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Antidiabetic Potential of Different Solvent Extractions of *Chenopodium botrys*

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ABSTRACT

Aims and objectives The current study was designed to explore the antidiabetic effect of different solvent extracts of *Chenopodium botrys* (*C. botrys*). ***Material and methods*** Chemicals and equipment used in the experiment were of analytical grade. Glucometer (Mode lAccu-Chek Performa) was used for glucose assessment. C. *botrys* was collected in August from the area of Lower Dir, Khyber Pakhtunkhwa, Pakistan. The collected plant materials were washed with tap water and air-dried at room temperature for twenty days. Male mice of albino type, 8-10 week old, weighing 25-28 g were used. Diabetes was induced by injecting freshly prepared alloxan monohydrate (98%) in distilled water with injection volume 20ml/kg at a dose of 90mg/kg (I.P) to the study groups. ***Results*** Results revealed the presence of flavonoids, phenols, saponins and tannins in the crude extracts of *C. botrys*. Flavonoids, phenols and tannins were detected in all three extracts while the alkaloids test was negative for all three extracts. Similarly, saponins were only detected in methanol and ethanol extracts but absent in water extract. All the extracts on treated mice did not show any sign of toxicity during study period. Oral administration of extracts and standard drug significantly reduced the fasting blood glucose levels in methanol, ethanol, water and glibenclamide groups as compared to a diabetic control group. The blood-glucose-lowering effect was more pronounced in methanol extracts at a dose of 1g/kg when orally administered. ***Conclusion*** From this study it can be concluded that *C. botrys* methanolic extract exhibited the antidiabetic effect in alloxan-induced diabetic mice. This study had validated the folklore use of *C. botrys*for the cure of diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by persistent hyperglycemia either caused by a defect in insulin secretion, insulin action, or both, as well as disturbances of carbohydrate, fat, and protein metabolism. Polyuria, thirst, blurred vision, weight loss, and genital yeast infection are the most common symptoms associated with diabetes mellitus (World Health Organization, 2019). Diabetes mellitus exist in two main forms; Type I Diabetes Mellitus (T1DM) and Type II Diabetes Mellitus (T2DM). T1DM is the result of autoimmune β cells destruction characterized by lack of insulin secretion whereas T2DM is due to progressive loss of β cells function caused by insulin resistance (Katsarou et al., 2017). Both forms of DM is caused by a complex interplay of genetic, epigenetic, environmental factors as well as concurrent illness, autoimmunity, insulin resistance, inflammation, proteomic and metabolic process (Alonso-Magdalena, Quesada, & Nadal, 2011; Mlinar, Marc, Janež, & Pfeifer, 2007; Stankov, Benc, & Draskovic, 2013). International Diabetes Federation (IDF) reported in diabetes atlas 9th edition that globally approximately 463 million adults are living with diabetes and this number will rise to 700 million by 2045 (International Diabetes Federation, 2019). Good glycemic control in DMs has shown to prevent macrovascular and microvascular complications (Feingold, 2019). Nonpharmacological therapy such as a healthy diet, regular physical activity and weight loss has crucial role in the treatment of diabetes (Nagi & Gallen, 2010). Pharmacological therapy is essential in the treatment of diabetes and prevention of microvascular and macrovascular complications and includes insulin preparation, sulfonylureas, glinides, biguanides, thiazolidinedione, α-glucosidase inhibitors, dipeptidyl peptidase-IV inhibitors, sodium-glucose transporter-2 (SGLT-2) inhibitor (Brunton, Hilal-Dandan, & Knollmann, 2018).

In recent years, herbal medicines due to less side effects and affordable prices have attracted health practitioners and people to use them for the treatment of DM. In addition, research on hypoglycemic agents from plant has gained importance throughout the world. Since ancient times, peoples used folklore medicine of plants origin for the cure of DM (Ríos, Francini, & Schinella, 2015). Within this context, *Chenopodium botrys* (*C. botrys*) is a flowering plant in the genus chenopodium. *C. botrys* is an annual herb growing to 0.6 m (2ft). The plant is in flower from July to October and seeds ripen in this period. This plant is indigenous to the Europe, Asia, and much of North America (Morteza-Semnani, 2015). Review of different research articles has shown that *C. botrys* is utilized for the treatment of jaundice, diabetes, liver disease, fever, headache, itching, healing, stomachache, cough, expectorant,

