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Original Article

COMPARATIVE STUDY OF AQUEOUS EXTRACT OF *MOMORDICA CHARANTIA* SEEDS WITH SYNTHETIC INSULIN SENSITIZERS ON BLOOD GLUCOSE LEVELS AND BODY WEIGHT IN ALBINO RATS

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ABSTRACT

Objective: To evaluate the effect of aqueous extract of *Momordica charantia* seed in combination with an insulin sensitizer metformin on blood glucose levels and body weight compared to metformin plus pioglitazone.

Methods: Rats were divided into 5 groups of 6 animals each. Metformin and Pioglitazone were given at doses of 1250mg/kg b. w/day and 75mg/kg b. w/day. *Momordica Charantia* Seed Extract (MCSE) was administered at a dose of 2500mg/kg b. w/day. All these drugs were administered orally once daily for a period of 7 days (total study duration). Injection Dexamethasone (8mg/kg) was given intraperitoneally to all the 3 test groups during the last 3 days of study to induce a state of insulin resistance. Blood glucose levels and body weight were measured before starting the study, before starting Dexamethasone (Day 5), and at the end of the study (Day 7). Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet test.

Results: In case of Dexamethasone induced diabetic model, the combination of Metformin and MCSE exhibited significant reduction in blood glucose ($P = 0.019$) and body weight ($P = 0.004$) as compared to Metformin and Pioglitazone.

Conclusion: The aqueous extract of *Momordica charantia* seed has potent blood glucose lowering activity in dexamethasone induced diabetes in rat.

Keywords: MCSE, Metformin, Pioglitazone, Insulin resistance, Dexamethasone induced diabetes.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a serious, chronic, and progressive disease that is rapidly increasing in prevalence. It accounts for up to 90% of DM cases and is usually characterized by the presence of both insulin resistance and relative insulin deficiency. Insulin resistance is manifested by increased lipolysis and free fatty acid production, increased hepatic glucose production, and decreased skeletal muscle uptake of glucose. β-Cell dysfunction is progressive and contributes to worsening blood glucose control over time. T2DM occurs when a diabetogenic lifestyle (excessive calories, inadequate exercise, and obesity) is superimposed upon a susceptible genotype.

Pioglitazone is the only drug available with a strong effect on insulin resistance, although metformin also has some effect in improving peripheral insulin sensitivity [1]. Pioglitazone is known to improve glycemic control, dyslipidemia, hypertension, and microalbuminuria in patients with T2DM. Pioglitazone has been shown to increase HDL cholesterol, decrease fasting triglycerides, and decrease fasting plasma free fatty acids.

A large randomized controlled trial (RCT) showed that both pioglitazone and metformin reduced HbA1c by 1.5% from baseline [2]. In contrast to the more commonly prescribed sulfonylureas, pioglitazone showed a significantly better durability of diabetes control in patients with T2DM [3]. Similarly, pioglitazone was superior to the dipeptidyl peptidase (DPP) -4 inhibitor sitagliptin in reducing HbA1c levels in drug-naïve patients [4].

However, the clinical use of pioglitazone is limited by the risk of adverse events, including weight gain, congestive heart failure, bone fractures, macular edema, and possibly bladder cancer. Concern exists about an association between pioglitazone and bladder cancer [5]. The PROactive trial first reported the finding of an increased risk of bladder cancer (14 vs. 5) in patients taking pioglitazone to those in the placebo arm [6]. A more recent update on the same study reported no observed increase in bladder cancer risk in the pioglitazone arm [7]. Another study from the Kaiser Permanente Northern California diabetes registry reported that use of pioglitazone for more than 2 years was weakly associated with increased risk for bladder cancer [8]. A large study from the United Kingdom reported no increase in bladder cancer with pioglitazone use [9]. The findings thus have been varied.

Based on the findings published in a retrospective study carried out by the French authorities, European Medicines Agency (EMA) put a ban on the sale of pioglitazone in France and Germany in May 2011 [10]. The United States Food and Drug Administration (USFDA) in June 2011 mandated the introduction of information on the increased risk for bladder cancer in the pioglitazone label [11]. On June 18, 2013, the Drug Controller General of India (DCGI) placed pioglitazone under suspension based on the actions taken by the above regulatory authorities [12]. Pharmacovigilance Program of India (PVPI) did not report any suspicious signals for pioglitazone. Hence, the nation was informed that the suspension was being revoked and pioglitazone would now be available again with an updated warning for the patients [13]. So the clinical use of pioglitazone is currently under scrutiny because of safety issues and because of the availability of newer drugs (DPP-4 inhibitors, glucagon-like peptide-1 receptor agonists, and sodium glucose cotransporter 2 inhibitors. However, none of these newer drug classes target insulin resistance.

Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, associated complications with synthetic drugs have led to a shift towards locating natural resources showing antidiabetic activity. Many Indian plants have been investigated for their potential in reducing blood sugar levels in animal models. Extracts from various parts of plants such as *Aegle marmelos, Allium cepa, Allium sativa, Caesalpinia bonducella, Capparis deciduas, Coccinia indica, Eriobotrya japonica, Eugenia jambolana, Ginseng species, Gymnema sylvestre, Mangifera indica, Momordica charantia, Ocimum sanctum, Phyllanthus amarus, Psidium guajava, Pterocarpus*

marsupium, Trigonella foenum graecum, Tinospora cordifolia, Stevia rebaudina, etc have shown blood glucose lowering activity. The plant constituents such as alkaloids, imidazoline compounds, ferulic acid, polysaccharides, saponin, triterpenoids, steroidal glycosides, and flavonoids among others were reported to have antidiabetic activity. However, their exact mechanism of action in lowering blood sugar is not clear enough to support therapeutic utility [14].

Momordica charantia also known as bitter melon, karela, balsum pear or bitter gourd is a popular plant used as an alternative therapy for diabetes among the indigenous population of Asia, South America, and Africa [15]. *M. charantia* has been proposed to contain bioactive components having antidiabetic properties such as charantin, vicine, and polypeptide-p, as well as other unspecific bioactive components including antioxidants. Metabolic and antidiabetic effects of bitter melon extracts have been demonstrated in cell culture, animal, and human studies [16]. *M. charantia* has shown to stimulate glycogen storage by liver and insulin secretion by islets of Langerhans [17]. *M. charantia* suppresses weight gain by decreasing the expression of leptin in white adipose tissue and has the potential to reduce adiposity [18]. Moreover, bitter melon supplementation lowers serum and hepatic triglyceride in normal rats [19].A recent study proved that bitter melon could upregulate the activity of glucose transporter 4 (GLUT-4), peroxisome proliferator-activated receptor γ (PPARγ) and phosphatidylinositol 3 kinase (PI3K) thereby augmenting the glucose uptake and homeostasis [20] This action of bitter melon is in synergistic with pioglitazone. *M. charantia* can also improve insulin sensitivity by increasing insulin-stimulated insulin receptor substrate-1 (IRS1) tyrosine phosphorylation in high-fat diet-fed mice/rats [21]. Recent extensive screening has identified triterpenoids to be the blood glucose lowering component present in *Momordica* that may be responsible for the activation of AMP-activated protein kinase [22].

Thus the present study was undertaken to evaluate any possible synergistic effects between aqueous extract of *M. charantia* seed in combination with an insulin sensitizer metformin on blood glucose levels and body weight compared to metformin plus pioglitazone.

MATERIALS AND METHODS

Plant material

Momordica charantia fruit available in the local market was used in the study.

Preparation of seed extract

The *Momordica charantia* fruit is cut and seeds were separated, dried under shade and then grounded into a fine powder by an electronic grinder. The powdered seeds were kept in a airtight container in a deep freeze maintained at 4°C until the time of further use. The seed extract was prepared by dissolving a known amount of seed powder in distilled water. An aqueous suspension was prepared to facilitate easy handling. The drug solutions were administered intragastrically. The dosage schedule for the drug was once a day.

Experimental animal

Healthy adult rats of Wistar strain weighing around 200±10 gms were used in the present study. The animals were housed in clean polypropylene cages and maintained in a well ventilated temperature controlled animal house with constant 12 h light\dark schedule. The animals were fed with standard rat pellet diet and clean drinking water was made available *ad libitum*. All animal procedures have been approved and a prior permission from the Institutional Animal Ethical Committee was obtained as per prescribed guidelines (KSHEMA/IAEC/20/2013).

Experimental design

The animals were divided into five groups with 6 animals in each group.

Group I: Normal control

Group II: Rats treated with Metformin (1250mg/kg b. w/day) in aqueous solution orally for 7 days along with intraperitoneal Dexamethasone (8mg/kg) for the last 3 days.

Group III: Rats treated with Metformin (1250mg/kg) + Pioglitazone (75mg/kg) in aqueous solution orally for 7 days along with intraperitoneal Dexamethasone (8mg/kg) for the last 3 days.

