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PHYTOCHEMICAL DETERMINATION AND EXTRACTION OF MOMORDICA CHARANTIA FRUIT AND ITS HYPOGLYCEMIC POTENTIATION OF ORAL HYPOGLYCEMIC DRUGS IN DIABETES MELLITUS (NIDDM)

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Abstract : *Momordica charantia* (MC) fruit was subjected to phytochemical and pharmacological interaction studies with oral hypoglycemis in NIDDM patients. Phytochemical, chromatographical analysis and extraction of methanolic MC fruit soft (semi-solid form) in CCl_4 + C_6H_6 solvent system yielded 15 diverse chemical constituents – alkaloids, glycosides, aglycone, tannin, sterol, phenol and protein. The CCl_4 + C_6H_6 MC soft extract was used orally in a dose of 200 mg twice daily (BD) for pharmacological interactions with two diversely acting oral hypoglycemic agents- 1) metformin BD and 2) glibenclamide BD in 15 patients of either sex (52–65 years of age) of NIDDM. It was observed that with CCl_4 + C_6H_6 MC soft extract plus half doses of metformin or glibenclamide or both in combination caused hypoglycemia greater than that caused by full doses used in the study with 7 days treatment. Conclusively the extract acts in synergism with oral hypoglycemics and potentiates their hypoglycemia in NIDDM.

Key words : *momordica charantia* (MC) NIDDM hypoglycemic potentiation metformin glibenclamide

INTRODUCTION

Since antiquity *Momordica charantia* (MC – Karela) fruit has been an edible vegetable item in Indian food and has been known to exhibit blood sugar lowering potential. Diabetic patients use it in various forms eg. juice of MC as home remedy against diabetes mellitus. The hypoglycemic effect of MC in alloxan induced diabetes mellitus, in experimental rat and rabbit model has been documented (1–5).

The present study was undertaken with two objectives - 1) To explore the chemical constituents in MC fruit extract

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phytochemically and 2) To study the influence of MC fruit extract on the hypoglycemic response of two differently acting oral hypoglycemics metformin and glibenclamide.

METHODS

Phytochemical studies

The *Momordica charantia* (MC) fruit was procured from south India (Bangalore) and authenticated by a pharmacognosy expert before subjecting it to phytochemical analysis, chromatographical analysis, separation and extraction. The whole MC fruit comprising pericarp pulp and seeds was, ground in a mixer to get homogenized powdered mass. The powdered mass weighing 400 g was subjected to refluxing with water and methanol separately, followed by vaccum distillation. The vaccum distilled aqueous and methanolic extracts were subjected to evaporation on water bath to obtain aqueous and methanolic softs (semisolid residue containing substances which were soluble in respective solvents). Thin layer chromatography (TLC) of aqueous and methanolic softs was performed separately, by using solvent system comprising of methanol 2.5 ml and chloroform 47.5 ml. Here libermann burchard (LB) solution was used as a chromatographic spots detecting reagent.

A spot each of aqueous and methanolic softs was applied on two different thin layer chromatographic plates. The plates were dried at 100°C, sprayed with LB reagent and observed under 365 nm. The methanolic soft indicated presence of more number of chemical constituents than aqueous soft. Therefore, for further investigations methanolic soft was subjected to column chromatography.

Column chromatography was performed with different solvents in accord with their increasing polarity. Petroleum ether (PE) 600 ml, PE 300 ml + CCl_4 (carbon tetrachloride) 300 ml mixture, CCl_4 600 ml only, CCl_4 300 ml + C_6H_6 (benzene) 300 ml solvent mixture were passed through the column. TLC of soft obtained from CCl_4 300 ml + C_6H_6 300 ml solvent mixture after chemical treatment, showed 15 spots on chromatogram with different Rf values. Many of the above chemicals were inferred to be glycoside and aglycone in nature as their Rf values range from 0.1 to 0.4 and 0.5 to 0.9 respectively. As more number of spots were found in chromatogram of CCl_4 + C_6H_6 MC soft than in any other chromatogram obtained, therefore CCl_4 + C_6H_6 soft was selected for the present exploration in NIDDM.

Clinical studies

The institutional medical ethics committee passed the research project. The volunteer patients of noninsulin dependent diabetes mellitus (NIDDM) were explained the research protocol and their written consents obtained for the study.

CCl_4 + C_6H_6 MC soft was selected for pharmacological interaction study with metformin and glibenclamide in 15 patients of NIDDM, with ages ranging 52–65 years. The patients were divided into 3 groups. Group A (n = 5) received oral metformin (Glyciphage) tablet 0.5 g BD, AC (antecibum or before meal) for 7 days, the group B (n = 5) received oral glibenclamide (Daonil) tablet 5 mg BD, AC for 7 days and the group C (n = 5) received combined oral metformin 0.5 g tablet BD, AC plus glibenclamide 5 mg tablet BD, AC for 7 days. The fasting

and 2 hours post prandial (post lunch) blood glucose levels were determined by the standard orthotoluedine method, after diagnosis and before treatment (Control group) ie. on day 0 forming the base line and, after 7 days drug treatment on the 7th day. From the 8th day to 14th day, (7 days) the 3 groups (A, B, C) were treated with half the doses of respective drugs with the addition of CCl_4 + C_6H_6 MC soft orally 200 mg BD, AC. The fasting and postprandial blood glucose was determined again on 14th day. All the patients took routine diet avoiding sweets and sugar during the course of study and all the patients did routine physical work to which they were accustomed.

