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Study of Hypoglycemic Properties of Aqueous Fresh Leaves Extracts of *Azadirachta Indica*, *Bryophyllum Pinnatum*, *Carica Papayan*& *Mikania Cordata* Prepared by Ultrasound Assisted Extraction Method

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Abstract:- Diabetes mellitus is a metabolic disorder associated with chronic hyperglycemia caused by inherited or acquired deficiency of insulin production or resistance to action of the produced insulin. In the present study fresh leaves extracts of *Azadirachta indica, Bryophyllum pinnatum, Carica papaya*and *Mikania cordata*were selected on the basis of traditional behavior of local people for the treatment of diabetes in Bangladesh. Crude extracts prepared by aqueous UAE method was used in this study on hormone induced diabetic mice by FPG Test. Most of the plants showed marked anti-diabetic properties comparison to the standard antidiabetic drug Glibenclamide.

Keywords:- *Diabetes, Glucocorticoid, Dexamethasone, FPG, Ultrasound.*

I. INTRODUCTION

Diabetes mellitus is a metabolic disorder associated with chronic hyperglycemia and imbalance of carbohydrate, protein and fat metabolism (Adenowo *et al.,* 2014). It is an age-long disease (Ogundele *et al.,* 2017) caused by inherited or acquired deficiency of insulin production or resistance to action of the produced insulin (Setter *et al.,* 2000; Fakeye *et al.,* 2007). The distinguishing symptoms of diabetes are polyuria, polydypsia, polyphagia, and unexpected weight loss (Altan 2003; Sinha *et al.,* 2018). Long term complications arising from diabetes are major causes of diabetes morbidity and mortality (Akhere *et al.,* 2013).

Most of the drugs for example insulin and oral hypoglycaemic agent such as sulfonylureas, metformin, α-glucosidase inhibitors, troglitazone, are currently used to treat diabetes mostly target the lowering of blood glucose concentrations to normal levels (Gy *et al.,* 2005; Akhere *et al.,* 2013, Sinha *et al.,* 2018). However, marked drawbacks such as resistance and side effects ranging from liver toxicity, increased cardiovascular risk, abdominal discomfort, flatulence, diarrhea etc. are also observed (Cheng and Fantus, 2005; Adenowo *et al.,* 2014). Natural medicine may be the good source for treating diabetes and associated diseases. Worldwide 306 to 400 plants or fruits have been identified as herbal remedies for diabetes (Andrade-Cetto and Heinrich, 2005; Rao *et al.,* 1997; Sinha *et al.,* 2018).

Ethnobotanical study in Bangladesh conducted by Ocvirk *et al.,* (2013) and Haque *et al.* (2017) observed the popular uses of *Azadirachta indica* in rural and urban people for the remedial purpose of diabetes. Similarly remedial use of *Bryophyllum pinnatum (*Rahmatullah *et al.,* 2011a), *Carica papaya* (Haque *et al.* 2017) and *Mikania cordata* (Uddin *et al.,* 2019) were also observed on diabetes patients by separate study. In the present study *Azadirachta indica, Bryophyllum pinnatum, Carica papaya* and *Mikania cordata* were selected for hypoglycemic study on dexamethasone induced diabetic mice.

A comparatively noble green extraction procedure was used in the present study for extraction purpose. Ultrasound was used to breakdown the cell wall for driving out all polar and non-polar compounds present in the plant's cell. Simple distill water was used as solvent make it environment friendly and cost effective.

II. MATERIALS AND METHODS

Collection and Identification Plant Materials

Fresh leaves of *Azadirachta indica*, *Bryophyllum pinnatum*,*Carica papaya* and*Mikania cordata* were collected from the Botanical Pesticide Garden of Institute of Environmental Science of Rajshahi University, Bangladesh. All plant's parts were identified by the Taxonomist of the Department of Botany, University of Rajshahi. Herbarium specimens of all plants were duly preserved in the Botanical Pesticide and Environmental Microbiology Lab, IES, RU for further reference.

Preparation of Crude Extracts

Fresh leaves of *Azadirachta indica*, *Bryophyllum pinnatum*, *Mikania cordata*and *Carica papaya* were washed properly by running tap water followed by distilled water for removing debris, and placed in a shade for drying out the

surface water. Within 6 hours 50 gm of each plant's was taken in a conventional juice blender machine by addition of 250 ml distilled water i.e., material: water ratio was 1:5 (Sadat *et al.,* 2019). 10-15 minutes blending were sufficient to get fine particle in the leave juice which was easily passed through a 20 mesh size net. Juice was transferred to a 500 ml conical flask and placed in an ultrasonic bath for ultrasound treatment. Total 30 minutes ultrasound treatments were done in the following manner 15 minutes treatment + 15 minutes rest + 15 minutes treatment at 40^0C bath temperature. The mixture was then filtered by three layer of cloth and dried at 55^0C temperature in a water bath. The dried extract was collected in a vial and preserved in the refrigerator (for further use). The process was previously validated in the Botanical Pesticide and Environmental Microbiology Lab, University of Rajshahi.

