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In vitro antioxidant and hypolipidemic activity of *Momordica cymbalaria Fenzl* in wistar rats

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ABSTRACT

The main aim of the study is to investigate the vitro antioxidant and hypolipidemic activity of Momordica cymbalaria Fenzl. against high cholesterol diet induced hyperlipidemia in rats for 30 days.Rats were fed with ethanolic extract of Momordica cymbalaria (250mg/kg and 250mg/kg p.o) and atorvastatin (30 mg/kg, p.o) along with hyperlipidemic diet for 30 days. Momordica cymbalaria *and atorvastatin were found to lower the serum cholesterol, triacylglyceride, VLDL, LDL, and atherogenic index, but were found to increase the HDL as compared to the corresponding high fed cholesterol diet group (control). The hypolipidemic activity of Momordica cymbalaria can be ascribed to its inhibitory effect on the liver HMG CoA reductase activity. Thus, the study demonstrates that* Momordica cymbalaria *possesses a hypolipidemic effect.*

Keywords: *Momordica cymbalaria, HMG Co-A reductase, hypolipidemic effect, lipid profile.*

INTRODUCTION

Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death (Grundy 1986; Davey1993). Hyperlipidemia characterized by elevated serum total cholesterol , low density, very low densit y lipoprotein and decrease high density lipoprotein are the risk factor for coronary heart diseases. Hyperlipidemia associated lipid disorders are considered to cause the atherosclerotic cardiovascular disease (Kaesancini et al., 1994).

Disorders of lipid metabolism, following oxidative stress are the prime risk factors for initiation and progression of these diseases (Sharma et al.,2009:Kumar et al., 2008). The known lipid lowering drugs, such as fibrates, statins and bile acid sequestraints have many side effects in patients (Chattopadhyaya et al.,1996).

Momordica cymbalaria Fenzl (MC) (Cucurbitaceae) is a species found in Karnataka and Andhra Pradesh, India. Its tuber is traditionally used as an abortifacient and also for the treatment of diabetes mellitus. Its fruit powder and extract were reported to have antidiabetic activity in experimental type 1 diabetic models (Kameswara et al., 2003, Rao et al., 2001, Rao et al., 1999,). We have previously reported the antidiabetic activity of saponins of *M. cymbalaria* (SMC) possibly due to reversal of the atrophy of the pancreatic islets of β-cells, resulting in increased insulin secretion and hepatic glycogen levels which may attenuate hyperinsulinemia. The alpha-adrenergic blocking effect may also contribute to their insulin secretion and sensitizing effects (Raju et al., 2008).

Consumption of much fat may lead to the production of VLDL leaves increases, resulting in the formation of large amounts of LDL which may stick to the walls of the blood vessels if the quantity of HDL is insufficient, causing blockages for the normal flow of blood. Dietary fibres is highly recommended for disease prevention. The medicinal plants play a major role in hypolipidemic activity (Muramatsu et al., 1986).

MATERIALS AND METHODS

Collection of Plant Material:

Fresh leaves of *Momordica Cymbalaria* were collected from the forests of Tirupathi in Andhra Pradesh. The plant material was identified and authenticated by Dr. K. Madhav Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University.

Preparation of plant material and ethanolic extract:

The leaves were dried under shade at room temperature for seven days and powdered by the means of grinder and were sieved through sieve no.40 to get the coarse powder (750gm) and was extracted with ethanol by Soxhlet apparatus and obtained extract was concentrated and stored in vacuum desiccator. The obtained yield was calculated. Then the ethanolic extract of *M. Cymb*alaria was subjected to qualitative and phytochemical analysis.

Preliminary Phytochemical Screening:

The ethanolic extracts of *M. Cymb*alaria were subjected to preliminary phytochemical screening for their presence or absence of active phytochemical constituents by the following methods.

Experimental Animals:

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee) with registration number.

In-*Vitro* Antioxidant activity by DPPH Method (1, 1 diphenyl 2, picryl hydrazyl):

To 1 ml of DPPH dissolved in methanol (0.33%), 1 ml of (1.25-10 µl/ml) essential oil/ascorbic acid was added. After the incubation for 30 min, at 37°C, the absorbance at 517 nm was measured using UV-spectrophotometer. Corresponding blanks were taken for the same. The experiment was performed in triplicate. The absorbance of DPPH as control was obtained at 518 nm. Lower absorbance of the reaction mixture was an indication of higher radical scavenging activity of essential oil/standard antioxidant. DPPH become a stable diamagnetic molecule by accepting an electron. The methanolic solution of DPPH (violet colour) has got a strong UV absorbance at 517 nm. The presence of a reducing environment in the solution pairs the odd electrons of DPPH radical and the solution in turn losses its colour stoichiometrically and also decreases the absorbance at 517 nm (Brand-Williams et al., 1995) .

