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mg/dL) and random plasma glucose ≥11.1 mmol/L (200 mg/dL) considered as diabetic (hyperglycemia or a hyperglycemic crisis) symptoms [4,5]. Majority of diabetes cases are classified as either type-1 (10-15%) or type-2 (85-90%), where abnormal insulin secretion is either caused by destruction of insulin-secreting pancreatic β-cells or, due to peripheral resistance of insulin [5]. Diabetes may also result from the imbalance of carbohydrate, protein and fat metabolism [6] responsible for characterized symptoms of diabetes including polyuria, polydipsia, polyphagia, and unexpected weight loss [7,8]. Lowering elevated blood glucose concentrations to the normal is the mechanism of action of most of the popularly administered oral hypoglycaemic drugs such as sulfonylureas, metformin, α-glucosidase inhibitors, troglitazone [8-10]. However, many of the above synthetic drugs have many side effects ranging from liver and cardiovascular toxicity, abdominal discomfort, flatulence, diarrhea etc. [6,11]. In that circumstance, natural medicine may be the good source for treating diabetes and associated diseases.

Several ethnobotanical studies on Garo [12,13], Koch [12], Hajong [12], Santal [14] Tribe in Bangladesh observed the popular uses of *Moringa oleifera* leaves for the treatment or minimizing the diabetes-like symptoms. Moringa oleifera is locally known as "Sajna or Sojna or sometime Khonjhon" [12] in different parts of Bangladesh. The plant is widely known as cabbage tree, drumstick tree, horseradish tree, ben-oil tree, benzoil tree etc. [15]. *M. oleifera*, is one of about thirteen species that belong to the Moringaceae Family popularly recognized as the Miracle Tree as it has wide range of beneficial effects [16]. Several studies reported that intraperitoneal administration with ethanol extract of *M. oleifera* leaf reduced blood glucose levels in fasted streptozotocin-induced diabetic animals [17]. In the present study crude extracts obtained from the fresh leaves of *M. oleifera* were treated by the ultrasound [18] was selected for hypoglycemic study on glucocorticoid hormone (created by dexamethasone) induced [19] diabetic mice.

Materials and Methods:

Study protocol:

Ultrasound Assisted Extraction (UAE) is a comparatively noble approach in the field of bioactive compound extraction from plant materials introduced at the end of the 20th century. In this method high-intensity and high-frequency sound waves are applied on the plant materials to facilitate the extraction process. Cell disruption, improved penetration, enhanced swelling, capillary effect, hydration process are the probable mechanisms for ultrasonic enhancement of extraction [20]. The popularity of this extraction method is increasing due to its green (environmental friendly) approach [21]. Several studies successfully replaced the hazardous organic solvents (generally used in the conventional extraction method) by the water [22-24]. In the present study, the hypoglycemic properties of *M. oleifera* obtained from fresh leaves through ultrasound assisted extraction by water (600 mg/kg-bw/dose/day) and conventional ethanolic extract (600 mg/kg-bw/dose/day) were compared to the glibenclamide (600 µg/kg-bw/dose/day) as an standard anti-diabetic drug (Table-1). The study was conducted on the glucocorticoid hormone (which was created by dexamethasone) induced diabetes mice [25].

Table 1: Enrolment of animal and study design

Treatment group	Treatment Dose (Once daily)	Enrolment of mice (Male+Female)	Study of diabetic profiling
Group I: Diabetic control	Distill water	2 + 2	1. FPG at '0' h 2. OGTT (after administration of 1st dose)
Group II: Glibenclamide	600 µg/kg-bw	2 + 2	
Group III: Aqueous UAE extract of *M. oleifera* leaves	600 mg/kg-bw	2 + 2	

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Group IV: Ethanolic extract of *M. oleifera* leaves	600 mg/kg-bw	2 + 2	3. FPG at 8th day (after 7 days treatment)

Collection and Identification of Plant Materials:
Fresh leaves of *Moringa oleifera* was collected from the Botanical Pesticide Garden of Institute of Environmental Science (IES) of Rajshahi University (RU), Bangladesh and duly identified by the taxonomist of the Department of Botany, RU and the herbarium specimens was preserved in the institute for further reference.

Preparation of Crude Extracts:
Healthy leaves of *Moringa oleifera* were collected from the previously selected plant before sunrise and immediately washed by the running tap water followed by distilled water. After shade drying of the surface water, the leaves were divided into Part A and Part B. The fresh leaves of *M. oleifera* of Part-A were blended in a conventional juice machine with distilled water (material solvent ratio 1:5) [21-24]. The juice was transferred to a 500 ml conical flask and placed in an ultrasonic bath (Power Sonic 405) for 30 minutes treatments at 40^{o}C bath temperature [22]. In Part-B, the week-long dried leaves powder (500 g) was macerated in 70% ethanol [17] at room temperature for 72 hours with intermittent shaking as per standard method. In both cases filtration was done by using three layers of polyester cloth followed by a filtered paper (Whatmann size no.1). The filtrate was dried at 60^{o}C in a conventional water bath. Dried crude extract of Part-A and Part-B was then stored in an air tight bottle and preserved in cold a chamber for further use.