Hypoglycemic Study of Crude Extracts on Diabetic Mice

The objective of this study was to measure the hypoglycaemic effect of crude extracts on overnight fasted animals. 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 (ADA 2014; TUEPG 2015). Hormone (glucocorticoid created by dexamethasone) induced diabetes mice were enrolled in the present study. 6 groups including 4 mice of either sex were selected from the dexamethasone induced diabetic mice which were created by intraperitoneal injections of 10 mg/kg/day of dexamethasone on normal *Swiss albino* mice for around 7 days (Ogawa *et al.,* 1992). Group I mice were considered diabetic control were served only distil water. Group II mice were served as diabetic treatment by standard anti-diabetic drug glibenclamide (600 µg/kg-bw/dose) and Group III to VI were served as diabetic treatment by extract (300 mg/kg-bw/dose). After overnight fasting, a 0-min blood samples were taken from the tail tip of all mice under mild ether anesthesia. Blood samples were taken after 2 hours of administration of test drugs. All experimental procedures were performed in compliance with institutional and international policies governing the humane and ethical treatment of experimental animals as contained in the United States National Institutes of Health (NIH) guidelines (NIH 1985) after ethical approval by the Ethical Committee of University of Rajshahi.

III. RESULTS AND DISCUSSION

In the present study anti-diabetic (hypoglycaemic) properties was measured on artificially developed diabetic animal (mice) model by using the aqueous UAE crude extracts of *Azadirachta indica*, *Bryophyllum pinnatum*, *Carica papaya* and *Mikania cordata*. It was observed that *A. indica*, *B. pinnatum* and *C. papaya* successfully reduced the plasma glucose level below 126 mg/dl and showed significant ($p<0.05$) glucose lowering effect compared with the diabetic control (Table 1). The effect was observed almost similar to the standard anti-diabetic drug glibenclamide ($p>0.05$). Though crude extracts of *M. cordata* failed to reduce plasma glucose level below 126 mg/dl, but significant ($p<0.05$) reduction was observed than the diabetic control (Chart 1), statistically similar to the standard drug glibenclamide ($p>0.05$). Results indicated the distinguished anti-diabetic properties of those crude extracts.

Table 1: Effect of Crude Extracts on FPG Level of Experimented Mice

Treatment Group	Plasma Glucose Level (mg/dl) (Values are mean ± SEM for n=4)		P value (After treatment)	
	0 Hour (Before treatment)	2 Hour (After treatment)	Control Vs. Treatment	Treatment Std. drug Vs. Extract
Group I: Diabetic control	233.10±7.28	229.95±7.54	-	-
Group II: Glibenclamide	227.70±13.663	100.35±12.32	0.000	-
Group III: *A. indica*	229.95±28.46	119.70±14.28	0.000	0.089
Group III: *B. pinnatum*	234.00±18.30	120.15±22.12	0.001	0.288
Group IV: *C. papaya*	239.85±15.38	113.40±12.02	0.000	0.130
Group VI: *M. cordata*	226.35±13.32	147.60±24.10	0.013	0.078

*At fasting condition, ≥7 mmol/l (>126mg/dl) considered diabetic situation (ADA 2014).
P<0.05, indicate significant difference at 5% level of significance
P>0.05, indicate no differenceat 5% level of significance

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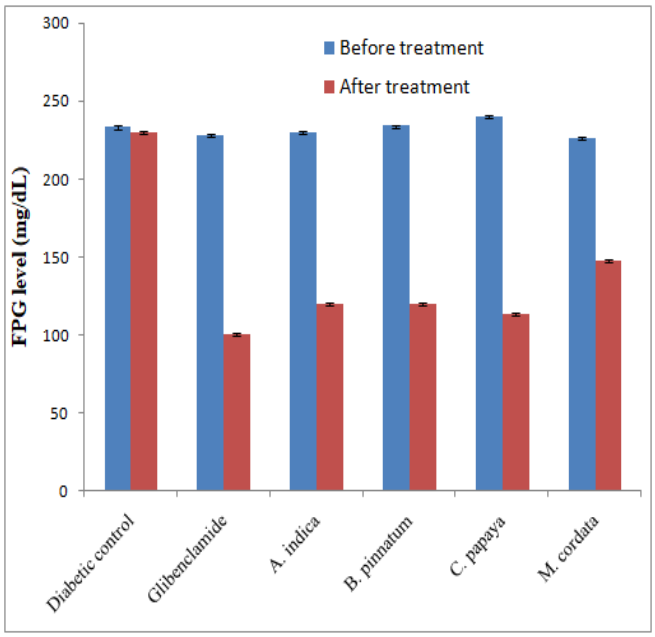

Chart 1: Comparison of hypoglycemic effects of crude extracts with standard anti-diabetic drug.

