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RESEARCH ARTICLE

Attenuation of Chemically Induced Diabetes in Rabbits with Herbal Mixture (*Citrullus colocynthis* and *Cicer arietinum)*

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ABSTRACT

The present study was carried out to investigate the hypoglycemic potential of herbal mixture (*Citrullus Colocynthis* and *Cicer arietinum)* in normal and alloxan induced diabetic rabbits. Hypoglycemic potential was evaluated through curative and preventive modes of treatments. Blood glucose and lipid level was measured in serum of experimental rabbits. Significant ($P<0.05$) increase was observed in glucose level (466.33±9.07 mg/dl) in diabetic control group after the injection of alloxan (120 mg/kg) as compared to normal group (103.67±4.51 mg/dl). Treatment of diabetic rabbits with herbal mixtures at the dose of 150 mg/kg for three weeks significantly decreased (126±9.17 mg/dl) glucose level of the alloxan induced. The oral administration of herbal mixture in curative and preventive groups showed significant reduction in plasma glucose, and low density lipoprotein (LDL), total cholesterol levels, and significant ($P<0.05$) increased was observed in the level of high density lipoprotein (HDL). The phytochemical studies revealed the presence of alkaloids, flavonoids, saponins and steroids in herbal mixture. The results of this study indicated that antidiabetic potential of herbal mixture (*Citrullus Colocynthis* and *Cicer arietinum)* may be due to presence of bioactive phytochemical constituents. It can be concluded that herbal mixture has strong hypoglycemic and antilipidemic potential which is comparable with standard drug.

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INTRODUCTION

Diabetes mellitus is a clinical syndrome which is characterized by chronic hyperglycemia and disturbances in carbohydrate, lipids and protein metabolism (Jafri *et al*., 2011; Hussain *et al*., 2012). The disease may result from defects in insulin secretion, insulin action or both. It is considered seventh among the leading causes of death, and third when it's chronic situations are taken in to account (Trivedi *et al*., 2004; Raghavendra *et al*., 2011; Patil *et al*., 2011; Aslam *et al*., 2012). The chronic hyperglycemia of diabetes leads to long term damages including improper functioning and failure of multiple organs (Lyra *et al*., 2006). Two main types of diabetes based on their clinical manifestation are identified as type I diabetes- known as juvenile onset or insulin sensitive diabetes and type II diabetes or non insulin dependent diabetes mellitus (NIDDM) (Sudha *et al.,* 2011). Type I diabetes is caused by improper functioning of beta-cells of pancreas leading to insulin deficiency, so it is treated by exogenous insulin source (Cook and Plotnick, 2008). On the other hand Type II diabetes, in which patients are unable to respond to insulin, is treated by changing diet, exercise and hypoglycemic agents (Raghavendra *et al*., 2011).

In developing countries the prevalence of diabetes is increasing because its treatment is more expensive. Pakistan is the seventh country in the list of diabetes prevalence, 6.9 million people are affected and this number will grow to 11.5 million by 2025 unless the control measurers achieved (Hayat and Shaikh, 2010). Many synthetic hypoglycemic agents such as biguanides, sulfonylureas and insulin are commonly used for treatment of diabetes but serious side effects are associated with synthetic hypoglycemic drugs. These associated problems demand the exploration of plants for better drugs with fewer side effects. Few natural hypoglycemic medicines derived from medicinal plants are available to combat with diabetes (Loew and Kaszkin, 2002; Joseph and Jini, 2011).

According to ethnobotanical information, 800 plants possess antidiabetics effect (Aguilara *et al*., 1998; Ahmad

et al., 2012a, 2012b). However limited reports are available on hypoglycemic potential of combination of understudy plants. Herbal remedies are deliberated to be more efficacious when used in combinations. In combination bioactive phyto-constituents of medicinal plants impart synergic therapeutic potential. When herbs are used in combination it helps the body to better control possibly adverse effects of any one and each herb in the combination/formulation plays a curative potential. It is therefore better to use herbal combinations instead of depending on single herbs.

In this study two medicinally important plants *Citrullus colocynthis* belongs to Family Cucurbitaceae and *Cicer arietinum* belongs to Family Fabaceae were used. *Cicer arietinum* is commonly known as chickpea. Its leaves and seeds showed hypoglycemic potential and it has been used in the treatment of dyspepsia, constipation and snakebite. They are also very high in dietary fiber and hence a healthy source of carbohydrates for persons with insulin sensitivity or diabetes. *Cicer arietinum* also improve lipid metabolism, decrease blood glucose level, prevent body weight loss, and reduce impairment of diabetic related spatial learning and memory. It elevated serum insulin in treated diabetic rats, which is probably one important reason for the hypoglycemic effect (Ahmad *et al*., 2009).