Enrolment of mice in the Study:
Four weeks old healthy *Swiss albino* mice (weight 20-30 gm of either sex) were collected from the animal house of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR'B) and they were kept in plastic cages under laboratory condition of temperature and humidity and placed on standard diet and allowed free access to water with 12 hours light/dark cycle. For the experiments, a diabeic state was induced by intraperitoneal injections of dexamethasone (10 mg/kg/day) on healthy mice for around 7 days [25]. Grouping (4 groups) of mice was made on the basis of average weight and average FPG at '0' hour including 2 male and 2 female in a group (Table-1). Group I mice were considered diabetic control and were served only distilled water. Group II was treated by standard anti-diabetic drug glibenclamide (600 µg/kg-bw/dose) whereas Group III and VI were treated by aqueous UAE extract from fresh leaves (500 mg/kg-bw/dose) and ethanolic extract (500 mg/kg-bw/dose) respectively. Oral Glucose Tolerance Test (OGTT) was performed immediately after administration of the first dose of drug/extract and Fasting Plasma Glucose (FPG) level test was performed after 7 days of maintenance dose of drug/extract. As per WHO guideline, OGTT was performed by administering oral glucose solution (equivalent to 75 gm anhydrous glucose dissolved in water) to the mice and recording the glucose level at two hours after administration. Before OGTT, a 0-hour blood samples were taken from the tail tip under mild ether anesthesia from each animal of overnight fasting mice group. Drugs or extracts were administered one hour before performing OGTT. After OGTT, the maintenance dose of drug/extract continued till 7 days and FPG was performed in the 8th day morning at their overnight fasting condition. During the study time, all participating groups were placed in separate cages and supplied the same composition and quantity of food and same source of water.

Results and Discussion:

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Hypoglycemic properties of ultrasound assisted crude extract and ethanolic extract of *Moringa oleifera* leaves were examined on glucocorticoid hormone induced diabetic mice by using OGTT and FPG test procedure. The FPG level at '0' hour (Table-2) and IBW (Table-3) indicate the enrolled mice groups were similar in diabetic condition and weight. After 1 hour of administration of standard drug and extracts, the OGTT study was performed on the diabetic mice showed the hypoglycemic properties of the both extracts of *M. oleifera* (≥11.1 mmol/L) which were almost similar to the standard antidiabetic drug glibenclamide (Chart 1). In the OGTT study, the extracts and drug showed a significant ($p<0.05$) (Table-2) glucose lowering effect compared to the diabetic control and became successful to reduce the plasma glucose level in the normal range (≥11.1 mmol/L). A drastic change in FPG level was observed after a 7 days maintenance dose of extracts and drugs. Though the extracts failed to lower the FPG below the diabetic range (≥7 mmol/L) which was observed incase of standard drug, the change was significant compared to the diabetic control group (Table-2). Though the average body weight of the treated groups were increased compared to the diabetic control groups (Chart-2), the changes were not statistically significant (Table-3).
From the above study, it was observed that the aqueous UAE crude extracts from fresh leaves and ethanolic extract of *Moringa oleifera* had significant hypoglycemic properties almost similar to the standard anti-diabetic drug glibenclamide (Chart-1). Previous study also supported the blood glucose levels lowering effects of aqueous extract of *M. oleifera* leaves after long (6 weeks) term administration on streptozotocin induced diabetic rats [26]. They also observed increased body weight after administration of aqueous extract [26] which was also observed in the present situation. Paula *et al.*, [27] found that 56.2% reduction in the blood glucose level on the 7th day after intraperitoneal administration of extracted protein from leaf extract of *M. oleifera.* Ndong et al. [28] observed that glucose tolerance tests significantly decreased the blood glucose for Goto-Kakizaki rats and Wistar rats. Chinedu et al. [29] observed that the extract of *M. oleifera* significantly lowered the fasting blood glucose at days 7 and 14 compared to controls. The results of the present study also supported the previous studies and proved the hypoglycemic properties of *M. oleifera* leaves.

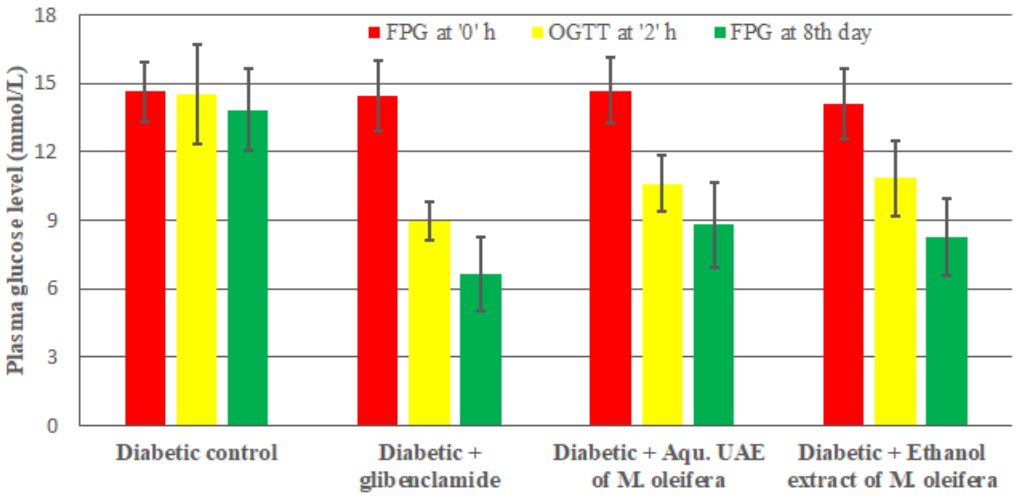

Chart 1: Effects of crude extracts of *M. oleifera* on glucocorticoid induced diabetic mice