hypertension, disturbed menstruation, digestive disorder, asthma, abdominal problem, uterus problem, and as well as anthelmintic, diuretic, laxative, tonic and anticonvulsant (Morteza-Semnani, 2015). Different studies conducted on phytochemical screening of *C. botrys* revealed the presence of alkaloids, flavonoids, tannins, terpenoids, anthraquinone, saponins, and phenols compounds (Kokanova-Nedialkova, Nedialkov, & Nikolov, 2009; Morteza-Semnani, 2015). A review of a research study has shown that *C. botrys* exhibited antibacterial, antioxidants, antidiabetic, and antinociceptive properties as well as enzyme inhibitory properties including acetylcholinesterase, α amylase, α glucosidase, and butyrylcholinesterase (Uddin, Rauf, Siddiqui, Khan, & Ullah, 2016). Therefore it is believed that components in *C. botrys* do have the antidiabetic potential that can be used against DM. Therefore the current study was designed to explore the antidiabetic effect of different solvent extracts of *C. botrys.* We believe that the activity (if reported) can shape as a potential treatment option for DM for healthcare system and a proposal for future research.

MATERIALS AND METHODS

Chemicals

Chemicals used in the experiment were of analytical grade purchased from standard commercial sources. Alloxan monohydrate (Lot No.SVBR77455) was purchased from sigma Aldrich. Glibenclamide tablets (batch No.WC040) were obtained from Sanofi Aventis. Methanol, ethanol, deionized water and all other chemicals were provided by Center for Advanced Study and Vaccinology (CASVAB), University of Baluchistan Quetta.

Instruments

Glucometer (Mode lAccu-Chek Performa), and strips from Accu-Chek Performa (Lot No.47366) from purchased from Roche, Germany. Rotary Evaporator (STRIKE-202, Italy) and all other instruments were provided by CASVAB, University of Baluchistan Quetta.

Plant materials

C. botrys was collected in August from the area of Lower Dir, Khyber Pakhtunkhwa, Pakistan. The plant was identified by Wisal Muhammad Khan, Department of Botany, Islamia College Peshawar. The plant was submitted to the herbarium of the same University for future reference.

Preparation of plant extracts

The collected plant materials were washed with tap water and air-dried at room temperature for twenty days. The dried plant materials were then minced and homogenized properly. For the preparation of methanol extract, *C. botrys* powder was soaked in 80% methanol and filtered through muslin cloth. Filtrates were mixed and evaporated under reduced pressure at 45^{o}C temperature using rotary evaporator and residue was acquired (Ullah et al., 2017). Similarly, *C. botrys* powder of 5g was soaked in ethanol and deionized boiled water. After filtration through muslin cloth, ethanol extract was dried by using rotary evaporator at 40^{o}C. After evaporation of ethanol, *C. botrys* ethanolic (CBE) extract was obtained. Similarly, after filtration through muslin cloth *C. botrys* water (CBW) extract was obtained by freeze-drying the filtrates (Ozer, Sarikurkcu, & Tepe, 2016).

Animals

Male mice of albino type, 8-10 weeks old, weighing 25-28 g were obtained from the animal house of CASVAB, University of Baluchistan, Quetta.

Induction of experimental diabetes in mice

Male mice weighing 25-28 g were housed in a clean cage at 24C^{o} ±1 and 55±5% humidity, with a 12-hrs light-dark cycle. Animals were provided lab food and water. All mice were divided equally in experimental groups and control groups. After one week of acclimatization, all mice were kept in fasting conditions for 12-hrs. Diabetes was induced by injecting freshly prepared alloxan monohydrate (98%) in distilled water with injection volume 20ml/kg at a dose of 90mg/kg (I.P) to the study groups. Similarly, equal volume of distilled water was injected into the control group. After three days of injection, fasting blood glucose was measured. The mice with fasting blood glucose levels (BGL) higher than 200mg/dl were diabetic and used for the study (Mukundi et al., 2015).

Experimental design

In the experiment, mice were divided into six groups (Table 1). Group 1 (normal control) received 0.9% normal saline 5ml/kg, Group 2 (diabetic control) received 0.9% normal saline 5ml/kg, Group 3 received glibenclamide 10 mg/kg, Group 4 received *C. botrys* methanol (CBM) extracts 1g/kg, group 5, received CBE extracts 1g/kg and group 6 received CBW

extracts 1g/kg. Treatment was given once daily for 10 consecutive days. All the mice had free access to normal lab food and water during the treatment period.

