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Compare the Hypoglycemic Properties of Ultrasound Assisted Aqueous Extract of *Argemone mexicana* with methanol extract

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Abstract

Argemone mexicana is locally known as shial kata, is widely grown in abandoned fields all over Bangladesh. Ethnobotanical study witnessed that roots, latex, stems, leaves etc. all are medicinally rich and widely prescribed in different communities of Santal tribe for the different diseases along with diabetes. The present study was designed to scan the anti-diabetic properties of the whole plants of *A. mexicana*. The study was also emphasized on developing a green extraction method by introducing ultrasound assisted extraction in aqueous medium from fresh plants. The intended crude extracts were then compared to the conventional extraction methods such as aqueous decoction and the methanol cold extraction method. Hypoglycemic study was conducted by using glucocorticoid hormone dependent diabetes mice and the potency was compared to the standard antidiabetic drug glibenclamide. Results indicated that chronic administration of the crude extracts obtained from *A. mexicana* plants significantly reduced the fasting glucose level of the diabetic animal model. Even a slight weight gaining effect was also observed during the treatment by *A. mexicana* plant's extract.

Keywords: *Argemone Mexicana*, Prickly Poppy, Green Extraction, Ultrasound

Introduction

The prickly poppy [1] (Fig 1) is locally known as "Shial Kata" in Bangladesh [2] recognized as an extremely growing weed commonly observed in the cultivated and abandoned land. The scientific name of "Shiail Kata" is *Argemone mexicana* L. and belongs to the family Papaveraceae [3] which has more than 30 species. The plant has prickly stems, leaves, capsules, glabrous (check meaning) and branching herb with eye-catching yellow flowers [4]. Along with Bangladesh, the plant is widely grown in many tropical and subtropical regions in the world such as India, Ethiopia, United States etc. [3, 5]. It is a popular medicinal plant and widely prescribed by the traditional and the folk medicinal practitioners for the treatment of several ailments [6-8]. From ethnobotanical study it was observed that the Santal tribal practitioners at the Village Jamtala of Chapai Nawabganj District, Bangladesh prescribed latex juice for the treatment of jaundice [6]. They also widely prescribed root paste and latex for the treatment of skin cracks [6]. Tribal practioners of Santal tribe of Joypurhat District, Bangladesh, also widely prescribed juice of roots, latex and the curry of stems for the treatment diuretic, diabetes, jaundice, itches, skin disease etc. [7]. Ethnobotanical studies also observed that the folk practioners of Sabgram village of Bogra, Bangladesh also prescribed different parts of Shiail kata for the treatment of skin cracks, dropsy, jaundice warts, tumours, cancer, cutaneous affections etc. Several studies also described its analgesic [9], anti-bacterial [9, 11], anti-diabetic [12], anti-helminthic [11], anti-inflammatory [11], anti-malarial [9], anti-oxidant [10], anti-spasmodic [9], cold sores [13], dropsy [13], jaundice [13], sedative [9], narcotic [9], skin diseases such as cutaneous infections, itches, warts etc. [13], effects and even sometime as an antidote to snake poisoning [14-15].

In the present study, the whole plant of *A. mexicana* was used to prepare crude extract by using an optimized green extraction method known as "Aqueous Ultrasound Assisted Extraction (UAE) from Fresh Plants'' as per the process proposed by Sadat *et al*. [13]. The basic principle of the UAE method is to utilize ultrasonic waves on the vegetal material that breaks the cells and releases the cells' contents into the extraction medium [14]. This method is capable of reducing the use of extraction solvents, processing time, and therefore, energy consumption enhancing during the extraction of the desired biocomponents [15-18].

In the present study anti-diabetic properties of *A. mexicana* was studied by using the crude extract prepared by using a green extraction method named "aqueous Ultrasound Assisted Extraction (UAE) from fresh plants". In the previous studies, the

above-mentioned green extraction method by using *A. mexicana* was designed and validated compared to the conventional popular extraction method [16]. From the previous studies it was observed that the aqueous UAE crude extract are rich in different potential phytochemicals such as alkaloids, anthraquinones, flavonoids, glycosides, saponins, steroids, terpenoids, tannins etc. and showed promising antimicrobial activities on *Staphylococcus aureus* (Gram +ve) and *Salmonella typhi* (Gram –ve). The present study was solely designed to evaluate the hypoglycemic properties of aqueous UAE crude extracts obtained from fresh plants of *A. mexicana* on artificially developed diabetic animal models.

