Antidiabetic Evaluation of *Momordica charantia* L Fruit Extracts

S Tahira[1] F Hussain[2]

ABSTRACT

To investigate hypoglycaemic, hypolipidaemic and pancreatic beta cell regeneration activities of Momordica charantia L fruits *(MC). Alloxan-induced diabetic rabbits were treated with methanolic and ethanolic MC extract. Effects of plant extracts and the drug glibenclamide on serum glucose, lipid profile and pancreatic beta cell were determined after two weeks of treatment. Serum glucose and lipid profiles were assayed by kit methods. Pancreatic tissue histopathology was performed to study pancreatic beta cell regeneration.* Momordica charantia *extracts produced significant hypoglycaemic effects (*$p < 0.05$*). Hypolipidaemic activity of MC was negligible.* Momordica charantia *supplementations were unable to normalize glucose and lipid profiles. Glibenclamide, a standard drug, not only lowered hyperglycaemia and hyperlipidaemia but also restored the normal levels. Regeneration of pancreatic beta cells by MC extracts was minimal, with fractional improvement produced by glibenclamide. The most significant finding of the present study was a 28% reduction in hyperglycaemia by MC ethanol extracts. To determine reliable antidiabetic potentials of MC, identification of the relevant antidiabetic components and underlying mechanisms is warranted.*

Keywords: Diabetes mellitus, hyperglycaemia, hyperlipidaemia, *Momordica charantia* L

Evaluación Antidiabética de los Extractos Frutales de la *Momordica charantia* L

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RESUMEN

Investigar las actividades hipoglicémicas, hipolipidémicas, y de regeneración de las células betas pancreáticas, de los frutos de la Momordica charantia L *(MC). Conejos con diabetes inducida por aloxana fueron tratados con extractos de MC metanólicos y etanólicos. Tras dos semanas de tratamiento, se determinaron los efectos de los extractos de plantas y el medicamento, la glibenclamida en glucosa sérica, el perfil lipídico, y las células beta pancreáticas. Los perfiles de glucosa y lípidos séricos fueron analizados mediante métodos de ensayo con kits. Se llevó a cabo una histopatología del tejido pancreático con el fin de estudiar la regeneración de las células beta pancreáticas.* Los extractos de Momordica charantia *produjeron efectos hipoglicémicos significativos (*$p < 0.05$*). La actividad hipoglicémica de MC fue insignificante.* Los suplementos provenientes de la Momordica charantia *no fueron capaces de normalizar los perfiles de lípidos y glucosa. La glibenclamida, un fármaco estándar, no sólo redujo la hiperglicemia y la hiperlipidemia, sino que también restauró los niveles normales. La regeneración de las células beta pancreáticas por los extractos de MC fue mínima, con una mejoría fraccional producida por la glibenclamida. El hallazgo más significativo del presente estudio fue la reducción en un 28% de la hiperglicemia por extractos etanólicos de MC. Para determinar los potenciales antidiabéticos confiables de la MC, se garantiza la identificación de los componentes antidiabéticos y mecanismos subyacentes pertinentes.*

Palabras claves: Diabetes mellitus, hiperglicemia, hiperlipidemia, Momordica charantia L

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INTRODUCTION

There is an increasing need for effective, economical and accessible natural antidiabetic agents, as diabetes mellitus has led to serious morbidity and mortality worldwide (1, 2). Several studies have acknowledged the antidiabetic effects of *Momordica charantia* (MC) which has insulin secretagogue and insulinomimetic activities. *Momordica charantia* affects glucose and fatty acid transport and modulation of insulin secretion (3−6).

In diabetes mellitus, malfunction and apoptosis of beta cells lead to deficient insulin secretion. It is believed that beta cell function is severely compromised before the disease appears and then continues to decrease linearly with time. Therapeutic components that can restore pancreatic beta cells would be useful to develop a new treatment approach. Several bioactive metabolites are known to suppress islet fibrosis in diabetic rats. It has been reported that MC restores the altered histological architecture of the islets of Langerhans and thereby enhances insulin secretion. Another sugges-tion is that polypeptide-k and seed oil from MC are potent potential hypoglycaemic agents (7−10). Despite widespread use, limited empirical data on the efficacy of MC regarding restoration of the beta cells of pancreatic islets are available.

The intent of this study is to provide valuable insight regarding hypoglycaemic, hypolipidaemic and pancreatic beta cell regeneration properties of ethanol and methanol extracts of *Momordica charantia* fruits.

