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MOMORDICA CHARANTIA FRUIT EXTRACT AND GLIBENCLAMIDE SYNERGISTICALLY AMELIORATE CORTICOSTEROID INDUCED DIABETES MELLITUS IN MICE

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ABSTRACT

As fruit extract of bitter gourd is known to be anti-hyperglycemic and anti-oxidative in nature, in this study we have investigated the possible synergistic effects of *Momordica charantia* (MC) fruit extract and glibenclamide (GLB) in corticosteroid-induced hyperglycemic mice. Four different groups of animals were treated with dexamethasone alone for first 7 days and then with either MC extract, GLB or MC + GLB along with dexamethasone for next 15 days. On 23rd day, animals were sacrificed; liver and kidney were excised for the estimation of lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). Alterations in serum glucose as well as liver histology were also studied. While dexamethasone administration increased the concentration of serum glucose ($p<0.001$) and LPO (for both renal and hepatic tissues, $p<0.001$) with a concomitant decrease in SOD, CAT and GSH; administration of MC (150 mg /kg BW) and GLB (500 µg /kg BW) reversed all these indices in -induced diabetic animals. Although, individual treatment with MC or GLB also exhibited positive effects, simultaneous administration of both the test drugs ameliorated hyperglycemia and oxidative stress more effectively. The test extract also exhibited high antioxidative activity in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and in 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assays. Our findings indicate the synergistic benefits of the combined use of Momordica extract and glibenclamide in regulating diabetes mellitus. HPLC analysis suggests the involvement of gallic acid, quercetin and rutin, present in the test extract in the mechanism of observed synergistic action.

KEYWORDS: **Synergistic effects, *Momordica charantia,* Glibenclamide, Diabetes mellitus**

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INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder caused by insulin deficiency and /or insulin resistance is considered as one of the major health problems.[1] Most of the conventional hypoglycemic drugs , currently in use are known to exhibit side effects.[2-4] In fact, management of diabetes without any side effect is still a challenge to the medical system and this has led to an increasing demand for natural products with antidiabetic activity and fewer side effects.[5] Hence there is a need to search for a better strategy for the treatment of diabetes mellitus (DM) with less or no side effects. Since herbal extracts are believed to exhibit no adverse effect,[6] very often they are preferred over the conventional drug. *Momordica charantia* (MC), commonly known as bitter gourd (Family- Cucurbitaceae) is a known anti-diabetic agent with additional anti-peroxidative property.[7,8] However, in most of the earlier studies MC extract was used as single component in therapeutic studies. Whether in combination with conventional drug it can alter the beneficial effect was not evaluated by any one earlier. Similarly, glibenclamide(GLB) is a commonly prescribed antidiabetic conventional drug.[9] Although some studies are available on its hypoglycemic affects, no investigation was made so far in corticosteroid-induced diabetic subjects, despite the fact that the chronic treatment of glucocorticoids invariably causes hyperglycemia.[10,11]Although, ample work has been done on the herbal regulation of DM in general,[12,13] scientific report on the amelioration of corticosteroid induced diabetes by plant extract is negligible.[11] Although literature is available on the use of herbal formulations,[14] on the combined effects of plant extract and a conventional drug, practically no trial had been made earlier against corticosteroid-induced diabetes. The present investigation is an attempt to explore this possibility.

MATERIALS AND METHODS

Chemicals

Trichloro-acetic acid, sodium dodecyl sulphate, ellman's reagent, diethyl triamine penta acetic acid, tris buffer, methanol, acetonitrile and HPLC grade water were purchased from E. Merck Ltd., Mumbai, India, while thiobarbituric acid and gallic acid were purchased from Hi-media, Mumbai, India. Dexamethasone (Decdan, Merind Co., Bombay, India) and the test drug glibenclamide (Aventis, India) were procured from a registered local pharmacy shop. Quercetin and rutin were purchased from Sigma-Aldrich chemicals, St. Louis, MO, USA.