Fig 1: The Prickly Poppy or Shial Kata (*Argemone mexicana* L)

Materials and methods
Preparation of Crude Extracts
Healthy fresh plants including roots, stems, and leaves of *Argemone mexicana* were collected from the field before sunrise and immediately washed by the running tap water and distilled water. The clean plants were placed under air for drying the surface water and equally divided into three (A, B, C) parts for getting extracted through (A) ultrasound treatment method, (B) decoction method and (C) methanol cold extraction method respectively.

Processing of Part-A: Within 6 hours 100 gm of whole plants were taken in a conventional juice blender machine by addition distilled water q.s. to 500 ml [17]. The whole juice was passed through a 20-mesh size net for getting fine particles and transferred to a 500 ml conical flask. Juice was placed in the ultrasonic bath (Power Sonic 405) for 30 minutes of ultrasonic treatments at 40^{oC} bath temperature. The mixture was then filtered by three layers of cloth and dried at 55^0C temperature in a water bath.

Processing of Part-B: Juice of 100 gm whole plants in 500 ml distilled water was prepared and transferred to a 1000 ml beaker and boiled 10 minutes before filtration by three layers of cloth and dried at 55^0C temperature in a water bath [16].

Processing of Part-C: 100 gm of whole plants were allowed for shade drying and prepared fine powder by blender. The powder was mixed in methanol at the ratio 1: 5 for 72 hours [16]. The mixture was then filtered by three layers of cloth and dried at 55^0C temperature in a rotary evaporator.

All the above dried crude extracts were collected in the glass vial and preserved in the refrigerator before conducting hypoglycemic study on animal models.

Enrolment of animal in the Study
Two weeks old *Swiss albino* mice were collected from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR'B) and they were kept in a plastic cage under laboratory condition of pleasant temperature and humidity with 12 hours light/dark cycle. They were supplied standard diet and allowed free access to the water [17]. After six weeks nursing and adjustment with the condition, healthy mice (average weight 25±3 gm) for control group were separated including four mice of either sex (Table-1) and maintain the ideal condition. Rest mice were administered intraperitoneal injections of dexamethasone (to induce glucocorticoid hormone dependent diabetes) at 10 mg/kg/day basis for around 7 days [18]. Diabetic conditions and intensity were measured by Oral Glucose Tolerance Test (OGTT) as per WHO guideline [19-20]. A 2 hours plasma glucose value during an OGTT of ≥11.1 mmol/l (200 mg/dl) considered as diabetic condition [19-20]. Diabetic mice were segregated into 5 groups (Table-1) including four mice of either sex in each on the basis of their average weight (25±3 gm) and average OGTT level (250±50 mg/dl). All groups were treated as per Table-1 and allowed the ideal condition, food & water similar to the healthy control. Fasting Plasma Glucose (FPG) test was performed periodically for detecting the hypoglycemic nature of the crude extract on animal model. Fasting is defined as no calorie intake for the last eight hours and fasting plasma glucose (FPG) levels ≥7 mmol/l (126 mg/dl) is considered as diabetic condition [19-20]. During the study maximum 15 hours fasting were allowed. The test drugs were administered orally by gavaging method after 8 hours fasting of taking morning feed. Blood samples were drawn 2-hours after administration of drug by punching the tail-tip of the mice. Determination of the blood glucose level was done by the glucose-oxidase principle using the ONE TOUCH basic instrument and results were reported as mmol/L (which was manually converted to mg/dl). Paired t-tests among the groups were conducted by SPSS software to compare the variation at 5% level of significance.

Table 1: Enrolment of animal and study design

Treatment group	Treatment Dose (Once daily)	Enrolment of mice (M+F)	Study of diabetic profile
Group I: Healthy control	Distill water	2 + 2	1. OGTT (for enrollment of diabetic mice in the study)
Group II: Diabetic control	Distill water	2 + 2	2. FPG at '0' hour (before treatment)
Group III: Glibenclamide	600 µg/kg-bw	2 + 2	3. FPG at 1st day
Group IV: Aqueous extract of *A. mexicana* by ultrasound	600 mg/kg-bw	2 + 2	4. FPG at 7th day
Group V: Aqueous extract of *A. mexicana* by decoction	600 mg/kg-bw	2 + 2	5. FPG at 14th day
Group VI: Methanolic extract of *A. mexicana*	600 mg/kg-bw	2 + 2	

