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ANTIDYSLIPIDEMIC, ANTIATHEROGENIC AND ANTIOXIDANT ACTIVITY OF *MOMORDICA CHARANTIA* IN WISTAR ALBINO RATS

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ABSTRACT

The aim of present study was to provide the pharmacological basis for the medical use of *Momordica charantia* in diabetes and dyslipidemia using the powder of its fruit, to prevent atherosclerosis and other cardiovascular diseases. The lipid lowering activity of *Momordica charantia* has been studied in triton treated and high fat diet fed hyperlipemic rats (*in vivo*). It has also been studied for antioxidant activity *in vitro*. Serum lipids were lowered by feeding *Momordica charantia* (200 & 400 mg/kg, b.w.) in triton WR-1339 induced hyperlipemic rats. Chronic feeding of the powder at above doses in animals simultaneously fed with high fat diet for 30 days caused lowering in the lipids and apolipoprotein levels of very low density lipoprotein and low density lipoprotein as well as atherogenic index. The *M. charantia in vitro* inhibited the generation of superoxide anion and hydroxyl radicals in both enzymatic and non-enzymatic systems. The results of the present study demonstrate the lipid lowering and antioxidant activities in extract of *M. charantia*, which could help in prevention of cardiovascular diseases, particularly atherosclerosis.

INTRODUCTION

Dyslipidemia is one of the major modifiable risk factor for cardiovascular diseases including atherosclerosis[1]. Furthermore, disorders of lipid metabolism are associated with increased oxidative stress and overproduction of oxygen free radicals[2]. Free radicals are implicated in etiology of several lifestyle-related diseases such as atherosclerosis, stroke, diabetes, and cancer[3]. The treatment of dyslipidemia reduces cardiovascular events[4]. The modern pharmacological therapy for hyperlipidemia is effective but associated with side effects leading to patient incompliance[5]. Moreover, the lipid lowering drugs viz. fibrates, statins and bile acid sequestraints do not possess antioxidant property[6]. Therefore, a drug having dual property of antidyslipidemic and antioxidant activities from natural products is the most preferred option.

Bitter melon (*Momordica charantia*) is a vegetable cultivated in the tropical or sub-tropical regions of South America and Asia. It has been used as a traditional remedy for various illnesses, particularly diabetes mellitus[7, 8]. Clinical investigations in humans have also reported beneficial effects of bitter melon in preventing and/or relieving hyperglycemia in human type II diabetes[9, 10]. Animal experiments have further substantiated these claims, and it has been shown there are hypoglycemic effects in alloxan or streptozotocin induced diabetic animals [11-14]. However, research on bitter melon has mainly focused on its purported antidiabetic properties, in spite of the possibility that bitter melon might affect lipid metabolism as well, due to the interconnection between carbohydrate and lipid metabolism.

On the other hand, it was recently reported that longterm feeding of bitter melon juice resulted in a significant reduction in the concentration of serum lipids in the streptozotocin-induced hyperlipidemic rats, but only total cholesterol was reduced in the non treated normal rats fed bitter melon juice [15]. Chen et al. [16] recently reported that feeding of freeze-dried bitter melon juice resulted in improved insulin resistance and lower visceral adipose tissue weight, serum insulin and leptin, but that it raised serum-free fatty acid concentrations in rats fed a high-fat diet. These observations suggest that the edible portion of bitter melon contains some components that influence lipid metabolism, probably through hormonal regulation.In the present study, we examined the lipid-lowering activity of *M. charantia* in rats fed a diet with or without 02% cholesterol supplementation. Rats are a useful model system for studying lipid metabolism as they share many similarities with humans in lipid metabolism and in cholesterol and atherogenic lipoprotein cholesterol (LDL and VLDL) responses to atherogenic diets [17].