Table 1: Group allocation for the experimental animals

S. No	Groups	Group properties
1.	Group 1	Normal mice untreated
2.	Group 2	Diabetic mice untreated
3.	Group 3	Diabetic mice treated with glibenclamide 10mg/kg
4.	Group 4	Diabetic mice treated with 1g/kg of CBM extracts
5.	Group 5	Diabetic mice treated with 1g/kg of CBE extracts
6.	Group 6	Diabetic mice treated with 1g/kg of CBW extracts

Phytochemical screening

Preliminary qualitative phytochemical screening of CBM, CBE, and CBW extracts was carried out according to the following methods:

Flavonoids: Plant extracts of 0.2 g were mixed with sodium hydroxide and then small amount of hydrochloric acid was added. Discoloration of yellow solution showed the existence of flavonoids (Uddin et al., 2016).

Phenols: Plant extracts were mixed in a test tube with 1ml of distilled water followed by the addition of ferric chloride solution. The development of blue, green or red color revealed the presence of phenol contents (Tepal, 2016).

Alkaloids: Plant extracts of 50 mg dissolved in 10 ml of dilute hydrochloric acid with continuous shaking. Extracts was filtered and added two drops of Mayer's reagent to the solution. The appearance of white precipitates indicated the presence of alkaloids (Khan, 2019).

Saponins: Dried plant extracts of 200 mg was shaken and boiled with 5ml of distilled water. The formation of frothing revealed the existence of saponins (Uddin et al., 2016).

Tannins: Dried plant extract of 500 mg mixed with 20 ml water and heated on water bath. After filtration, few drops of ferric chloride (0.1%) solution were added. The formation of brownish-green color or bluish-black color showed the presence of tannins (Uddin et al., 2016).

Acute oral toxicity

Acute oral toxicity test in albino mice of both gender was carried out followed the method of with slight modification (Vaghasiya, Shukla, & Chanda, 2011). Animals were divided equally into seven groups and each group contained 4 mice (Table 2). After dosing all the mice were continuously observed daily for 2 hours up to one weak for behavioral changes (such as hypo activity, ataxia, irritability, stereotypy, Straub tail and convulsion) and other toxicity symptoms such as diarrhea, coma and mortality. Change in food habit and water consumption was also observed (Vaghasiya et al., 2011).

Table 2: Acute toxicity test for all mice

S. No	Groups	Dosing
1.	Group A	Normal mice received normal saline 0.9% 5ml/kg
2.	Group B	Normal mice received CBM extracts 500 mg/kg
3.	Group C	Normal mice received CBE extracts 500 mg/kg
4.	Group D	Normal mice received CBW extracts 500 mg/kg
5.	Group E	Normal mice received CBM extracts 1000 mg/kg
6.	Group F	Normal mice received CBE extracts 1000 mg/kg
7.	Group G	Normal mice received CBW extracts 1000 mg/kg

Measurement of Blood glucose levels (BGL)

Fasting BGL were measured in overnight 12-hr fasting mice before the beginning of treatment on first day and after treatment on day 10^{th} i.e. last day of treatment. Blood samples were collected 2-hr after last administration. Blood glucose was determined with an Accu-Chek Performa glucometer.

RESULTS AND DISCUSSION

RESULTS

Phytochemical analysis

The preliminary qualitative phytochemical test of CBM, CBE and CBW extracts were conducted as per the prescribed procedure (Table 3). Results revealed the presence of flavonoid, phenols, saponins and tannins in the crude extracts of *C. botrys*. Flavonoids, phenols and tannins were detected in all three extracts while the alkaloids test was negative

for all three extracts. Similarly, saponins were only detected in methanol and ethanol extracts but absent in water extract.

Table 3: Qualitative phytochemical test of CBM, CBE and CBW extracts of *C. botrys*

S. No	Phytochemicals	CBM extracts	CBE extracts	CBW extracts
1.	Flavonoids	Present	Present	Present
2.	Phenols	Present	Present	Present
3.	Alkaloids	N/D	N/D	N/D
4.	Saponins	Present	Present	N/D
5.	Tannins	Present	Present	Present

N/D: not detected

Oral toxicity analysis

In acute oral toxicity test, all three plant extracts were given in two different doses (500 mg/kg and 1000 mg/kg) to designed group of normal mice. All the extracts on treated mice did not show any sign of toxicity during study period. No mortality and coma was observed up to one week in either sex. All the extracts treated mice had normal food and water consumption when compared to control mice. Results indicated that CBM, CBE and CBW extracts were harmless to a dose level of 1000mg/kg (Table 4).