RESULTS

CCl_4 + C_6H_6 MC soft significantly increases the hypoglycemic effect of – half dose (0.25 g) of metformin by 10% (F) and 17% (PP), half dose (2.5 mg) of glibenclamide by 11% (F) and 15% (PP) and half doses both of metformin and glibenclamide in combination by 13% (F) and 21% (PP) in comparison to the hypoglycemic effect obtained by their full doses. CCl_4 + C_6H_6 MC soft on phytochemical testing was found to contain glycoside and aglycone mainly. However, traces of alkaloid, tannin, sterol, phenol, protein and carbohydrate were also detected. Therefore, the observed hypoglycemic effect of CCl_4 + C_6H_6 MC was due to the presence of these detectable chemical

TABLE I: Shows change in the blood glucose level following treatment with (a) Metformin (b) Glibenclamide (c) Metformin plus Glibenclamide separately & in combination with *Momordica charantia.*

Groups/Treatment	*Mean blood glucose level in mg % ± SEM*					
	(a) Control group (Before treatment)		*(b) After 7 days treatment with oral hypoglycemic (OH)*		*(c) After 7 days treatment with ½ dose of OH & CCl_4 + C_6H_6 MC soft 200 mg BD AC*	
	Fasting sugar (F)	*Post prandial sugar (PP)*	*Fasting sugar (F)*	*Post prandial sugar (PP)*	*Fasting sugar (F)*	*Post prandial sugar (PP)*
(A) Metformin 0.5 g BD, AC (n=5)	122.6±2.66	220.4±3.41	109.4±3.06	165.4±7.22	96.4±2.36	128.0±1.84
P value	–	–	>0.02 (a) (F) Vs. (b) (F)	>0.001 (a) (PP) Vs. (b) (PP)	>0.01 (b) (F) Vs. (c) (F)	<0.001 (b) (PP) Vs. (c) (PP)
(B) Glibenclamide 5 mg BD, AC (n=5)	122±1.70	196.6±5.34	106.8±1.07	156.4±4.06	93.8±1.36	128.4±2.66
P value	–	–	>0.001 (a) (F) Vs. (b) (F)	>0.001 (a) (PP) Vs. (b) (PP)	>0.001 (b) (F) Vs. (c) (F)	>0.001 (b) (PP) Vs. (c) (PP)
(C) Metformin 0.5 g BD, AC plus glibenclamide 5 mg BD, AC (n=5)	127±1.52	220.8±2.46	101.8±2.63	177.8±4.35	85.4±1.21	133.2±2.82
P value	–	–	>0.001 (a) (F) Vs. (b) (F)	>0.001 (a) (PP) Vs. (b) (PP)	>0.001 (b) (F) Vs. (c) (F)	>0.001 (b) (PP) Vs. (c) (PP)

It is evident that half dose 0.25 g of metformin with 200 mg of CCl_4 + C_6H_6 MC soft caused hypoglycemia more than that caused by full dose 0.5 g of metformin alone. Likewise half dose 0.25 mg of glibenclamide with 200 mg CCl_4 + C_6H_6 MC soft caused hypoglycemia more than that caused by 5 mg full dose of glibenclamide alone. Potentiation is apparent since after 7 days treatment with metformin (Ab), glibenclamide (Bb) and their combination (Cb), there is lowering of blood sugar levels as compared with their corresponding blood sugar levels before treatment – Control groups (Aa, Ba, Ca). These lowered blood sugar levels are further lowered by 7 days treatment with half doses of OH in presence of MC (Ac, Bc, Cc).

constituents which may be acting in synergism.

DISCUSSION

Momordica charantia causes hypoglycemia per se owing to hypoglycemic principle (3, 4). Many more diverse chemical constituents are presumed to be present in MC fruit. Under this presumption a detailed phytochemical and chromatographical exploration of MC fruit was undertaken and ahead in line, it was thought worthwhile to investigate its influence on the hypoglycemic activity of oral hypoglycemic drugs – metformin and glibenclamide in NIDDM patients.

Phytochemical and chromatographic exploration revealed that CCl_4 + C_6H_6 MC soft contained 15 diverse chemical constituents falling in the categories of alkaloid, glycoside, aglycone, tannin, sterol, phenol, protein and carbohydrate.

It was observed that with the 50% reduced doses of metformin and glibenclamide the CCl_4 + C_6H_6 MC soft showed an augmented hypoglycemic activity in NIDDM patients and fasting and post prandial blood glucose levels were 10% to 21% reduced more as compared to values obtainable with their full doses of metformin and glibenclamide.

The above augmented hypoglycemic activity may be attributable to the hypoglycemic activity *per se* of CCl_4 + C_6H_6 MC soft, facilitation of metformin activated extrapancreatic mechanism of tissue glucose uptake (6) and facilitation of glibenclamide activated pancreatic mechanism of insulin release (6). The interaction is a clinical synergism in terms of objective parameter of blood glucose reduction in NIDDM. It is hoped that prolonged use of CCl_4 + C_6H_6 MC soft with oral hypoglycemic drugs may effect retrogression of pathogenetic changes in NIDDM patients with restoration of euglycemia as reported earlier (1). This hope is based on the hitherto detected 15 diverse natural chemical constituents in CCl_4 + C_6H_6 MC soft that may have inherent potential to interact with molecules in cell milieu for homeostasis (2).

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