Citrullus colocynthis also known as bitter apple found in desert plant its fruits are widely used as herbal medicine. It contained flavonoids, caffeic acid derivatives and terpenoids that are responsible for its antioxidants and hypoglycemic potential (Tannin-Spitz *et al*., 2007). Therefore, in present study the aim was to examine the synergistic antidiabetic activity of herbal mixture comprising of *Citrullus colocynthis* and *Cicer arietinum* in alloxan induced diabetic rabbits.

MATERIALS AND METHODS

Herbal mixture preparation and phytochemical analysis: The hypoglycemic herbal mixture was prepared by following the routine procedure performed by the alternative/traditional practitioners in Pakistan. Juice from fresh fruits of *Citrullus colocynthis* was extracted with Juice extractor. A total 5 kg of *Cicer arietinum* was taken and soaked over 72 hours in the juice of *Citrullus colocynthis.* The possibility of adsorption of phytoconstituents may be maximum during this period. After that the material was dried, ground into fine powdered form and stored in air tight jars. Qualitative phytochemical screening of herbal mixture was performed according to standard methods given by (Yadav *et al*., 2010).

Animal selection and blood sampling: The study was conducted on eighteen rabbits with average weight of about 1.25kg. Throughout the investigation, animals were housed in animal room in the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad at internationally recommended conditions of environment. Blood was taken from the jugular vein of overnight fasted rabbits. The blood samples were collected in centrifuged glass tubes, and then serum was separated and stored at -4ºC.

Hypoglycemic activity of herbal mixture: Hypoglycemic activity of herbal mixture was determined in alloxan induced diabetes in rabbits (n=3) by pre- and post-treatment of herbal mixture. In curative way of treatment alloxan (120 mg/kg) was administered for 10 days and then these diabetic rabbits were treated with herbal mixture for 12 days. While in preventive group rabbits (n=3) were pretreated with 150 mg/kg of herbal mixture for three weeks and then alloxan was administered for the induction of diabetes.

After one week acclimatization the rabbits were divided into five groups comprised of three each and experiment duration was 23 days. The rabbits of group 1 (control) were received only normal diet. Group 2 (positive control) were administrated herbal mixture once daily by oral gavage. Group 3 (diabetic control) rabbits were injected with alloxan (120 mg/kg) for 10 days and blood samples were taken after every 2nd day. Group 4 (Preventive group) rabbits were pre-treated with herbal mixture (150 mg/kg) by oral gavage. After 23rd day the rabbits were injected alloxan (120 mg/kg) for 10 days to induce diabetes. The blood samples were taken after every 2nd day. Rabbits of group 5 (Curative group) were treated with alloxan (120 mg/kg) to induce diabetes for 10 days. Then, post-treatment of herbal mixture (150 mg/kg) was given to rabbits for 23 days and after every 5th day blood sample were collected. Group 6 (Glibenclamide group) were given glibenclamide (25 mg/kg) orally for experimental duration after induction of diabetes with injection of alloxan (120 mg/kg) then blood samples were collected after every 5th day.

Blood glucose levels were compared in all groups by taking glibenclamide as standard drug. Lipid profiles including low density lipoprotein, high density lipoprotein and total cholesterol were also evaluated. Serum was separated from blood samples of experimental animals and all analysis were performed with commercially available kits using chemistry analyzer.

Statistical analysis: Each sample was evaluated in triplicate and data has been expressed in the form of mean ±SD. Data was analyzed using analysis of variance (ANOVA) in SPSS 15 software. Turkey's Multiple Comparison test was used for comparison of means of different experiments ($P<0.05$) (Steel *et al*., 1997).

RESULT AND DISCUSION

Phytochemistry of herbal mixture showed the presence of flavonoids, alkaloids, glycosides, saponins and steroids. The literature review also revealed that the leaves and roots of *Cicer arietinum* and *Citrullus colocynthis* fruit showed the presence of alkaloids. Some alkaloids present in the plants function as spasmolytic, anti-cholinergic and anesthetic agents. Secondary metabolites of *Cicer arietinum* including cucurbitacins, flavonoids, saponins, cardiac glycosides caffeic acid derivatives and terpenoids showed antidiabetic potential (Tannin-Spitz *et al*., 2007).