Group IV: Rats treated with Metformin (1250mg/kg) + *Momordica charantia* seed extract (2500mg/kg) in aqueous solution orally for 7 days along with intraperitoneal Dexamethasone (8mg/kg) for the last 3 days.

Group V: Rats treated with intraperitoneal Dexamethasone (8mg/kg) for 7 days.

Dexamethasone induced diabetes model

Dexamethasone-induced hyperglycemia is a useful experimental model to study the activity of antidiabetic agents in reducing insulin resistance. The mechanism by which dexamethasone brings about a diabetic state includes an increase in serum glucose levels and fats by promoting gluconeogenesis and lipolysis and by inhibiting glucose uptake in muscle and adipose tissue. Increased serum glucose levels under the influence of glucocorticoids stimulates insulin release. Eventually the beta cells of pancreatic islets are exhausted and diabetes mellitus, also called steroid diabetes results. Due to direct inhibition of peripheral glucose uptake by skeletal muscle, glucocorticoids cause insulin resistance. Increased proteolysis under the influence of dexamethasone results in decreased muscle mass and thinning of the skin.

Blood glucose estimation

The blood sample was obtained through tail-flick method and Random Blood Sugar (RBS) levels were estimated on the1st, 5th, and the 7th day by ONETOUCH Select Simple Glucometer.

Body weight determination

Weight of rats was recorded on the1st, 5th, and 7th day of the study period.

Statistical analysis

All experimental results were represented as the mean ± SD for 6 animals per group. Statistical analyses were performed using analysis of variance (one-way ANOVA) followed by Dunnett's test. Statistical significance was set at $P < 0.05$.

RESULTS

The effect of aqueous extract of *Momordica charantia* seeds on blood glucose level on the 1st, 5th and 7th days of the study period was shown in fig. 1. All the treatment groups showed a significant antidiabetic effect on 5th and 7th day of treatment. Data shows that after 7 days of treatment blood glucose reduction in metformin + MCSE group ($P = 0.019$, $P < 0.05$) was more than a Metformin + Pioglitazone group. Blood glucose levels were increased in the Dexamethasone control group due to increase insulin resistance.

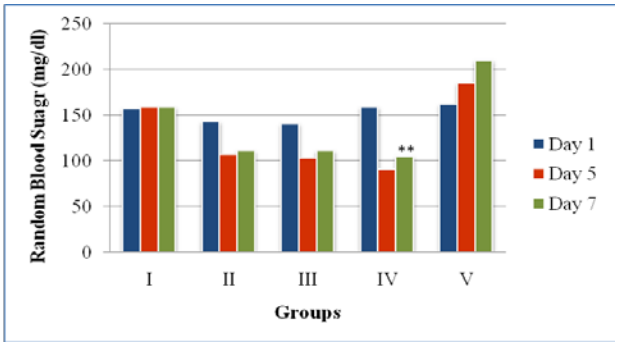

Fig. 1: All values are expressed as mean ± S. D (n = 6). **P = 0.019 (p is significant), as compared to Metformin plus Pioglitazone group. One-way ANOVA followed by Dunnet test

The effect of aqueous extract of *Momordica charantia* seeds on body weight on the 1st, 5th and 7th days of the study period was shown in fig. 2. The body weight of diabetic control rats decreases during the study period, as administration of dexamethasone results in decreased muscle mass and thinning of the skin. All the treatment groups showed significant reduction in body weight on 5th and 7th day of treatment. Data shows that after 7 days of treatment body weight reduction in metformin + MCSE group ($P = 0.004$, $P < 0.05$) was more than a Metformin + Pioglitazone group.

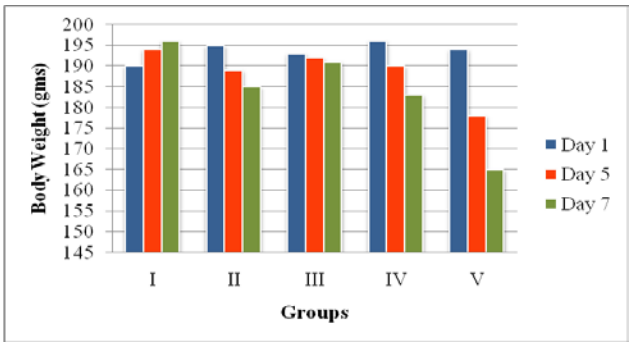

Fig. 2: All values are expressed as mean ± S. D (n = 6)

DISCUSSION

The increased admiration of herbal medicines for diabetes may be due to the side-effects associated with the conventional antidiabetic drugs. The World Health Organization (WHO) has also substantiated the utilization of herbal remedies for the management of diabetes [23].