MATERIALS AND METHODS

Plant material

Momordica charantia Linn (Cucurbitaceae) fruits procured from the local retail markets were identified by Dr Mansoor Hameed, Associate Professor, Department of Botany, University of Agriculture, Faisalabad, Pakistan, and a voucher specimen was kept at the laboratory.

Preparation of plant extracts

Momordica charantia fruits were dried at room temperature, powdered in an electric grinder and stored at 5 °C until further analysis. Fruit powder was extracted with solvents (ethanol and methanol) by soxhlet extraction. Solvents were evaporated in a rotary evaporator at 40°−50 °C. With final yields of 3.2% and 3.6% for ethanolic and methanolic extracts, respectively, their suspensions were prepared in 0.5% w/v carboxymethylcellulose in normal saline solution.

Chemicals

Alloxan monohydrate (Sigma Chemical Co, St Louis, MO, USA), glibenclamide (benclamide, Valor Pharmaceuticals, Islamabad, Pakistan) and all other chemicals were of analytical grade.

Animals

Male rabbits (1−1.5 Kg weight) were housed at the Institute of Microbiology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan, at room temperature with a 12-hour light and dark schedule with free access to standard feed and tap water *ad libitum.* The institutional Advanced Studies and Research Board (ASRB) approved the study.

Experimental design

Rabbits were divided into five groups (seven animals per group) as: healthy non-diabetic – negative controls (NC), diabetic rabbits that received no plant extract or drug except saline treatment – positive control (DC), diabetic rabbits supplemented with 100 mg/kg body weight/day oral doses of MC fruit ethanol extract (DE), diabetic rabbits supplemented with 100 mg/kg body weight/day oral doses of MC fruit methanol extract (DM) and diabetic rabbits supplemented with 2.5 mg/kg body weight/day oral dose of glibenclamide, a standard antidiabetic drug (DG).

Induction of diabetes

Diabetes mellitus was induced by administering alloxan monohydrate (80 mg/kg body weight, dissolved in normal 5% saline solution) through marginal ear vein intravenously. Those with 200 mg/dL blood glucose were considered diabetic.

Bioassay

After two weeks of experiment, fasting glucose (FG), total cholesterol (TC), triacylglycerides (TAG), and high-density lipoprotein cholesterol (HDL-C) were measured by (Ecoline Diasys GmbH Merck, Germany) kit methods. Low-density lipoprotein cholesterol (LDL-C) was assessed as described earlier (11).

Pancreatic histopathology

Animals were sacrificed under anaesthesia and their pancreas was placed in 10% formalin. The paraffin sections were prepared in an automatic tissue processor and sliced into 5 mm thick sections in a rotary microtome and then stained with haematoxylin-eosin dye (Merck) and mounted with Canada balsam. Histopathological inspection was performed with photomicroscope (12).

Statistical analysis

Results are presented as mean ± standard deviation (SD) of three observations. All the data were evaluated with SPSS (version 12.0, 2003 ® SPSS Inc, Chicago, IL, USA). *P*-values < 0.05 were considered significant.

RESULTS

There was a significant elevation in FG in all diabetic groups after alloxan injection in comparison to the control group. Figure 1 illustrates the variation in blood glucose levels of

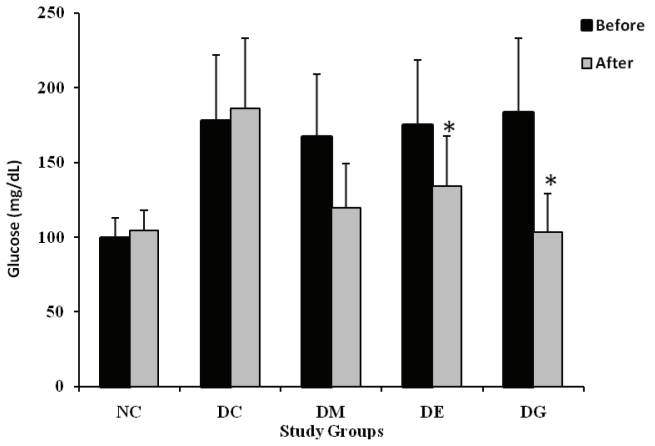

Fig. 1: Hypoglycaemic effect of *M charantia.*

NC: normal control (non-diabetic); DC: diabetic control (positive control); DM: diabetic group given *M charantia* methanol extract; DE: diabetic group given *M charantia* ethanol extract; DG: diabetic group given glibenclamide. * $p < 0.05$.

normal control, diabetic control and treated rabbits before and after the study. Supplementation of methanolic and ethanolic MC extracts resulted in significant reduction of FG (23−28%; $p < 0.05$) compared with diabetic control rabbits that continued to exhibit elevated glucose levels. However, these extracts did not cause FG to settle to the control levels. The antidiabetic drug normalized the FG by a 43.7% reduction ($p < 0.05$) as compared to the initial levels.