Hypoglycemic effects of herbal combination (*Cicer arietinum* and *Citrullus colocynthis*) and standard drug has been shown in table 1. The results showed that there was a significant ($P<0.05$) elevation in blood glucose

Table 1: Effect of herbal mixture on blood glucose and lipids level in experimental groups of rabbits

Parameter	Sample* no.	Groups					
		1	2	3	4	5	6
Glucose (mg/dl)	1	120.3±5.5	124.3±4.0	297.7±2.5	173.7±7.8	175.3±3.1	163.0±4.6
	2	125.0±0.5	128.7±6.5	342.7±4.2	176.0±6.6	157.3±1.2	145.3±3.2
	3	117.0±4.6	120.0±3.6	377.7±7.1	157.0±2.9	160.3±2.1	137.0±3.6
	4	97.3±6.1	115.7±3.2	413.7±1.6	170.3±6.0	134.3±1.1	123.7±5.5
	5	103.7±4.5	111.7±4.0	466.3±9.1	147.7±6.5	126.0±9.2	95.7±6.8
HDL (mg/dl)	1	42.0±3.6	55.7±4.2	68.0±2.6	37.3±8.6	43.0±4.2	---
	2	46.3±3.8	51.7±2.5	69.3±3.5	48.3±4.5	34.3±5.7	---
	3	51.3±2.5	56.3±3.5	48.0±0.5	63.0±0.4	48.7±5.0	---
	4	50.3±2.5	60.7±5.0	38.7±7.5	68.7±3.5	55.3±4.0	----
	5	55.3±3.5	65.3±5.5	26.3±4.3	58.3±1.5	54.7±5.5	--
LDL (mg/dl)	1	151.7±4.9	177.7±3.5	196.3±8.4	233.0±3.6	202.3±9.5	---
	2	164.7±5.9	178.7±4.7	224.0±5.3	223.0±3.0	210.3±9.6	--
	3	168.0±8.5	166.0±2.0	239.7±4.0	201.7±8.0	192.3±3.5	--
	4	142.3±3.7	151.7±4.0	244.7±4.9	199.0±3.6	193.3±6.6	--
	5	159.0±9.2	146.3±3.5	254.7±3.5	193.3±0.6	185.0±6.0	--
Cholesterol (mg/dl)	1	199.7±3.5	238.0±0.3	242.7±6.1	268.3±2.5	249.7±8.1	-
	2	208.3±4.0	232.3±6.1	259.7±3.1	258.7±2.5	249.3±1.5	--
	3	176.3±6.3	224.3±4.1	273.3±4.5	246.7±6.1	246.7±8.1	---
	4	221.3±4.1	219.7±3.7	289.0±4.0	241.3±2.5	240.7±5.9	--
	5	220.3±5.1	209.3±8.5	291.67±8.7	236.7±7.0	229.3±8.0	-----

*Each sample is a combination of three readings. Group 1: Control Group, 2: Positive control, Group 3: Diabetic control, Group 4: Preventive group, Group 5: Curative group, Group 6: Glibenclamide group.

level in control diabetic rabbits as compared to normal group due to alloxan. Alloxan is one of the usual substances used for induction of diabetes mellitus. It induces the free radical production that has destructive effect on the beta cells of pancreas (Raghavendra *et al*., 2011; Patil *et al*., 2011). The plasma glucose levels in standard drug (glibenclamide) and herbal combination treated (curative and preventive) groups was significantly ($P<0.05$) low as compared to diabetic control group. The herbal mixture showed hypoglycemic potential comparable to standard drug glibenclamide. Mixture of both plants demonstrated efficacious hypoglycemic potential through both preventive and curative mode of treatment. This antihyperglycemic effect of herbal mixture is attributed to phytoconstitutes like flavonoids and saponins that may interact with several metabolic pathways or insulin metabolism and influence directly and indirectly on glucose homeostasis (Prabhakar *et al*., 2008). It is well documented in the literature that Charantin, a steroidal saponin, isolated from *Momordica charantia* L. (Cucurbitaceae) possess an insulin-like activity by enhancing the release of insulin and slow down the glycogenesis (Ng *et al*., 1986). In previous study the ethanol extract of leaves of *Melothria maderaspatana* also belongs to family Cucurbitaceae showed its effects on intestinal glucose absorption and insulin secretion. The results demonstrated a significant reduction in glucose in plasma glucose level in a dose dependant manner (Balaraman *et al*., 2011).