Plant Material

M. charantia fruits were purchased from local market, seeds were removed and an alcoholic extract was prepared according to the method of Panda and Kar. [15] In brief, about 1 kg seedless vegetable was blended with 1500 ml of 95 % ethanol and left at room temperature (RT) with occasional shaking. Suspension was filtered and filtrate was evaporated at 50˚C to remove alcohol.

Animals

Swiss colony bred albino mice (28 ±2 g) were acclimatized for 7 days in a temperature (27 ±1˚C) and light controlled (14 hr light: 10 hr dark) room with the provision of food (Gold Mahur mice feed, Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. Animals were maintained in accordance with the guidelines of committee for the purpose of control and supervision on experiments in animals, Ministry of Social justice and Empowerment, Govt. of India, India. Permission was also taken from the Institutional Animal Ethics Committee (Reg. No, 779) of Devi Ahilya University, Indore, India.

Experimental design

Thirty five healthy male mice were divided into 5 groups of seven each. While group I animals received normal saline (0.1 ml/d) and served as

control, rest all were treated with dexamethasone at 1 mg /kg for 7 consecutive days[10] to render them diabetic. Animals of group III, IV and V received test plant extract at 150 mg /kg,[16] GLB at 500 µg /kg[11] or equivalent doses of both the drugs respectively, for a further period of 15 days. In these three groups simultaneous administration of dexamethasone at 1 mg /kg was also continued. On 23rd day, overnight fasted animals were sacrificed by cervical dislocation under mild anesthesia. Blood samples were collected from each animal, allowed to clot and centrifuged to get a clear serum and were assayed for glucose. Liver and kidney were quickly removed and washed with phosphate buffered saline (PBS) and immediately processed for the estimation of lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). Serum glucose level was measured by the glucose oxidase / peroxidase method based on the protocol of Trinder [17] as followed earlier in our laboratory.[18] LPO was determined by the method of Ohkawa *et al* [19] and SOD activity was estimated using the protocol of Marklund and Marklund. [20] While catalase activity was estimated by the method of Aebi, [21] for GSH content the protocol of Ellman[22] and for protein content, method of Lowry *et al* [23] were followed.

A portion of liver tissue was fixed in 10% buffered formaldehyde and kept for 24 hrs. After fixation was accomplished, the excess of fixative was washed out under running tap water. The tissues were then dehydrated in the descending grades of alcohol, finally cleared in xylene and then embedded in molten paraffin wax. Sections were cut at 5-6 µm thickness, stained with hematoxylin and eosin, and viewed under light microscope to study the histopathological changes.[24] Following the procedure of Makawi *et al*, [25] the C18 column was rinsed with 3 mL methanol, 3 mL acetonitrile and 3 mL deionized water (adjusted to pH 2 with 1N HCl) at a flow rate of 1 mL /min. The prepared test extract (1 g) was dissolved in 10 mL deionized water, and the solid phase extraction (SPE) was processed. In brief, samples (500 mg each) were applied at top of the octadodecyl whatman SPE cartridges preconditioned with 3 mL deionized water (at pH 2) + 10 mL deionized water. The adsorbed materials were rinsed by 2 mL methanol + 1 mL acetonitrile. Elutes were then filtered through a 0.45 µm membrane syringe filter, and subjected to HPLC analyses.