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The DPPH scavenging activity (%) was measured using the following formula
DPPH radical scavenging activity (%)

$$= [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Where,
$Abs_{control}$ is the absorbance of DPPH radical + methanol
Abs_{sample} is the absorbance of DPPH radical + essential oil/standard

Acute Toxicity studies:

The acute oral toxicity of ethanolic extract of leaves of the *M. cymbalaria* was carried out as per OECD 423 – guidelines

Evaluation of Anti-Hyperlipidemic activity:

Animals - Wistar rats of either sex weighing 200-250 g
Animals are to be divided into **5** groups containing **6** in each group

Group I	**Administered with normal saline along with normal diet**
Group II	Administered with normal saline along with cholesterol diet (2% cholesterol, 1% sodium cholate and 2% arachis oil) for 30 days
Group III	Administered with atorvastatin (30 mg/kg, p.o) along with high cholesterol diet for 30 days.
Group IV	Administered with ethanolic extract of *M. cymbalaria* (250mg/kg p.o) along with high cholesterol diet for 30 days.
Group V	Administered with a different effective dose of ethanolic extract of *M. cymbalaria* (500mg/kg p.o) along with high cholesterol diet for 30 days.

On the 31st day, blood samples were collected from the retro orbital sinus and serum samples were analyzed for serum total cholesterol (TC), triglyceride (TG) and HDL-C using diagnostic kits. Very low density lipoprotein (VLDL), Low density lipo-protein (LDL), HDL ratio was analyzed.

Parameters of Evaluation:

- Body weight
- Blood Lipid Profile

Total Cholesterol	**(TC)**
Triglycerides	(TG)
High Density Lipoprotien	(HDL-C)
Low density Lipoprotien	(LDL-C)
Very Low density Lipoprotien	(VLDL-C)

RESULTS

Preliminary phytochemical studies (**Table 1**) of ethanolic extract *of M. cymbalaria* revealed the presence of flavanoids, triterpenoids, steroids and carbohydrates.

Table No. 1: Preliminary phytochemical screening

Phytochemical	Present/Absent
Alkoloids	-
Carbohydrates	+
Flavanoids	+
Aaponins	-
Cardiac glycosides	-
Tannins	-
Proteins	-
Steroids	+
Triterpenes	+

- = Absent; + =Present

The acute toxoicity studies were conducted according to OECD 423 guidelines. The ethanolic extract *of M. cymbalaria* found to be non toxic up to 2000 mg/kg.
The in vitro antioxidant activity of ethanolic extract *of M. cymbalaria* was also studied by DPPH methods. Ethanolic extract *of M. cymbalaria* .The evaluation of antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method showed significant results. DPPH is one of the stable organic nitrogen free radicals, which is widely used for testing preliminary radical scavenging activity of a compound or a plant extract. It has a maximum absorbance at 517 nm. Absorbance decreases when antioxidants donate protons to DPPH, thereby reducing the latter. The % scavenging of *M. cymbalaria* was found to be 64.68±0.324% at a dose of 100 µg mL^{-1} as compared to the standard ascorbic acid in case of DPPH free radical scavenging activity (**Table 2**).

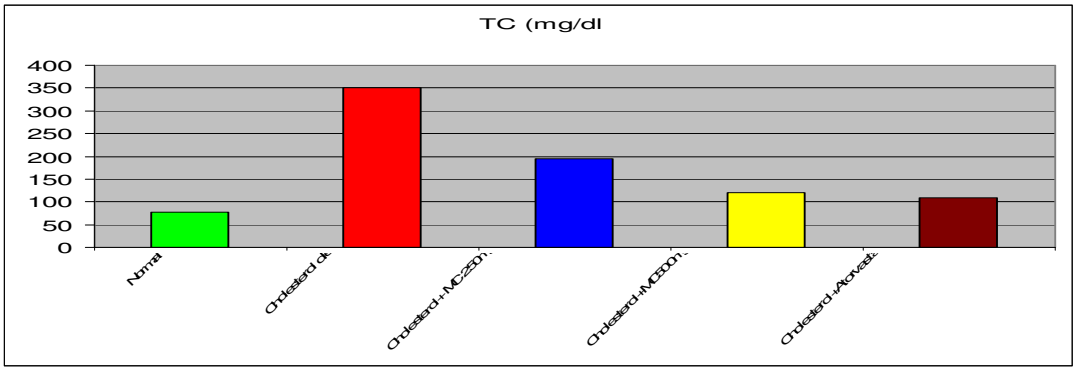
Table No. 2: Percentage scavenging of DPPH radical

Concentration (µg mL^{-1})	Scavenging of DPPH (%)	Ascorbic Acid
25	44.32±0.521	53.43±0.054
50	55.42±0.873	64.86±0.455
100	64.68±0.324	76.54±0.563