Form the above study, it was observed that the aqueous UAE crude extracts from fresh leaves of *Azadirachta indica*, *Bryophyllum pinnatum*, *Carica papaya*and *Mikania cordata* had significant hypoglycemic properties almost similar to the standard anti-diabetic drug glibenclamide (Chart 1). Leaf extract of *A. indica*was also previouslyproved hypoglycemic on alloxan induced diabetic animals (Nagashayana *et al.,* 2014) and streptozotocin induced diabetic animals(Morshed *et al.* 2011). Similarly, Kpomah and Arhoghro (2012) reported significant reduction of serum glucose of the alloxan induced diabetic albino rats by *B. pinnatum* extracts whereas Aransiolav *et al.* (2014) found the enhanced performance of the existing drugs (glibenclamide) with the use of the aqueous extract *B. pinnatum* leaves.Previous study found significant activity of *C. papaya* leaves extracts on alloxan induced diabetic mice (Sinha *et al.,* 2018) and streptozotocin induced diabetes rats(Juárez-Rojop *et al.* (2012).Crude extracts of *Mikania cordata* fresh leaves had marked anti-diabetic effect found in the present study but comparatively very less than the standard anti-diabetic drug glibenclamide. However, previous study found a beneficial effect in the treatment of diabetes mellitus on alloxan-induced diabetic rats and proposed for the therapy of diabetes (Nurhayati *et al.,* 2013).

IV. CONCLUSION

Most of the selected plants showed significant hypoglycemic effect on animal which was almost similar to the standard anti-diabetic drug glibenclamide. Aqueous UAE extract (free from hazardous organic solvents) of the above plants may be suitable for applying human trial for evaluating anti-diabetic properties on human.

REFERENCES

[1]. ADA (American Diabetes Association). 2014. Diabetes and driving. *Diabetes Care*, 37(1): 97-103.

[2]. Adenowo AF, Ilori MF, Balogun FO and Kazeem MI. 2014.Protective Effect of Ethanol Leaf Extract of *Carica papaya* Linn (Caricaceae) in Alloxan-induced Diabetic Rats.*Tropical Journal of Pharmaceutical Research November*, 13 (11): 1877-1882.

[3]. Akhere AO, Iyere OO, Afolabi FA, Lekan OA and Ayodeji RA. 2013. Long Term Anti-Diabetic, Anti-Hyperlipidaemic and Anti-Atherogenic Effects of *Carica papaya* Leaves in Streptozotocin Diabetic Rats. *European Journal of Medicinal Plants*, 3(4): 508-519.

[4]. Altan VM. 2003. The pharmacology of diabetic complications. *Current Medicinal Chemistry*, 10: 1317-1327.

[5]. Andrade-Cetto A and Heinrich M. 2005. Mexican Plants with hypoglycaemic effect used in the treatment of diabetes. *Journal of Ethnopharmacology*, 99: 325-348.

[6]. Aransiolav EF, Daramola MO, Iwalewa EO, Seluwa AM and Olufowobi OO. 2014. Anti-Diabetic Effect of *Bryophyllum pinnatum* Leaves. *International Journal of Biotechnology and Bioengineering*, 8(1): 89-93.

[7]. Cheng AYY and Fantus IG. 2005. Oral antihyperglycemic therapy for type 2 diabetes Mellitus. *Canadian Medical Association Journal*,172(2): 213-226.

[8]. Fakeye TO, Oladipupo T, Showande O and Ogunremi Y. 2007. Effects of Coadministration of Extract of *Carica papaya* Linn (family Cariaceae) on Activity of Two Oral Hypoglycaemic Agents.*Tropical Journal of Pharmaceutical Research*, 6(1): 671-678.

[9]. Gy SY, Cisse A, Nongonierma RB, Sarr M, Mbodj NA and Faye B. 2005. Hypoglycaemic and antidiabetic activity of acetonic extract of *Vernonia colorata* leaves in normoglycaemic and alloxan induced diabetic rats. *Journal of Ethnopharmacology.*, 98: 171-175.