Serum TC and TAG levels were significantly elevated in the diabetic groups in comparison to the non-diabetic control (Fig. 2). Methanol and ethanol MC extracts were

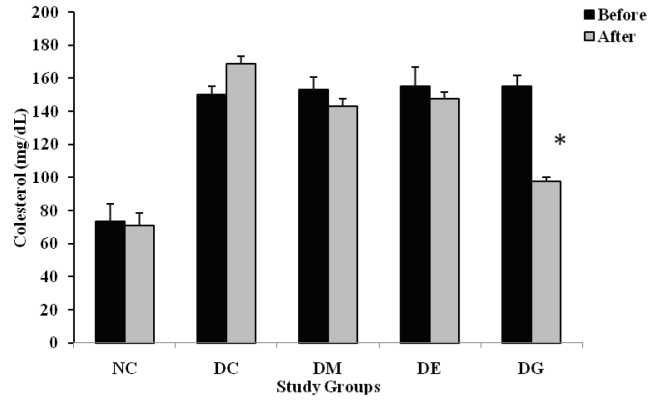

Fig. 2: Hypocholesterolaemic effect of *M charantia.*

NC: normal control (non-diabetic); DC: diabetic control (positive control); DM: diabetic group given *M charantia* methanol extract; DE: diabetic group given *M charantia* ethanol extract; DG: diabetic group given glibenclamide. * $p < 0.05$.

equally effective (6.56% and 6.37%) in decreasing TC. Nonetheless, the reductions were non-significant as compared to the non-diabetic control. On the other hand, a 27.29% reduction ($p < 0.05$) in TC was produced by glibenclamide. None of the treated groups, except the DG group, attained TC values close to the non-diabetic control group.

Similarly, only 7.3−7.9% amendments were observed in hypertriacylglyceridaemia in extract-treated rabbits (Fig. 3). In the DG group, TAG were shifted to the normal range,

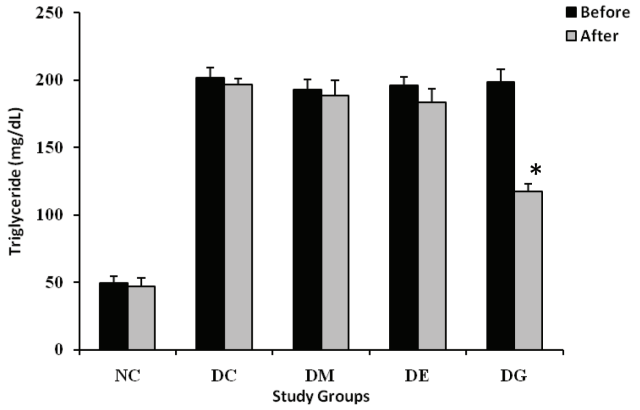

Fig. 3: Effect of *M charantia* on triacylglyceride.

NC: normal control (non-diabetic); DC: diabetic control (positive control); DM: diabetic group given *M charantia* methanol extract; DE: diabetic group given *M charantia* ethanol extract; DG: diabetic group given glibenclamide. *$p < 0.05$.

but these were higher than the TG levels in the non-diabetic group (51%, $p < 0.05$). High-density lipoprotein cholesterol decreased in diabetic animals compared to the normal controls (Fig. 4). Their levels improved by 7.5−7.7% following

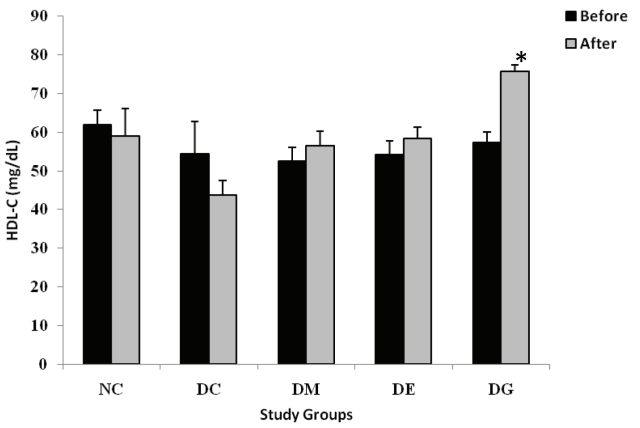

Fig. 4: Effect of *M charantia* on high-density lipoprotein cholesterol.