Although considered natural, many of these herbal therapies can interact with other medications, causing either potentially dangerous side effects and/or reduced benefits from the medications. Currently, there is very little information published on herb-drug interactions whilst the use of herbs is progressively growing across the world [24]. The present study was undertaken to evaluate the effect of *M. charantia* seed aqueous extract on blood glucose levels and body weight along with a well known insulin sensitizer metformin in comparison with a combination of metformin plus pioglitazone.

Metformin is recognized as a first-line antidiabetic agent for the management of type 2 diabetes mellitus. It is suitable, irrespective of age, body weight, severity of hyperglycemia and provides a convenient pharmacological base for combined therapy with other antidiabetic agents. Metformin has a lower mortality and cardiovascular risk as compared with most other antidiabetic agents. Another benefit of metformin is that it does not produce hypoglycemia because it does not stimulate insulin secretion when it is given alone in patients with type 2 diabetes mellitus. Metformin is also renowned to facilitate modest weight loss in type 2 diabetic patients.

Currently, the cellular mechanisms involved in the antidiabetic effects of *M. Charantia* are not yet fully established. However, a number of studies have suggested that *M. charantia* may either have insulin like Secretagogue effect, it can stimulate peripheral glucose utilization or it may inhibit key gluconeogenic enzymes such as glucose-6-phosphatase and fructose biphosphatase. A recent study done by Tripathi Poonam et al., reported that *M. Charantia* fruit juice showed significant reduction in blood glucose levels and body weight when combined with low and high dose of metformin in a streptozotocin-induced diabetes model [25].

Many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another. Interactions between herbs and drugs may increase or decrease the pharmacological or toxicological effects of either component. Synergistic therapeutic effects may complicate the dosing of long-term medications e. g., herbs traditionally used to decrease glucose concentrations in diabetes could theoretically precipitate hypoglycemia if taken in combination with conventional drugs [26].

The result of the current study showed that MCSE (2500mg/kg) potentiate the blood glucose lowering effect of metformin in dexamethasone induced diabetic rats i. e., MCSE along with metformin showed synergistic effect. This synergistic effect may be helpful in reducing the dose of metformin in the treatment of diabetes, which will be helpful in minimizing the adverse effect related to metformin.

Even though pioglitazone remains an effective and useful antidiabetic drug with a strong effect on insulin resistance, its clinical use is limited by the risk of adverse events. Metformin also has some effect in improving peripheral insulin sensitivity. In contrast to pioglitazone and metformin combination, treatment with metformin plus MCSE may provide a better reduction in insulin resistance and improve glycemic control with an additive effect on body weight. The MCSE may substitute pioglitazone in the management of T2DM patients, provided its safety and efficacy be proven in well controlled randomized trials in humans.

CONCLUSION

Administration of MCSE with metformin for the treatment of dexamethasone induced insulin resistance showed synergistic effect. Because diet is one of the approaches in the management of diabetes mellitus, the antidiabetic potency of *M. Charantia* is to be utilized to the maximum extent. As both MCSE and metformin are associated with weight loss, MCSE could be an add-on therapy for treatment of T2DM in obese patients. With the advent of MCSE being used as an effective blood glucose lowering agent in our study, it can be used as an effective alternative to pioglitazone provided its safety and efficacy is being proved in human studies.

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CONFLICTS OF INTEREST

Declared None

REFERENCES

1. Natali A, Ferrannini E. Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: a systematic review. Diabetologia 2006;49:434-41.
2. Schernthaner G, Matthews DR, Charbonnel B, Hanefeld M, Brunetti P. Efficacy and safety of pioglitazone versus metformin in patients with type 2 diabetes mellitus: a double-blind, randomized trial. J Clin Endocrinol Metab 2004;89:6068-76.
3. Charbonnel B, Schernthaner G, Brunetti P, Matthews DR, Urquhart R, Tan MH, *et al*. Long-term efficacy and tolerability of add-on pioglitazone therapy to failing monotherapy compared with addition of gliclazide or metformin in patients with type 2 diabetes. Diabetologia 2005;48:1093-104.
4. Russell-Jones D, Cuddihy RM, Hanefeld M, Kumar A, Gonzalez JG, Chan M, *et al*. Efficacy and safety of exenatide once weekly versus metformin, pioglitazone, and sitagliptin used as monotherapy in drug-naive patients with type 2 diabetes(DURATION-4): a 26-week double-blind study. Diabetes Care 2012;35:252-8.
5. Takeda Pharmaceuticals. Highlights of prescribing information. [Internet]; Available from http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021073s035lbl.pdf [Last accessed on 2014 May 09].
6. Dormandy J, Bhattacharya M, van Troostenburg de Bruyn AR. Proactive investigators. Safety and tolerability of pioglitazone in high-risk patients with type 2 diabetes: an overview of data from proactive. Drug Saf 2009;32:187-202.
7. Erdmann E, Song E, Spanheimer R, van Troostenburg de Bruyn AR, Perez A. Observational follow-up of the Proactive Study: a 6-year update. Diabetes Obes Metab 2014;16:63-74.
8. Lewis JD, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry CP Jr, *et al*. Risk of bladder cancer among diabetic