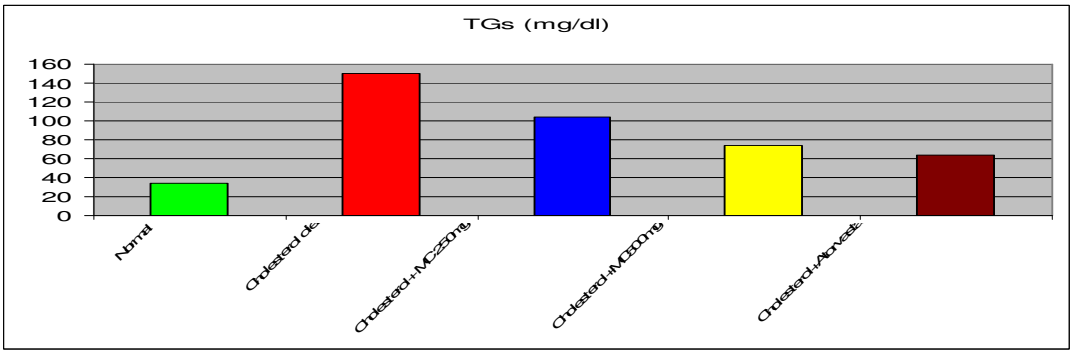
The effect of ethanolic extract of *M. cymbalaria* on TC, TGs, HDL-C, VLDL and LDL in rats are summarized in **Table 3**. There was a significant increase in TC, TGs, VLDL and LDL in Cholesterol diet group II rats, when compared to the normal control group. The HDL-C levels were significantly decreased to 14.3712.46 mg/dl in Cholesterol diet rats from the level of 41.29 mg/dl in normal group. On the other hand the group with received both leaves extracts 250mg/kg and 500mg/kg + Cholesterol diet (**Group III & Group IV**) and Cholesterol+Atorvastatin (**Group V**) showed significantly decreased the elevated TC , TGs , VLDL and LDL when given orally and reversed the altered HDL-C to almost normal level (**Table 3 & Graph 1-5**).

Table No. 3: The effect of *M. cymbalaria* on TC, TGs, HDL-C, VLDL and LDL

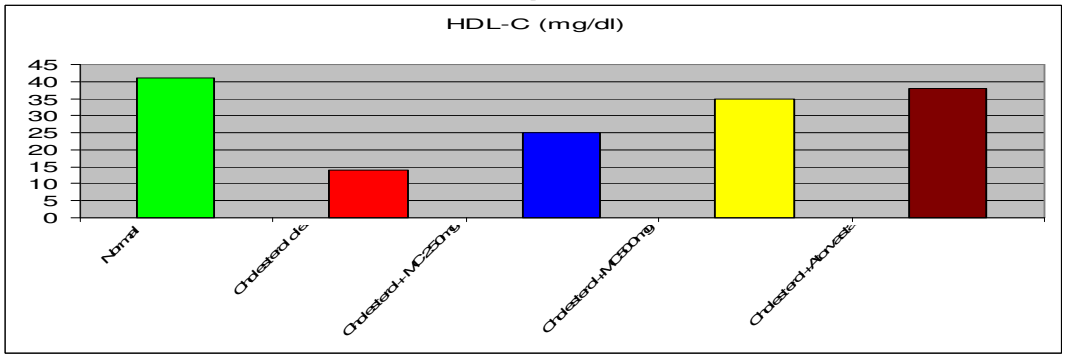
Groups	TC (mg/dl	TGs (mg/dl)	HDL-C (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Normal	78.34 ±1.76	34.76± 2.12	41.29±1.12	7.86±0.11	27.62±2.11
Cholesterol diet	350.8±6.12	150.98±2.91	14.37±0.12	28.24±1.14	242.46±3.16
Cholesterol+ *M. cymbalaria* 250mg/kg	195.56±2.33	104.82±1.33	25.36±1.06	20.25±1.01	162.42±3.12
Cholesterol+ *M. cymbalaria* 500mg/kg	119.24±2.12	74.28±1.42	35.56±1.20	11.39±1.08	82.25±3.10
Cholesterol+Atorvastatin	109.29±2.16	64.48±2.01	38.16±1.40	9.08±0.98	52.01±0.84