HPLC analysis was done in a Jasco, PU-1580 instrument with HSS-1500 monitoring system, using a Hypersil ODS (5µm) reverse phase C-18 column (250 x 4 mm). The mobile phase consisted of acetonitrile and water (7:3). The flow rate was 0.5 ml /min, while the injection volume and the monitoring wavelength were 20 µl and 254 nm respectively. The quantities of three compounds in the extract were calculated by interpolation of the peak area of samples with that of standards in the calibration curve. Total polyphenolic contents of the test extract was estimated following the protocol of Leontowicz *et al*.[26] In brief, 0.125 ml of test extract of known concentration (100 mg/ml) was diluted with 0.5 ml of distilled water, and then 0.125 ml of Folin-Ciocalteu reagent was added to the mixture. The final volume was made up to 3.0 ml with distilled water and then was incubated at room temperature for another 90 min. Finally the absorbance was measured against the prepared blank at 765 nm in comparison with standard of known concentrations of gallic acid. The results were expressed in mg gallic acid equivalent / 100 g dry weight of the extract. The coefficient of determination was, R^2=0.9748. Total flavonoids were determined following the protocol of Leontowicz *et al*. [26] The test seed extract of 25, 50 and 100 ppm concentrations were prepared and (0.25 ml) each dilution was mixed with 1.25 ml distilled water. It was followed by the addition of 75 µl of 5% sodium nitrite solution and 150 µl of 10% aluminum chloride ($AlCl_3.6H_2O$) solution. After incubation of 5 min 0.5 ml of 1M NaOH was added. The total volume was made up to 2.5 ml with distilled water. Finally the absorbance was measured against the prepared blank at 510 nm in comparison with standards prepared similarly with known concentrations of quercetin. The results were expressed in mg quercetin equivalent / 100 g dry weight of the extract. The coefficient of

determination was, R^2=0.9748. The results are expressed as mg of quercetin equivalents / 100 g dry weight of the extract.

Different concentrations of the extract (5, 50 and 100 ppm) were taken in separate test tubes. One ml of the test extract was mixed with 0.5 ml of 0.15mM DPPH and allowed to stand at 20˚C for 30 min. Control tubes were prepared by adding all the chemicals, but without the extract. Ethanol was used for baseline correction. Changes in absorbance of the samples were measured at 517 nm. The radical scavenging activity was expressed in percent inhibition [% RSA = (control OD - sample OD / control OD) X 100], as described by Leontowicz *et al*.[26] ABTS radical cation decolorization assay was carried out using an improved method as described somewhere else.[27] ABTS was generated by oxidation of ABTS with potassium persulfate. ABTS was dissolved in deionized water to 7 mM concentration, and potassium persulfate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature for 24 h in the dark before use. The ABTS solution was diluted with ethanol to an absorbance of 0.7± 0.02 at 734 nm. After the addition of 1 ml of diluted ABTS solution to 10 µl of plant extract, absorbance was taken at 734 nm exactly 1 min after initial mixing up to 10 min. All determination was carried out in triplicate. The radical scavenging activity was expressed as % inhibition = [($Abs_{t=0}$ – Abs_{t-10}) / $Abs_{t=0}$ X 100]

Statistical analysis

Data are expressed as mean ± SE. Statistical analysis was done using analysis of variance (ANOVA) followed by student's t-test.

RESULTS

Following dexamethasone administration, a marked increase in serum glucose (Fig. 1) and in tissue LPO ($p < .001$) with a concomitant decrease in endogenous antioxidants such as SOD, CAT and GSH (Fig 2 and 3) was observed indicating a hyperglycemic and peroxidative condition. Although the test extract or the GLB alone decreased serum glucose as well as LPO and increased the levels of antioxidants to some extent; with simultaneous administration of MC extract and GLB in dexamethasone-induced animals, tissue LPO (both hepatic and renal tissues) and serum glucose were markedly decreased with a parallel increase in SOD, CAT and GSH (Fig. 1-3, $p < .001$for all). In fact, co-administration of both the drugs led to a greater decrease in serum glucose level (48 %) as compared to the treatments of MC and GLB alone where it was only 33 and 39 % respectively. A similar trend was also observed with respect to hepatic and renal LPO. Further, in this group, there was a greater increase in SOD, CAT and GSH in both hepatic (44 %, 49 % and 238 % respectively) and in renal (140 %, 68 % and 108 % respectively) tissues. Histological observations in liver also provided supportive evidence to the biochemical indices. While the liver of control mouse exhibited normal architecture with well preserved cytoplasm, nucleus and central vein; dexamethasone administration induced karyopyknosis and necrosis of the cell. The administration of drugs markedly ameliorated the changes induced by dexamethasone, which significantly reduced degenerated hepatocytes and markedly decreased the necrotic area (Fig 4). However, GLB was not found to be very effective in regaining the normal cell architecture. Here also the combined administration of both the drugs (MC and GLB) was found to ameliorate the toxic effects produced by dexamethasone in a better manner.