[10]. Juárez-Rojopa IE, Tovilla-Zárateb CA, Aguilar-Domíngueza DE, Roa-de la Fuentec LF, Lobato-Garcíac CE, Blé-Castilloa JL, López-Merazd L, Díaz-Zagoyae JC, Bermúdez-Ocañab DY. 2014. Phytochemical screening and hypoglycaemic activity of *Carica papaya* leaf in streptozotocin-induced diabetic rats. *Revista Brasileira de Farmacognosia*, 24: 341-347.

[11]. Kpomah ED and Arhoghro EM. 2012. Effects of doses of *Bryophylum pinnatum* and glibenclamide on serum glucose and lipid profile in alloxan-induced diabetic rats. *Indian Journal of Drugs and Diseases*, 1(5): 124-128.

[12]. Morshed MA, Fayzunnessa N , Haque MA, Uddin A , Bhoumik A , Ali L and Rokeya B. 2011. Comparative study of *Azadirachta indica* leaf powder and its different extracts on glycemic, insulinemic and lipidemic status of type 2 diabetic model rats.*International Journal of Pharmaceutical Sciences and Research*, 2(4): 954-960.

[13]. Nagashayana, Jagadeesh and Revankar SP. 2014.Evaluation of Hypoglycaemic Activity of Neem (*Azadirachta indica*) In Albino Rats.*IOSR Journal of Dental and Medical Sciences*, 13(9): 4-11.
[14]. NIH 1985.National Institute of Health. Guide for the care and use of laboratory animals. NIH publication no. 85-23, Bethesda, USA, 1985.
[15]. Nurhayati WHW, Arlizan TN and Nurdiana S. 2013.Effect of *Mikania micrantha* leaf extract on the level of blood glucose and hepatic glycogen in the normal and alloxan-induced diabetic rats.*Natural Products an Indian Journal*, 9(10): 398-402.
[16]. Ocvirk S, Kistler M, Khan S, Talukder SH, Hauner H. 2013. Traditional medicinal plants used for the treatment of diabetes in rural and urban areas of Dhaka, Bangladesh-an ethnobotanical survey. *Journal of Ethnobiology and Ethnomedicine*, 9(43): 1-8.
[17]. Ogawa A, Johnson JH, Ohneda M, Mcallister CT, Inman LR, Alam T, Unger RH. 1992. Roles of insulin resistance and beta-cell dysfunction in dexamethasone-induced diabetes. *Journal of Clinical Investigation*, 90: 497-504.
[18]. Ogundele AV, Otun KO, Ajiboye A, Olanipekun BE and Ibrahim RB. 2017. Anti-Diabetic Efficacy and Phytochemical Screening of Methanolic Leaf Extract of Pawpaw (*Carica papaya*) Grown in North Central Nigeria. *Journal of the Turkish Chemical Society Section A: Chemistry*, 4(1): 99-114.
[19]. Rahmatullah M, Mollik AH, Ali M, Abbas FB, Jahan R, Khatun A, Seraj S and Ahsan S. 2011a. An Ethnomedicinal Survey of Vitbilia Village in Sujanagar Sub-District of Pabna District, Bangladesh.*American-Eurasian Journal of Agricultural & Environmental Sciences*, 10: 106-111.
[20]. Rao BK, Giri R, Kesavalu MM and Apparao CH. 1997.Herbal medicine in the management of diabetes mellitus.*Manphar Vaidhya Patrika*, 1: 33-35.
[21]. Sadat AFMN, Mizan MRB, Sultana A, Rahman MM and Azad MAK. 2018. Comparative study of the Antimicrobial Activity of Methanol Extract and Ultrasound Assisted Water Extract of the Leaves of *Azadirachta indica*. *Rajshahi University Journal of Environmental Science*, 7: 40-47.
[22]. Setter SM, White JR and Campbell RK. 2000. Diabetes: Textbook of therapeutics, drugs and diseases management, Ed Herfindal E.T and Crowley D.R; Lippincott; Williams and Wilkins Publishing Company, United States of America: 45-50.
[23]. Sinha RK, Pratap R and Varma MC. 2018.Hypoglycaemic Activity of *Carica papaya* Leaf Aqueous Extract in Normal and Diabetic Mice.*International Journal of Advances in Scientific Research and Engineering*, 4(6): 12-16.
[24]. TUEPG.2015. TUE Physician Guidelines.Medical Information to Support the Decisions of TUE Committees Diabetes Mellitus, WADA- World Anti-Doping Program Version 3.0.
[25]. Uddin MZ, Mitu FY, Rifat AB and Kaium AA. 2019. Ethnomedicinal study focusing on anti-diabetic plants used by the community living in and around Dhaka. *Bangladesh Journal of Plant Taxonomy*, 26(2): 231-247.