Results and discussion

The whole plant of *Argemone mexicana* L. is occasionally used as diabetic remedy by the folk and tribal practitioners in Bangladesh. In the present study the hypoglycemic properties of *A. mexicana* whole plants were examined on artificially developed diabetic animals. The prime objective of this study was to assess the hypoglycemic properties of aqueous ultrasound assisted extract (Aq. UAE) of *A. mexicana* whole plants and subsequently compare the efficacy to the crude extract obtained from conventional extraction methods such as aqueous decoction and methanol cold extraction method. Hypoglycemic properties of crude extract *A. mexicana* whole plants prepared by Aq. UAE, decoction method and methanol cold extract method, were examined on glucocorticoid hormone induced diabetic animals by considering fasting plasma glucose (FPG) test procedure. Initially the OGTT test was performed to confirm the diabetes of the dexamethasone induced mice and classified them in the treatment groups on the basis of their average weight and average plasma glucose level. The results presented in the Table-2 indicated that all the treatment groups (G2, G3, G4, G5 & G6) were suffering almost similar diabetic condition (P>0.05 indicated by 'n') (Chart 1) and had plasma glucose level significantly higher (p<0.05) than the healthy control mice group. Similar conditions are also observed from the FPG data obtained immediately before administration of the first dose of treatment. After administration of the first dose of glibenclamide (standard anti-diabetic drug), a drastic change was observed. Whereas, very insignificant change compared to the standard drug was observed after the first dose of *A. mexicana* whole plant extract obtained by three different extraction methods. However, after regular administration of the Aq.UAE and methanol crude extract of *A. mexicana* whole plants showed significant reduction of plasma glucose level at 7th and 14th days which was almost similar to the standard antidiabetic drug glibenclamide. On the 14th day, it was observed that the intensity of the diabetic symptom was lowered in all the groups, which indicates the diminish effects of the dexamethasone on the animal. Nayak et. al., also stated the antidiabetic properties of the aerial parts of *A. mexicana* by using aqueous and ethanolic extract on alloxan induced diabetes rat model [21]. A significant (Table 2) change in the body weight was also observed during the study among all the treatment groups except the diabetic control (Group 2) and the aqueous decoction (Group 5). Though the difference of weight gain during treatment was not significant compared to the diabetic control, clear changes are observed in the Chart 2. The result indicates that the extract of *A. mexicana* may have some weight gaining properties which are normally lost in diabetic patients.
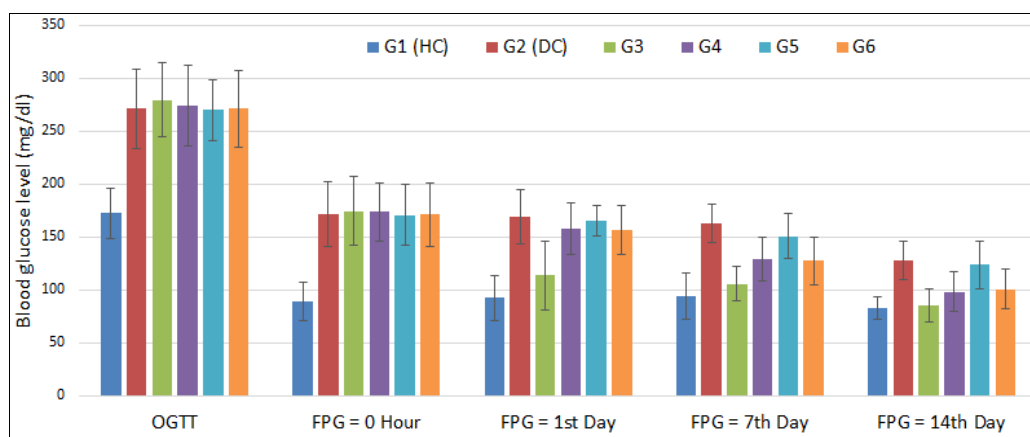

Chart 1: Effects of crude extracts of *A. mexicana* on glucocorticoid induced diabetic mice

Here, OGTT was performed to conform to the diabetic condition of the mice before enrolment in the study.
FPG at 0 level was performed to measure the intensity of diabetes before treatment.

FPG at Day 1, 7 & 14 were performed to measure the intensity of diabetes after treatment by single dose and multiple doses.