Abbreviations: MCP –Momordica charantia powder; TG –Triglycerides; TC – Total Cholesterol; TP – Total Protein; FFA – Free Fatty Acids; HDL – High density lipoprotein; LDL – low density lipoprotein; VLDL – Very low density lipoprotein; HFD – High Fat Diet

MATERIALS AND METHODS

Plant Material:

The fruits of *M. Charantia* was collected from the local market of Salepur and identified taxonomically by the Division of Botany, Revenshaw University, Cuttack, India. The fruits were dried in shade and grounded into fine powder. The dried powder of *M. Charantia* fruit, which was used for *in vivo* and *in vitro* studies.

Drugs and Standards:

Triton WR-1339 and standard drug Niacin along with other chemicals were procured from Sigma Chemical Company, St Luis, MO, USA.

Animals:

Male adult rats of *wistar* strain (100-150g) bred in the animal house of the institute were used for the study. The animal protocol was approved by Institutional Animal Ethical Committee of I. P. T., Salipur, Cuttack, Odisha, India with registration number 1053/ac/07/CPCSEA. All the experiments were performed as per the CPCSEA guidelines.

Triton-induced hyperlipidemia:

The animals were divided into five groups, group 1 – control, group 2 – triton treated, group 3 – MCP (200mg/kg b. w.), group 4 – MCP (400mg/kg b. w.) and group 5 – standard drug Niacin (100mg/kg b. w.). Each group contained six animals which were kept in controlled conditions of temperature (25-26 °C), relative humidity (60-80%) and 12/12h light/dark cycle (light from 8:00 am to 8:00pm) and provided with standard pellet diet (Lipton India Ltd) and water *ad libitum.* Hyperlipidemia in rats was induced by a single dose of triton WR-1339 (400mg/kg b. w.) intraperitoneally [18]. After dosing, the rats were fasted for 18h and then anaesthetized with sodium pentothal solution (50mg/kg *i.p.*) prepared in normal saline. Blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated tubes (3mg/ml blood). The blood was centrifuged at 2500Xg for 10min at 4°C and plasma was separated, which was used for biochemical analysis.

High Fat Diet-induced Hyperlipidemia:

The rats were randomly divided into five groups. All groups (except control) were injected a single dose of streptozotocin (*i.v.*) to induce type-II diabetes mellitus. The powder was given orally, simultaneously fed with high fat diet for 30 days [19]. At the end of treatment the animals were fasted for 24 hours, the blood of anesthetized animals was collected by cardiac puncture in EDTA coated glass tubes and centrifuged at 2500Xg for 10min in Sigma 3-30k centrifuge to obtain plasma.

Blood glucose and Glucose tolerance test:

Blood glucose was estimated by one-touch electronic glucometer and the oral glucose tolerance test was performed as reported by Vand & Karr[20].

Estimation of Lipid profile and total protein level:

The levels of serum cholesterol, triglycerides, phospholipids, free fatty acids, high density lipoproteins and total protein were estimated according to methods mentioned[21-26]. VLDL, LDL and atherogenic index were calculated according to following formulas [27]:

VLDL = TG/5
LDL = TC – HDL – TG/5
Atherogenic index = (TC – HDL)/HDL

Estimation of Antioxidant Activity:

Generation of Superoxide anions (O_2^-) was measured in an enzymatic system composed of Xanthine (0.122mg/ml), Xanthine oxidase (12µl/ml) and nitrobluetetrazolium (NBT) (0.74mg/ml) with or without addition of MCP (50-400µg) [28]. The amount of formazone formed as a result of reduction of NBT by O_2^- was measured at 560 nm on spectrophotometer. The system employed for non-enzymatic generation of O_2^- comprised of phenazine methosulphate (PMS) (0.28mg/ml), NADH (1.65mg/ml) and NBT (1.286mg/ml) in the absence or presence of MCP (50-400µg). After the incubation the amount of formazone formed was measured as above[29]. Generation of Hydroxyl free radicals and effect of MCP on the formation of hydroxyl free radicals (OH^-) was measured in an enzymatic system composed of hypoxanthine (0.4mM), $FeSO_4 \cdot 7H_2O$ (Fe^{2+}) (0.mM), sodium salicylate (5mM) and xanthine oxidase (0.07 units), 3,4-dihydroxybenzoate formed by OH^- mediated hydroxylation of salicylate was measured as reported by Richmond *et al*[30]. In another set of experiment OH^- were generated non-enzymatically by $FeSO_4$ (0.276mg/ml), sodium ascorbate (1.9mg/ml), H_2O_2 (5%) and