When total polyphenols and flavonoids were estimated, the amount of total polyphenols and flavonoids in the *M. charantia* fruit extract was calculated out to be of appreciable amount (44.66 ±3.06 mg gallic acid equivalent / 100 g dry weight and 37.59±0.81 mg / 100 g dry weight). The study of DPPH and ABTS radical scavenging activity revealed 71.4 % DPPH scavenging activity of *M. charantia* fruit extract as against 90 % for the standard antioxidant, ascorbic acid at the same concentration. Of course in case of ABTS radical scavenging assay the highest % inhibition of free radicals

shown by the test extract was 41.2 %. HPLC analysis. The test extract indicated the presence of gallic acid (23.8 µg/ml), quercetin (14.0 µg/ml) and rutin (8.9 µg/ml) in HPLC analyses (Fig. 5) .

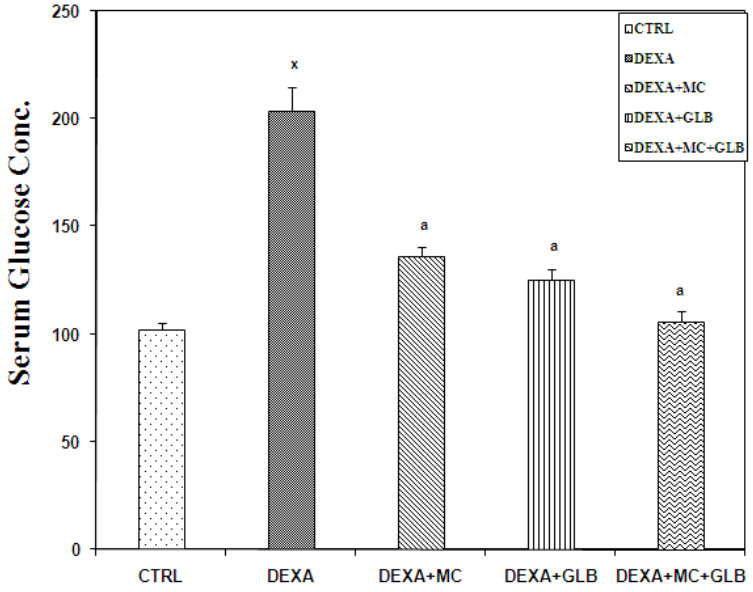

Fig. 1

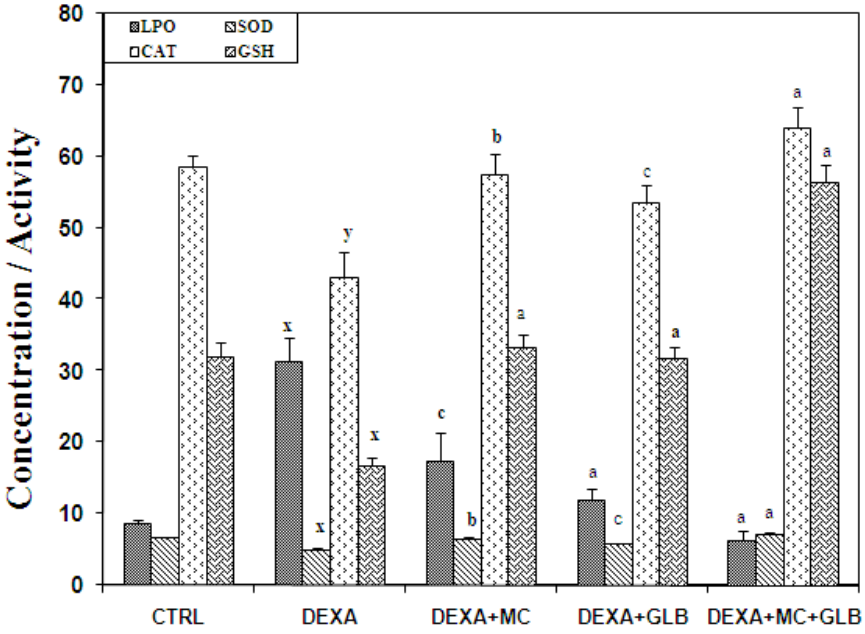

Fig. 2

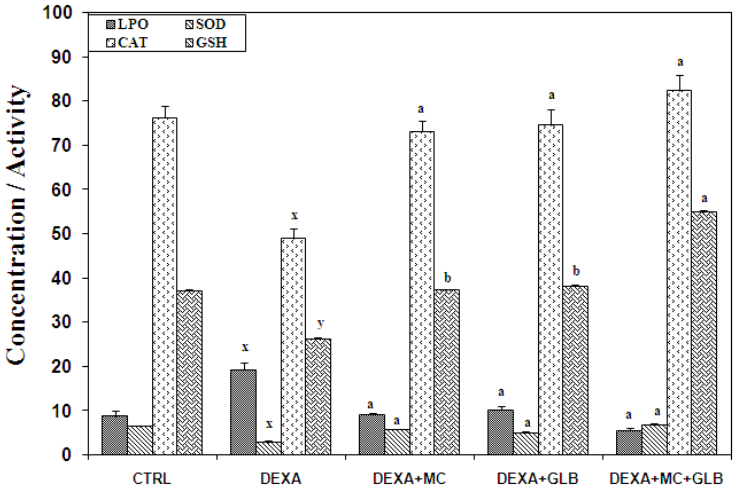

Fig. 3

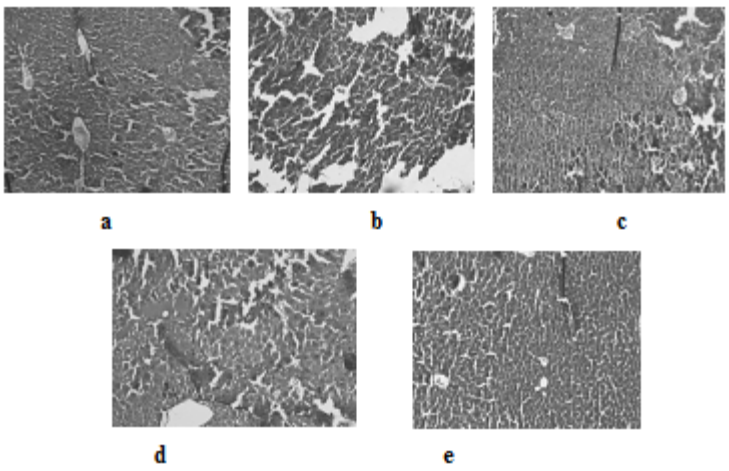

Fig. 4

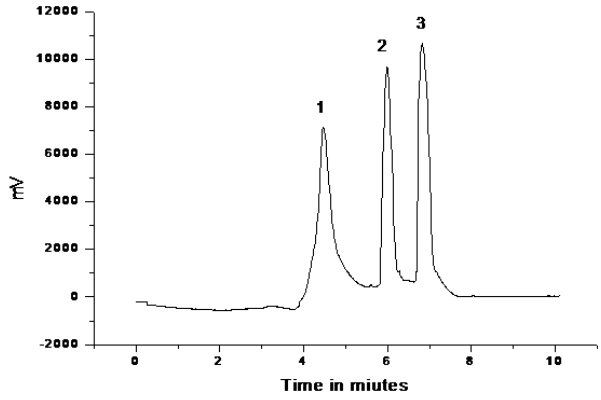

Fig. 5

DISCUSSION

The results clearly indicate that the administration of individual test drug, either MC extract or glibenclamide decreases serum glucose concentration as well as tissue lipid peroxidation with a concomitant increase in tissue SOD, CAT and GSH to some extent, as has also been observed by some other workers.[28] However, when both were administered together, additional beneficial effects were found in comparison to that of individual drugs, clearly indicating their synergistic effects. While a good number of reports are available on herbal formulations on the regulation of DM and LPO,[14,29,30] on the combined effect of a herbal drug and a conventional drug, investigations are extremely limited.[12,31] Practically not even a single one on corticosteroid induced diabetes is there. We for the first time report that a synergistic beneficial effect is possible when a herbal extract and a conventional drug are administered together for type II diabetes. The dexamethasone induced increase in serum glucose are reported to be caused by down regulation of glucose transporter protein and also by impairing the translocation machinery of GLUT 4 [32] indicating a diabetic condition. This could be a cause in the present study also. We also observed enhanced tissue LPO with a concomitant decrease in endogenous antioxidants such as SOD, CAT and GSH, as reported earlier by us and by some other workers.