patients treated with pioglitazone: Interim report of a longitudinal cohort study. Diabetes Care 2011;34:916-22.

9. Wei L, MacDonald TM, Mackenzie IS. Pioglitazone and bladder cancer: a propensity score matched cohort study. Br J Clin Pharmacol 2013;75:254-9.
10. Neumann A, Weill A, Ricordeau P, Fagot JP, Alla F, Allemand H. Pioglitazone and risk of bladder cancer among diabetic patients in France: a population-based cohort study. Diabetologia 2012;55:1953-62.
11. FDA Drug Safety Communication. Update to ongoing safety review of Actos (pioglitazone) and increased risk of bladder cancer. [Internet]; Available from http://www.fda.gov/ drugs/drugsafety/ucm259150. htm [Last accessed on 2014 May 06].
12. Gazette of India: New Delhi. [Internet]; Available from: http://cdsco.nic.in/writereaddata/GSR%20379E.pdf [Last accessed on 2014 May10].
13. Gazette of India: New Delhi. [Internet]; Available from: http://cdsco.nic.in/writereaddata/GRIjuly13.pdf [Last accessed on 2014 May10].
14. Khan V, Najmi AK, Akhtar M, Aqil M, Mujeeb M, Pillai KK. A pharmacological appraisal of medicinal plants with antidiabetic potential. J Pharm Bioall Sci 2012;4:27-42.
15. Basch E, Gabardi S, Ulbricht C. Bitter melon (*Momordica charantia*): A review of efficacy and safety. Am J Health-Syst Pharm 2003;60:356-9.
16. McCarty MF. Does bitter melon contain an activator of AMP-activated kinase? Med Hypotheses 2004;63:340-3.
17. Yibchok S, Adisakwattana S, Cheng YY, Sangvanich P, Roengsumran S, Hsu WH. Slow acting protein extract from fruit pulp of *Momordica charantia* with insulin secretagogue and insulinomimetic activities. Biol Pharm Bull 2006;29:1126-31.
18. Chen Q, Chan LLY, Li ETS. Bitter melon (*Momordica charantia*) reduces adiposity, lowers serum insulin and normalizes glucose tolerance in rats fed a high fat diet. J Nutr 2003;133:1088-93.
19. Senanayake GVK, Maruyama M, Shibuya K, Sakono M, Fukuda N, Morishita T, *et al*. The effects of bitter melon (*Momordica charantia*) on serum and liver triglyceride levels in rats. J Ethnopharmacol 2004;91:257-62.
20. Kumar R, Balaji S, Uma TS, Sehgal PK. Fruit extracts of *Momordica charantia* potentiate glucose uptake and up-regulate Glut-4, PPARγ and PI3K. J Ethnopharmacol 2009;126:533-7.
21. Nerurkar PV, Lee YK, Motosue M, Adeli K, Nerurkar VR. *Momordica charantia* (bitter melon) reduces plasma apolipoprotein B-100 and increases hepatic insulin receptor substrate and phosphoinositide-3 kinase interactions. Br J Nutr 2008;100:751-9.
22. Cheng HL, Huang HK, Chang CI, Tsai CP, Chou CH. A cell-based screening identifies compounds from the stem of *Momordica charantia* that overcome insulin resistance and activate AMP-activated protein kinase. J Agric Food Chem 2008;56:6835-43.
23. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. Diabetes Care 1989;12:553-64.
24. Gohil KL, Patel JA. Herb-drug interactions: a review and study based on assessment of clinical case reports in literature. Ind J Pharmacol 2007;39:129-39.
25. Tripathi P, Gupta PP, Lal Vijay K. Interaction of Aqueous Extract of Trigonella foenum-graecum Seeds with Glibenclamide in Streptozotocin Induced Diabetic Rats. Am J Pharmacol Toxicol 2013;8:102-6.
26. Adriane FB. Herb-drug interactions. Lancet 2000;355:134-8.