deoxyribose (0.94mg/ml) in absence or presence of ASE (50-400µg), and malondialdehyde produced was measured [31].

Statistical analysis:

All groups were compared by one way analysis of variance (ANOVA) & the significance of mean difference between different groups was done by Tukey's post hoc test. A two tailed (α=2) probability $p<0.05$ was considered statistically significant ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = *** and ns = not significant).

RESULTS

Effect of M. charantia powder on triton-induced hyperlipidemia:

Administration of triton caused a marked increase in serum cholesterol (4.8 folds), triglyceride (4.6 folds), phospholipids (4.04 folds) and total protein (2.7 folds) in treated rats as compared to control animals. The treatment of rats with MCP (200 & 400mg/kg b. w., orally) caused significant reversal of above effects [Table: 1; Figure: 1a & b].

Effect of M. charantia powder on high fat diet-induced dyslipidemia:

The HFD fed rats showed an increase in TC (2.1 folds), TG (4.6 folds), PL (2.2 folds), FFA (2.6 folds), TP (2.8 folds), plasma glucose (3.15 folds), LDL (4.4 folds) and atherogenic index (7.8 folds). The HDL showed 51 % decrease as compared to control. The oral administration of MCP (200 & 400mg/kg) caused decrease in TC (20 & 27%), TG (13 & 24%), PL (24 & 32.5%), FFA (15 & 21%), TP (21 & 33%), glucose (20 & 26%), LDL (45 & 49%) and atherogenic index (50 & 67%) respectively. [Table: 2, 3 & Figure 2(a,b,c,d)]

Glucose tolerance test:

The oral administration of glucose (3g/kg b. w.) caused a marked increase in postprandial plasma levels of blood glucose of the rats from 0min-120min.The treatments with MCP showed 30-32% improvement in oral glucose tolerance [Table: 4 & figure: 3a].

Antioxidant Activity:

Enzymatic generation of O_2^- anions in xanthine-xanthine oxidase system was inhibited to varying extent by MCP exhibiting 44% decrease at 400µg concentration. The MCP also inhibited the O_2^- anions generation non-enzymatic by 68.69% at 400µg concentration. MCP caused 42 & 61% inhibition in the formation of OH^- by enzymatic system and non-enzymatic system at 400µg concentration respectively [Table 5& figure: 3b].