The effect of herbal mixture on serum lipids in all experimental groups has been presented in table 1. A significant increase was observed in the serum triglycerides, total cholesterol and LDL levels, and decrease in HDL cholesterol levels were detected in alloxan induced diabetic rabbits. Hyperlipidemia is a recognized complication of diabetes mellitus and also reported by many researchers. There was significant ($P<0.05$) decrease in both total cholesterol and LDL levels while significant ($P<0.05$) increase in HDL levels was observed in rabbit treated with herbal mixture through both curative and preventive mode of treatments as compared to control diabetic groups. The combination of *Cicer arietinum* and *Citrullus colocynthis* was found to be as effective as glibenclamide (standard drug) in decreasing the lipid level in diabetic rabbits. These results indicated that the herbal mixture have efficient antilipidemic potential. These results are in agreement with many previous reports which indicated the hypoglycemic and hypolipidemic potential of *Citrullus colocynthis* in normal and hyperglycemic diabetic rabbits. Plasma high density lipoprotein, plasma total cholesterol, triglyceride and low density lipoprotein-cholesterol (LDL-C) were significantly ($P<0.05$) lower in the groups treated with the herbal mixture when compared with those untreated (Saeid *et al.* 2010). The basis of literature and from our experimental data, we assumed that *C. Colocynthis* may have many active constituents which regulate efficiently blood glucose in diabetic rabbits. They may exert their effect by potentializing the insulin-secretion of residual insulin cells or increase the ability of cells to keep glucose (Abdel-Hassan *et al*., 2000; Shetty *et al*., 2005; Abd El-Baky et *al*., 2009; Balaraman *et al*., 2011). The tendency of herbal combination to assist hyperlipidemia showed a potential beneficial effect on cardiovascular risk factors which is the major cause of death in diabetes mellitus (Raghavendra *et al*., 2011).

Due to the administration of alloxan significant changes occurred in different organs of rabbits. Liver was pale yellow and discolored, gallbladder was affected seriously having necrosis and hard consistency in diabetic control group, and however, it was normal in all other groups. In the previous histological studies of pancreas showed that there is shrinkage of cells of islets of langerhans in alloxan induced diabetic rats (Gohil *et al*., 2010). In diabetic control group there was also reduction in number of islets cells, however curative and preventive groups showed significant restoration of islets cells indicating the beneficial effect of herbal mixture.

Conclusion: This study supports that herbal mixture of *C. colocynthis* and *Cicer arietinum* possesses antidiabetic as well as antilpidemic potential. The fruits of this plant seem to have a promising value for the development of potent phytomedicine for diabetes.

REFERENCES

Abd El-Baky A, A Abdulla, H Abd El-Mawgoud and E Abd El-Hay, 2009. Hypoglycemic and hypolipidaemic action of bitter melon on normoglycemic and hyperglycemic diabeti rats. Res J Med & Med Sci, 4: 519-525.

Abdel-Hassan IA, JA Abdel-Barry and T Mohammeda, 2000. The hypoglycaemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. J Ethnopharmacol, 71: 325-330.

Ahmad M, Q Mahmood, K Gulzar, MS Akhtar, M Saleem and MI Qadir, 2012a. Antihyperlipidemic and hepatoprotective activity of *Dodonaea viscosa* leaves extracts in alloxan-induced diabetic rabbits (*Oryctolagus cuniculus*). Pak Vet J, 32: 50-54.

Ahmad M, R Qureshi, M Arshad, MA Khan and M Zafar, 2009. Traditional herbal remedies used for the treatment of diabetes from district Attock (Pakistan). Pak J Bot, 41: 2777-2782.

Ahmad N, ZU Rahman, N Akhtar and S Ali, 2012b. Effects of aqueous methanolic extract of flax seeds (*Linum usitatissimum*) on serum estradiol, progesterone, kidney and liver functions and some serum biochemical metabolites in immature female rats. Pak Vet J, 32: 211-215.

Alarcon-Aguliara FJ, R Roman-Ramos, S Perez- Gutierrez, A Aguilar-Contreras, CC Contreras-Weber and JL Flores-Saenz, 1998. Study of the antihyperglycemic effect of plants used as antidiabetics. J Ethanopharmacol, 61: 101-110.

Aslam N, MA Sheikh, KU Rehman and H Nawaz, 2012. Biochemical profile indicative of insulin resistance in nondiabetic and diabetic cardiovascular patients. Int J Agric Biol, 14: 126–130.

Balaraman AK, J Singh, TK Maity, VT Selvan and SB Palanisamy, 2011. Ethanol extract of *Melothria Maderaspatana* inhibits glucose absorption and stimulates insulin secretion in male C57BL/6 mice. Int conf Biosci Biochem Bioinform, 5: 429-434.

Cooke DW and L Plotnick, 2008. Type I diabetes mellitus in pediatrics. Pediatr Rev, 29: 374-384.