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

	Plasma Glucose Level (mmol/L) (Mean ± STD for n = 4)		
Treatment Group	FPG at "0" Hour	OGTT at "2" Hour	FPG at 8th day

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	(≥7 mmol/L indicate diabetic situation) [4]	(≥11.1 mmol/L indicate diabetic condition) [4,5]	(after 7 days of maintanence dose)
Diabetic control	14.63±1.28	14.50±2.17[c]	13.83±1.79[x]
Diabetic + glibenclamide	14.45±1.56[a]	8.98±0.85[b,d]	6.65±1.62[y,z]
Diabetic + Aqu. UA Extract	14.68±1.44[a]	10.60±1.23[b,d]	8.8±1.85[y,z]
Diabetic + Ethanol extract	14.13±1.55[a]	10.85±1.65[b,d,p]	8.275±1.69[q,y,z]

[a] The difference of FPG at '0' h is not significant compared to the diabetic control (p>0.05)
[b] The difference of OGTT at '2'h is significant compared to the FPG at '0' h (p<0.05)
[c] The difference of OGTT at '2'h is not significant compared to the FPG at '0' h (p>0.05)
[d] The difference of OGTT at '2'h is significant compared to the diabetic control (p>0.05)
[p] The difference of OGTT at '2'h is not significant compared to the FPG at '0' h (p<0.05)
[x] The difference is not significant compared to the FPG at '0' h (p<0.05)
[y] The difference is significant compared to the FPG at '0' h (p>0.05)
[z] The difference is significant compared to the FPG of diabetic control at 8th (p>0.05)

Table 3: Effect of Crude Extracts on body weight of experimented Mice

Treatment group	Body Weight Mean ± STD, for n = 4 (M+F)	
	IBW*	**EBW****
Diabetic control	26.03±3.16	25.30±3.19[b]
Diabetic + Glibenclamide	26.48±3.04[a]	27.15±2.71[b,c]
Diabetic + Aqu. UT Extract	26.50±3.45[a]	27.63±3.43[b,c]
Diabetic + Ethanol extract	26.38±3.77[a]	27.85±3.59[b,c]

*IBW: Initial Body Weight (considered during enrollment of the mice in different group);
**EBW: Experimented Body Weight (body weight after 7 days treatment)
[a] The difference of IBW is not significant compared to the diabetic control (p>0.05)
[b] The difference of EBW is not significant compared to the IBW (p>0.05)
[c] The difference of EBW is not significant compared to the diabetic control (p>0.05)

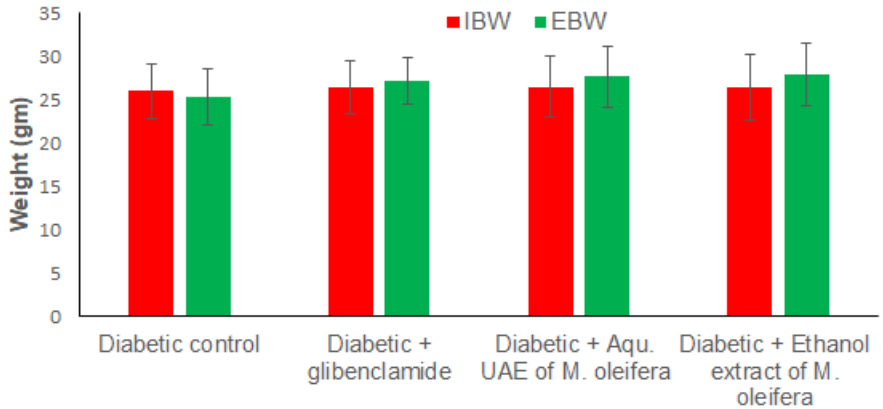

Chart 2: Effect on body weight after 7 days maintenance treatment (IBW: Initial body weight before treatment, EBW: Experimental body weight after treatment)

Conclusion:

The green extraction method named aqueous ultrasound assisted extract from fresh leaves and ethanolic extracts of *M. oleifera* provided similar hypoglycemic action on animal

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models. The intended results indicated that the *M. oleifera* leaves may be a good natural resource for diabetic treatment. People should be encouraged to intake *M. oleifera* leaves as a vegetable or dried form like green tea. The above results also indicated that the application of ultrasound on plant materials did not change the efficacy of the crude extracts. The method may be recommended for large scale production in the industry.

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