[10,33] However, *M. charantia* extract administration in dexamethasone-treated animals, not only decreased serum glucose but also the tissue LPO, suggesting its anti-diabetic role. Although, report is available on the hypoglycemic and antiperoxidative effects of MC extract,[16] the present finding on the anti-hyperglycemic and antioxidative effects of the test extract in dexamethasone induced diabetic animals, appears to be a new one. The supporting evidences with respect to antioxidant indices (SOD, CAT and GSH) in which MC extract brought significant reversible changes are also similar to the reports made with few other plant extracts.[11]

As expected administration of GLB alone to dexamethasone-treated mice also showed a decrease in serum glucose and LPO, which may be compared with an earlier report in streptozotocin induced diabetic animals.[34] Interestingly, following the administration of both the test plant extract and the conventional drug, there was a greater improvement in hyperglycemic conditions as compared to their individual treatments. In fact, co-treatment of MC and GLB not only led to a greater percent decrease in blood glucose and LPO, but also proportionately increased the status of SOD, CAT and GSH. This may be emphasized that nothing was investigated so far on the synergistic effect of Momordica extract and glibenclamide using a corticosteroid as diabetes inducing agent. Obviously, the present report is the first one that suggests the synergistic use of a herbal extract and a conventional medicine. Interestingly, beneficial effects of the test extract and GLB were also observed with respect to the changes in histological features. In liver sections of dexamethasone-treated mice a loss of cellular architecture, vacuolization, disarrangement of normal hepatic cells were observed (Fig. 