NC: normal control (non-diabetic); DC: diabetic control (positive control); DM: diabetic group given *M charantia* methanol extract; DE: diabetic group given *M charantia* ethanol extract; DG: diabetic group given glibenclamide. * $p < 0.05$.

MC extract use, even though normal HDL-C range was not achieved. In alloxan diabetic rabbits (DM and DE groups), abnormally high levels of LDL-C were observed despite treatment as compared to the vehicle controls. Though, in the

DG group rabbits, the drug managed to bring high LDL-C to normal LDL-C concentrations (Fig. 5).

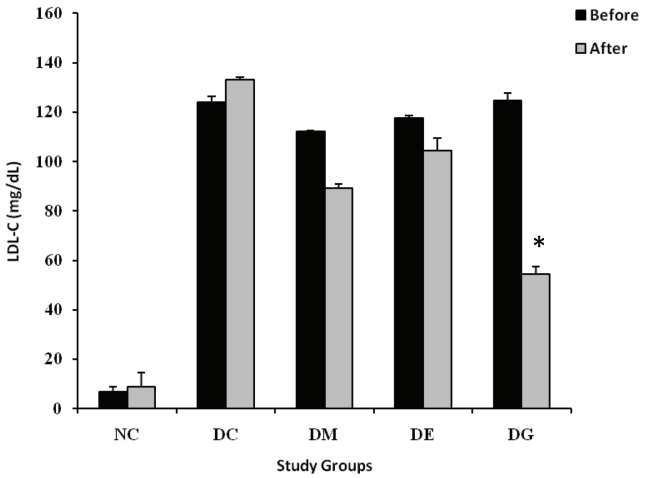

Fig. 5: Effect of *M charantia* on low-density lipoprotein cholesterol.

NC: normal control (non-diabetic); DC: diabetic control (positive control); DM: diabetic group given *M charantia* methanol extract; DE: diabetic group given *M charantia* ethanol extract; DG: diabetic group given glibenclamide. * $p < 0.05$.

The microscopic examination of stained pancreatic beta cells in control tissues showed normal appearance of the beta cells of the islet of Langerhans (Fig. 6a) which were severely impaired in diabetic pancreas (Fig. 6b). The overall architecture of damaged pancreatic beta cells was unaffected by ethanol and methanol MC extracts (Figs. 6c, 6d). Varied degree of atrophy of beta cells of the islet of Langerhans of the pancreas apparent in these extract treated groups was comparable to the untreated diabetic rabbits (DC group; Fig. 6b). The glibenclamide treated group showed partial regeneration of beta cells of the pancreas (Fig. 6e).

DISCUSSION

Previous studies indicated significant and consistent hypoglycaemic effect of MC (13−15). Similar antihyperglycaemic activity of MC evident in the present study can be justified by the multiple antihyperglycaemic mechanisms involved. From the review of the literature, it can be concluded that MC shows antidiabetic potential due to the presence of polyphenols, flavonoids, terpenoids, coumarins and other phytoconstituents which exhibit a lowering effect on glucose levels (13, 16). Similarly, MC is involved in protein tyrosine phosphatase 1B (PTP 1B) regulation (17). The presence of antihyperglycaemic proteins, other components or other mechanisms have already been confirmed and previously prominent hypoglycaemic potentials of MC were reported (18−20). In the current study, glibenclamide was a more effective hypoglycaemic agent than the MC extracts. The disparity in results can be justified by the fact