Table 2: Effect of drug or extracts on artificial diabetic mice

Experiment Group	Plasma Glucose Level (mmol/L) (Mean ± STD for n = 4)				
	OGTT at "2" Hour	FPG at "0" Hour	FPG at "1st" day	FPG at "7th" day	FPG at "14th" day
G1	172.4±24.1	88.7±18.1	92.3±21.5	94.1±21.3	82.8±10.6
G2	271.25±37.5[a]	171.5±30.4[a]	169.7±25.7[a]	162.5±17.9[a]	127.8±17.9[a]
G3	279.0±35.2[a,n]	174.2±32.6[a,n]	113.4±32.7[b,m]	105.8±16.5[b,m]	85.5±15.5[b,m]
G4	273.8±37.9[a,n,y]	173.5±27[a,n,y]	157.5±23.9[b,n,y]	129.3±20.4[b,n,y]	98.2±18.6[b,m,y]
G5	270.3±28.7[a,n,y]	170.8±28.9[a,n,y]	165.3±14.8[a,n,x]	150.7±21.6[b,n,y]	123.5±22.6[b,n,x]
G6	271.0±36.8[a,n,y]	171.3±29.8[a,n,y]	156.3±23.4[a,n,y]	127.3±22.4[b,n,y]	100.4±18.7[b,n,y]

Here, OGTT at "2" Hour ≥200 mg/dl indicate diabetic condition [19-20].
FPG level ≥ 126 mg/dl indicate diabetic situation [19]
[a]The difference compared to the healthy control (G1) was significant (p<0.05)

[b]The difference compared to the healthy control (G1) was not significant (p>0.05)
[m]The difference compared to the diabetic control (G2) was significant (p<0.05)

[n]The difference compared to the diabetic control (G2) was not significant (p>0.05)
[x]The difference compared to the standard drug (G3) was significant (p<0.05)
[y]The difference compared to the standard drug (G3) was not significant (p>0.05)

Table 3: Effect of Crude Extracts on body weight of experimented Mice after 14 days treatment

Treatment group	Body Weight Mean ± STD, for n = 4 (M+F)	
	Before treatment	After 14 days treatment
G1 (HC)	24.75±3.30	27.5±3.42[b]
G2 (DC)	24±2.94[a]	25.5±2.89
G3	24.25±2.75 [a]	28.75±2.50[b,c]
G4	24.5±2.65 [a]	28.5±4.2[b,c]
G5	24.25±3.30 [a]	24.5±4.2[b,c]
G6	24.75±3.09 [a]	27.75±3.5[b,c]

[a]The difference of average WBT is not significant (p>0.05) compared to the expected body weight (25 gm) analyzed by one sample t test. indicate that the weight variation among the test groups was minimum.
[b]The difference of WAT is significant (p<0.05) compared to the WBT analyzed by paired t test
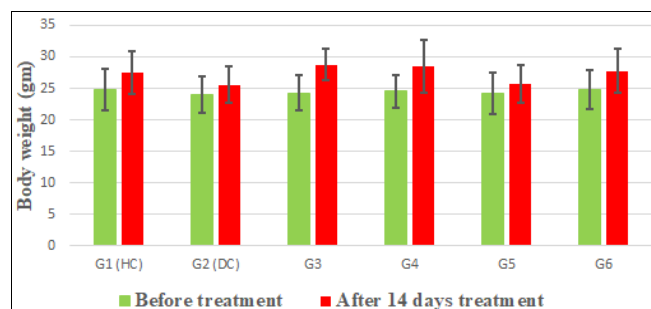[c]The difference of WAT is not significant (p>0.05) compared to the diabetic control analyzed by paired t test

Chart 2: Comparison of body weight after 14 days treatment

Conclusion
Crude extracts of *A. mexicana* were observed as potential hypoglycemic on diabetic animal animals compared to the standard antidiabetic drug. The extract also provides some weight gaining effects on diabetic animals. The study also indicated that the intended ultrasound extraction method was proved almost similar to the ethanolic cold extraction method and much better than the decoction method. No pharmacological changes were observed in the ultrasound assisted extract compared to the conventional ethanolic crude extract indicating the structural changes of the compounds did not happen after administration of ultrasound on natural products. Ultrasound assisted extraction occupied many points of green extraction methods such as cheap, rapid, hazards free, technically easy to operate etc. So, as a green extraction method the ultrasound assisted extraction may be recommended for larger scale production in the industry. From the above study, it was observed that the *A. mexicana* plants may be a promising source of natural drugs for the treatment of diabetes. In that case, the dried powder *A. mexicana* plants may be recommended for the people to consume as like green tea.

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