The phytochemical analysis also revealed that it contains Alkaloids. The phytochemical analysis indicated the presence of Glycosides and the previous studies have shown that the plant contains a phenolic glycoside named Chenoalbuside.

Seven lignans were isolated from the hydroalcoholic extract of leaves of *Chenopodium album* after precipitation with acetone. These were pinoresinol, lariciresinol and its derivative compound, syringaresinol and three sesquilignans. The phytochemical analysis also revealed that Lignans are present in the plant. The different extracts of the plant showed positive tests for the presence of Tannins and it has been already reported that the plant contains Tannins such as Catechins and Gallocatechins. 4-vinyl phenol was isolated from the aqueous extract of the plant. Previously phytochemical analysis showed that the plant contains saponins isolated from the roots of the plant. The phytochemical analysis of the whole plant indicated that it contains saponins. *Chenopodium album* contains high and balanced amounts of proteins and amino acids with high contents of Lysine (5.1-6.4%) and Methionine (0.4-1.0%) [18]. However all the three extracts gave a negative test for the presence of proteins and amino-acids. It has been reported that the variation in the climatic and environmental conditions may affect the different constituents present in plants. The three major flavonoids isolated from *Chenopodium album* are Quercitin, rutin and kaempferol. The plant extracts also gave positive results for the presence of flavonoids. These flavonoid containing medicinal plants are used as antifungal, anti-inflammatory and antibacterials.

Hypoglycemic studies showed that petroleum ether and chloroform extract were active biologically as both of them reduced sugar level significantly at the end of the treatment time. The statistical analysis revealed that there was no significant reduction in blood glucose level in the initial seven days using 100 µg/kg dose of the extracts, but afterward there was a significant decrease with the standard drug (Glibenclamide) P value 0.028 and with the petroleum ether extract showed a significant reduction in blood glucose level (P value 0.003). The chloroform extract showed significant reduction in blood glucose levels at day11 with the significance value 0.026. However, the methanol extract did not show any significant reduction in blood glucose levels even at the end of the treatment period (Table 2 and figure 1).

Double doses (200 µg/kg) of the petroleum ether extract and chloroform extracts showed comparatively rapid reduction in blood glucose levels as compared to initial dose of 100µg/kg. Significant reduction in the blood glucose level was observed on the 5th day of treatment with P value 0.001 and 0.002 with petroleum ether extract and chloroform extract of the plant respectively. The reduction in blood glucose level with petroleum ether and chloroform extracts while no reduction in case of methanol extract is justified as both these extract contain comparatively low polarity organic compounds that usually possess pharmacological activities [5]. In addition, these extracts contains phenolic compounds usually having antioxidant activity and can act against free radicals that contribute to the high glucose level.

Conclusions

This study concluded that the petroleum ether and chloroform extracts of the whole plant of *Chenopodium album* possess hypoglycemic potential. This may be due to the presence of glycosides, alkaloids, flavonoids or phenolic compounds, the presence of which was confirmed by the phytochemical analysis. Particularly the presence of flavonoids and phenolic compounds have been reported to be responsible for curing different diseases caused by oxidative stress including the diabetes. Further investigations are required to determine the active constituents that are responsible for the hypoglycemic activity of the plant. Column chromatography can be used to separate out the components from the biologically more active petroleum ether and chloroform extracts. Furthermore these isolated compounds may be characterized by spectral analysis of the constituents. It will lead to the establishment of structure activity relationship.

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Conflict of Interest

No conflict of interest associated with this study.

Contribution of Authors

It is declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the contents of this article will be borne by them.

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