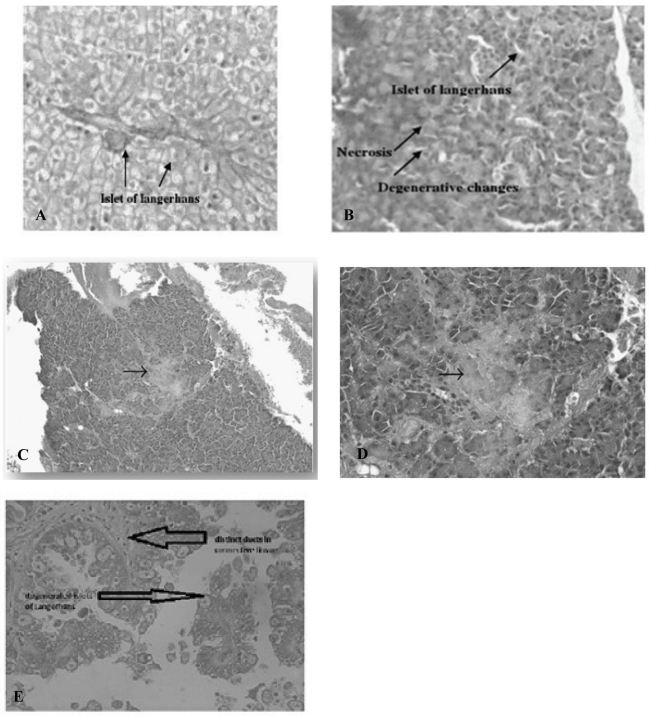

Fig. 6: Histopathological sections of pancreas stained by chromium haematoxyline-phloxine.

(A) Pancreas of normal healthy rabbit with normal appearance of islets of Langerhans. (B) Pancreas of diabetic rabbit showing degenerative changes and necrosis. (C) Pancreas of diabetic rabbit treated with *Momordica charantia L fruits* ethanol extract with aggregates (shrunken mass) of cells (k) and marked damage to beta-cells (→). (D) Pancreas of diabetic rabbit treated with *Momordica charantia L fruits* methanol extract with marked damage to beta-cells (→). The distorted beta cells of islets and degeneration are highlighted by deeply stained black and white areas, respectively. (E) Pancreas of diabetic rabbit treated with glibencla-mide, showing partial beta cells regeneration.

that various factors such as dose, treatment duration and solvents used, all shape the final impact of phytoconstituents in animal trials. Due to deficient evidence on the antihyperglycaemic effects of MC, further studies are required to address the issues of standardization and the quality control of preparations.

Hypocholesterolaemic results present in the current study were in accordance with the previous findings (21). Similarly, marginal effects of MC on serum lipids were reported (22). As studied formerly (23), MC lowered serum TAG by inhibition of hepatic fatty acid synthesis, by dampening sterol response binding protein 1c and fatty acid synthase mRNA, leading to reduction in TAG synthesis. That study demonstrated that MC ameliorates diabetic and hyperlipidaemic state in mice by regulation of hepatic PEPCK, 11beta-HSD1 and AMPK phosphorylation. The antihyperlipidaemic effect of MC can be justified by the fact that it enhances insulin secretion and promotes lipo-protein lipase activity. An indirect mechanism of lipid lowering by

MC might be its regulation of glucose levels, as hyperglycaemia affects circulating lipoprotein concentrations (24).

Bio-efficacy of the phytoconstituents depends on the dose provided as pure compound, plant extract, or whole food (25). *Momordica charantia* extracts were administered in a single dose of 100 mg/kg body weight/day. Repeated dosing with the same quantity of the extract over a 14-day period generated improvement in hyperglycaemia and hyperlipidaemia. This suggests the possibility of an effective dose of MC administration, above and below which beneficial physiological effects might be less likely. For chronic administration, MC content within the above range seems prudent. It is possible that other physiological parameters that benefit from a lower dose of MC extract might be exacerbated by a dose that is inappropriately high and/or sustained for prolonged periods of time. Conflicting prospects of dose response were presented in previous studies (9, 12), suggesting that this information should be useful for the design and interpretation of intervention studies investigating the health effects of MC.

After improvements in FG and lipid profile by MC extracts, it was expected that pancreatic damage might likewise be ameliorated in diabetic rabbit models. Many reports recommended that the fruit extract of *Momordica charantia* alleviated pancreatic damage and increased the number of β-cells in the diabetic rats (26−28). Our inferences regarding beta cell regeneration show discrepancy from documented information. The reason for this disparity may be ascribed to the fact that alloxan caused severe damage to the pancreas and the majority of the beta cells were destroyed with no chances of recovery from herbal treatment. The data available on the therapeutic use of MC suggest that it possesses blood glucose and lipid lowering effects, but at present, no firm conclusions as to its efficacy to restore pancreatic beta cells can be made. To assess reliable antidiabetic potentials of MC, identification of the relevant antidiabetic molecules and underlying mechanisms are warranted.

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