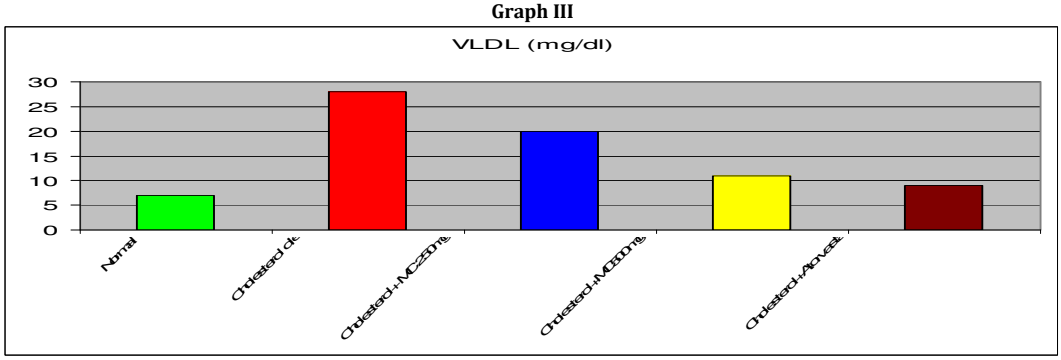
Graph I



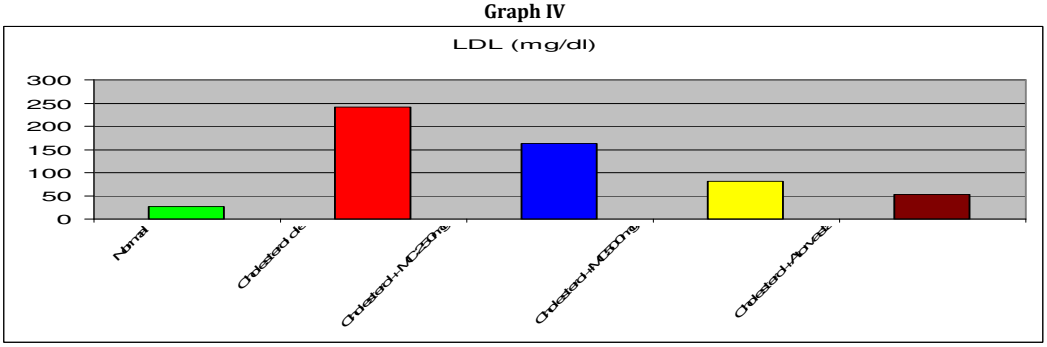
Graph II



Graph III



Graph IV



Graph V

DISCUSSION

Previous works on *Momordica cymbalaria* were carried out by many researchers and significance of MC in various disease treatment was illustrated through various animal models and presently by carrying out this experiment on antioxidant and anti hyperlipidemic activity of MC a direct approach of treating hypercholesterolemia in animal model is shown with various parameters. The previous work done on hyperlipidemia only show that the relation between blood glucose and lipid profile modifications. But from this research project this has been illustrated that MC could directly be effective in treating hyperlipidemia.

In the present preliminary phytochemical studies of ethanolic extract *of M. cymbalaria* revealed the presence of flavanoids, triterpenoids, steroids and carbohydrates and acute toxoicity studies *of M. cymbalaria* found to be non toxic up to 2000 mg/kg.

The possible mechanism involved in the atherogenesis in rat may be due to enhance cholesterol biosynthesis by increasing activity of HMGCoA reductase. In addition, this could be associated with a down regulation in LDL receptors by the cholesterol and saturated fatty acids in the diet(Bradley et al., 1995). The biochemical estimations shown that the extracts MEMD & FFMD increased the protective HDL-C level and decreased the atherogenic LDL and VLDL levels. The possible mechanism of test drug may involve increase of HDL-C, which can lead to the mobilization of cholesterol from peripheral cells to the liver(Khanna et al.,2002).

Flavonoids activate multi enzyme systems, such as citocrome P450 and b5(Lasker at al., 1984) and this action affects the whole metabolism, as these systems are involved in the metabolism of xenobiotics, including drugs, insecticides, and pollutants, that have great importance on pharmacology and toxicology. Due to this effect, flavonoids act on body lipid constituents like steroids and bile acids, and influence lipid metabolism. They increase bile acid excretion because cytocrome P-450 enzymes bind some compounds to the bile acids and therefore reduce cholesterol level in the body(Carlo at al., 1992) .

In the present study, the in vitro antioxidant activity of *M. cymbalaria* by DPPH methods showed significant results when compared to Vitamin C.The results shown suggest that the study carried out on *Momordica cymbalaria* is a effective against hyperlipidemia in reducing the levels of low density and very low density lipoproteins and increase in the HDL levels in the present model of research.

CONCLUSION

Chronic hyperlipidemia was induced by feeding male rats with high cholesterol diet for 30 days.Administration of *Momordica cymbalaria*(MC) (250mg/kg and 500mg/kg) for 30 days successfully prevented the elevation of serum TG, TC, LDL-C, VLDL and decrease of serum HDL-C in high cholesterol diet model rats.Ethanolic extract of *Momordica cymbalaria*(MC) also exhibited antioxidant effect in DPPH in vitro.In final conclusion the In vitro antioxidant and Antihyperlipidemic activity of *Momordica cymbalaria* might be due to flavonoids present in ethanolic leaves extract.

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