Gohil T, N Pathak, N Jivani, V Devmurari and J Patel, 2010. Treatment with extracts of *Eugenia jambolana* seed and *Aegle marmelos* leaf extracts prevents hyperglycemia and hyperlipidemia in alloxan induced diabetic rats. Afr J Pharm Pharmacol, 4: 270-275.

Hayat AS and N Shaikh, 2010. Barriers and myths to initiate insulin therapy for type 2 diabetes mellitus at primary health care centers of Hyderabad district. World Appl Sci J, 8: 66-72.

Hussain F, MA Sheikh, MA Maan and A Jamil, 2012. Advanced glycation endproducts (AGEs) in diabetic patients with systemic lupus erythematosus. *Int J Agric Biol*, 14: 440–444.

Jafri SA, S Abass and M Qasim, 2011. Hypoglycemic effect of ginger (*Zingiber officinale)* in alloxan induced diabetic rats (*Rattus norvagicus*). Pak Vet J, 31: 160-162.

Joseph B and D Jini, 2011. Insight into the hypoglycemic effect of traditional Indian herbs used in the treatment of diabetes mellitus. Res J Med Plant, 5: 352-376.

Loew D and M Kaszkin, 2002. Approaching the problem of bioequivalence of herbal medicinal products. Phytother Res, 16: 705-711.

Lyra R, M Oliveira, D Lins and N Cavalcanti, 2006. Prevention of type 2 diabetes mellitus. Arq Bras Endocrinol Metabol, 50: 239-249.

Ng TB, CM Wong, WW Li and HW Yeung, 1986. Insulin-like molecules in *Momordica Charantia* seeds. J Ethnopharmacol, 15: 107-17.

Owen PL, LC Martineau, D Caves, PS Haddad, T Matainaho and T Johns, 2008. Consumption of guava (*Psidium guajava L.*) and noni (*Morinda citrifolia L*) may protect betel quid-chewing Papua New Guineans against diabetes. Asia Pac J Clin Nutr, 17: 635-643.

Patil R, R Patil, B Ahirwar, D Ahirwar, 2011. Current status of Indian medicinal plants with antidiabetic potential: a review. Asia Pac J Trop Biomed, 1: 291-298.

Prabhakar PK and M Doble, 2008. A target based therapeutic approach towards diabetes mellitus using medicinal plants. Curr Diabetes Rev, 4: 291-308.

Raghavendra HG, S Sen, YSR Reddy, C Sridhar, R Chakraborty, B Suchitra, B Nagaiah, BA Jyothi, KA Babu, PM Babu, T Viswanadhan and D Sujatha, 2011. Synergistic activity of *Tribulus terrestris* and *Annona squamosa* extracts against alloxan induced diabetes and hyperlipidemia in rat. Pharm Sci Mon, 2: 12-23.

Saeid JM, AB Mohamed and MA AL-Baddy, 2010. Effect of aqueous extract of ginger (*Zingiber officinale*) on blood biochemistry parameters of broiler. Int J Poult Sci, 9: 944-947.

Shetty AK, GS Kumar, K Sambaiah and PV Salimath, 2005. Effect of Bitter Gourd (*Momordica charantia*) on glycaemic status in streptozotocin induced diabeticrats. Plant Foods Hum Nutr, 60: 109-112.

Stanely P, M Prince and VP Menon, 2000. Hypoglycemic and other related actions of *Tinospora cardifolia* roots in alloxan induced diabetic rats. J Ethnopharmacol, 70: 9-15.

Steel RGD, H Torrie and DA Dickey, 1997. Principles and procedures of Statistics. Mc Gram Hill Book Co. New York,USA.

Sudha P, SS Zinjard, SY Bhargava and AR Kumar, 2011. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC CAM, 11: 2-10.

Tannin-Spitz T, S Grossman, S Dovrat HE, Gottlieb and M. Bergman, 2007. Growth inhibitory activity of cucurbitacin glucosides isolated from *Citrullus colocynthis* on human breast cancer cells. Biochem Pharmacol, 73: 56-67.

Trivedi NA, B Mazumdar, JD Bhatt and KG Hemavathi, 2004**.** Effect of *Shilajit* on blood glucose and lipid profile in alloxan-induced diabetic rats. Ind J Pharmacol, 36: 373-376.

Yadav M, A Lavania, R Tomar, GB Prasad, S Jain and H Yadav, 2010. Complementary and comparative study on hypoglycemic and antihyperglycemic activity of various extracts of *Eugenia jambolana* seed, *Momordica charantia* fruits, *Gymnema sylvestre*, and *Trigonella foenum graecum* seeds in rats. Appl Biochem Biotechnol, 160: 2388-2400.