Table 4: Acute oral toxicity of CBM, CBE and CBW extracts in mice

Groups	Extract dose	Behavioral changes	Diarrhea	Coma	Mortality	Survival
A	N/S 5 ml/kg	0/4	0/4	0/4	0/4	All
B	CBM 500 mg/kg	0/4	0/4	0/4	0/4	All
C	CBE 500 mg/kg	0/4	0/4	0/4	0/4	All
D	CBW 500 mg/kg	0/4	0/4	0/4	0/4	All
E	CBM 1000 mg/kg	0/4	0/4	0/4	0/4	All
F	CBE 1000 mg/kg	0/4	0/4	0/4	0/4	All
G	CBW 1000 mg/kg	0/4	0/4	0/4	0/4	All

Data presented as sign of toxicity/No. of mice; N/S: normal saline

Antidiabetic profile assessment

Oral administration of extracts and standard drug significantly reduced the fasting blood glucose levels in CBM, CBE, CBW and glibenclamide groups as compared to the diabetic control group. The blood glucose-lowering effect was more pronounced in CBE extracts followed by glibenclamide, CBM and CBW extracts. Our result demonstrated that CBM extracts significantly reduced the blood glucose levels at a dose of 1g/kg when orally administered (Table 5).

Table 5: Antidiabetic profile of *C. botrys*

Extract	Pre blood group level Mean values	Post blood group level Mean values
Normal	653	661
Diabetic	2055	2068
Glibenclamide	2048	1015
Methanol	2078	1081
Ethanol	2050	1245
Water	2067	1206

DISCUSSION

It is essential to determine the phytochemicals of plant extracts before study their pharmacological activity. The phytochemical evaluation of CBM, CBE and CBW extracts were carried out for analysis of flavonoids, phenols, tannins, alkaloids and saponins as per the previously described method. The proposed study confirmed the presence of flavonoids, phenols, saponins and tannins in crude extract of *C. botrys*. The result of all extract for flavonoids, tannins and phenols is positive and resembles to the result reported by other researchers (Khan, 2019; Ozer et al., 2016; Uddin et al., 2016; Ullah et al., 2017). Our investigation of CBM extracts identified saponins similar to that reported by Uddin et al in the same extracts (Uddin et al., 2016). Ethanol extracts also reveal the existence of saponins similar to the results of (Ozer et al., 2016). Saponins were not identified in water extracts that resembled to that reported by Uddin,G et al (2016) but different from the result of Ozer, M.S et al (2016). This may be due to different geographical locations, method of test and sampling techniques. Alkaloids were not detected in all the three extracts this result is similar to (Uddin

et al., 2016) but showed deviation from the result of Khan (Khan, 2019). The reason of deviation might be due to different source of plant collection, method of extraction and environmental condition etc. Our result for acute oral toxicity test indicated that the crude extracts of *C. botrys* were harmless up to a dose of 1000 mg/kg which is consistent with the study of Khan (2019), in which it was reported that CBM extract up to a dose of 2000 mg/kg was safe for in vivo used in rodents.

The antidiabetic effect of CBM is consistent with that of (Khan, 2019) investigation, in which it was observed that CBM had markedly decline the blood glucose in alloxan-induced diabetic rats when administered i.p up to a dose of 400 mg/kg. The CBE extracts had shown highly marked reduction of fasting BGL at a dose of 1g/kg when orally administered in diabetic mice. As far as our literature review could find out, there was no data concerning the blood glucose-lowering effect of CBE extracts. It was reported that CBE extract exhibited alpha amylase and alpha-glucosidase inhibition activity (Ozer et al., 2016). Alpha-glucosidase inhibitor reduced postprandial hyperglycemia and used for the treatment of DM (Bischoff, 1994). Similarly, CBW extracts also shown a remarkable blood glucose-lowering effect as compared to diabetic control group. Unlike CBM extracts, CBW extracts blood glucose-lowering effect has not been previously reported in alloxan-induced diabetic mice. Our results indicated that glibenclamide orally given to alloxan diabetic mice at a dose of 10 mg/kg produced the significant hypoglycemic effect.

CONCLUSION

From this study, it can be concluded that *C. botrys* methanolic, ethanolic and water extracts exhibited the antidiabetic effect in alloxan-induced diabetic mice. This study had validated the folklore use of *C. botrys* for cure of diabetes. However further comprehensive pharmacological study is needed to isolate the active ingredients which are responsible for the reduction of blood glucose level and to study their mechanism that is responsible for antidiabetic effect.

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