4). While the administration of MC or GLB alone ameliorated these adverse effects to some extent,co-treatment of the two test drugs nearly normalized the hepatocyte architecture as indicated by healthy nucleus and reduction in necrosis, further supporting the additional beneficial effect of the test plant extract in GLB treated animals. Thus, histological observations of the liver further provided supportive evidence to the biochemical findings.

On estimating the major active principles, phenolic and flavonoid contents were found to be present in appreciable amount in the Momordica extract, indicating their possible involvement in exhibiting the strong antioxidant properties. This antioxidative / free radical scavenging property of test extract was further

supported through the findings with DPPH and ABTS systems.[35,36] HPLC analyses (Fig. 5) of the test extract indicated the contribution of gallic acid (23.8 µg/ml), quercetin (14.0 µg/ml) and rutin (8.9 µg/ml) ,which are well known for their antioxidative properties,[37-39] in the ameliorative action of Momordica extract. An earlier report [40] also indicated the presence of these active components, suggesting their possible involvement in the action of the test plant extract. Whatever may be the mode of action of test extract; our findings clearly indicate the synergistic effects of MC and GLB, suggesting their possible therapeutic use.

CONCLUSION

Keeping the observations in mind it is concluded that ethanol extract of *Momordica charantia* fruit, when administered along with a conventional antidiabetic drug, glibenclamide, markedly ameliorated the dexamethasone-induced diabetic condition. In fact, our findings for the first time suggest that MC and GLB, administered together may prove to be more advantageous and synergistic in comparison to their individual treatments. The additional benefit by the test extract could be the result of gallic acid and quercetin present in appreciable amount in the extract, resulting its free radical scavenging action.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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