DISCUSSION

In present study two different models viz., triton WR-1339 and high fat diet induced hyperlipidemia were used to evaluate the possible effects of *M. charantia* powder. Triton WR-1339 (tyloxapol) is a non-ionic surfactant being widely used to explore possible mechanism of lipid lowering drugs [18]. Triton causes drastic increase in serum TG and TC levels *due to* increase in 3-hydroxy, 3-methyl-glutaryl CoA (HMG-CoA) reductase activity and by inhibition of lipoprotein lipase responsible for hydrolysis of plasma lipids [32, 33]. In fasting condition the only source of serum lipid is by endogenous production. Significant inhibition of increase in serum lipid levels by MCP treatment in this model might be *due to* inhibition of HMG-CoA reductase. This enzyme plays a key role in controlling lipid levels in plasma and other tissues. High fat diet induces endothelial dysfunction, atherosclerosis[34] and increases oxidative stress by increasing the expression of oxidation-sensitive genes, such as Elk-1 and pCREB[35]. HFD containing cholic acid increases TC, LDL and decreases HDL by enhancing intestinal absorption and secretion of cholesterol likewise decreasing its catabolism[36]. Treatment with MCP caused a significant decrease in mean serum TC, TG, LDL in triton treated and HFD induced hyperlipidemia and increase in HDL levels. The MCP also caused significant reduction in atherogenic index, which is considered a better indicator of coronary heart disease risk than individual lipoprotein concentration [27]. Streptozotocin, an antibiotic produced by *Streptomyces achromogenes* var *streptozoticus*, is particularly toxic to β-cells of pancreas and inhibits the insulin production[37]. The administration of streptozotocin caused increased mobilization of free fatty acids from peripheral deposits, since insulin inhibits hormone-sensitive lipase[38]. The decrease in free fatty acid levels by MCP indicates that it inhibited the hormone-sensitive lipase. The results of our study showed that the MCP possesses the antioxidant property as it inhibited the *in vitro* generation of O_2^- and OH^- free radicals in both enzymatic and non-enzymatic systems. The oxidative stress due to HFD increases the oxidation of LDL resulting in development of atherosclerosis. Due to strong antioxidant property, *M. charantia* prevents the oxidation of LDL. The antidyslipidemic activity of *M. charantia* can be correlated to its inhibitory effect on lipid metabolism viz. biosynthesis, absorption and secretion. The antidiabetic, antioxidant and antidyslipidemic activities of *M. charantia* might be useful in reducing the development of diabetes and cardiovascular diseases.

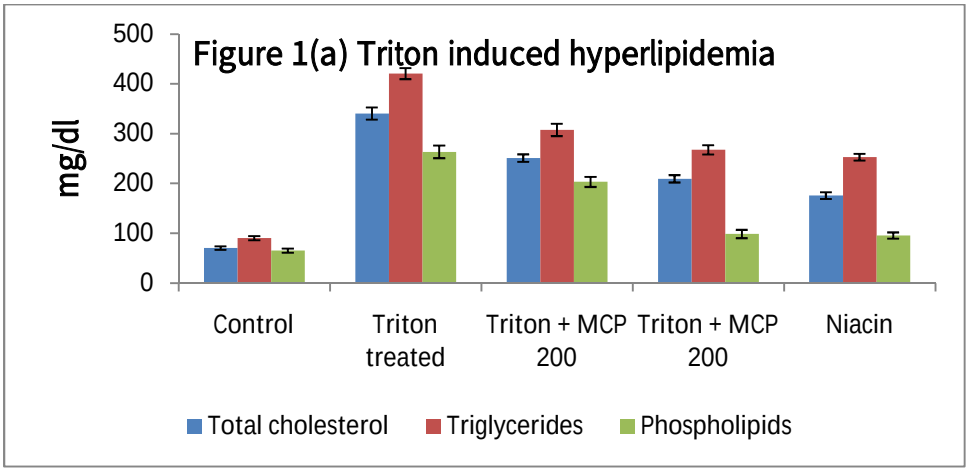
CONCLUSION

The finding of the study reveal that the fruit juice of *M. charantia* can effectively control the blood levels in dyslipidemic conditions by interfering with biosynthesis of cholesterol and utilization of lipids.

Table-1 Effect of MCP on total cholesterol, triglyceride, phospholipids and total protein in triton induced hyperlipidemia.

Group	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)	Total protein (g/dl)
Control	70.4±3.46	90.3±4.24	65.2±4.23	6.3±0.87
Triton treated	340.5***±12.09	420.7***±11.12	263.5***±12.61	16.2**±1.01
Triton+MCP200	251.1***±7.49	307.6***±12.31	203.2**±10.15	13.6*±0.54
Triton+MCP400	209.4***±7.43	267.5***±9.23	98.6***±8.34	11.4**±0.89
Niacin	175.7***±6.79	252.8***±6.57	95.4***±6.34	8.6***±0.75

Control group was compared with Triton treated; MCP treated with Triton treated. $p < 0.05$ was considered statistically significant. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). in HDL levels



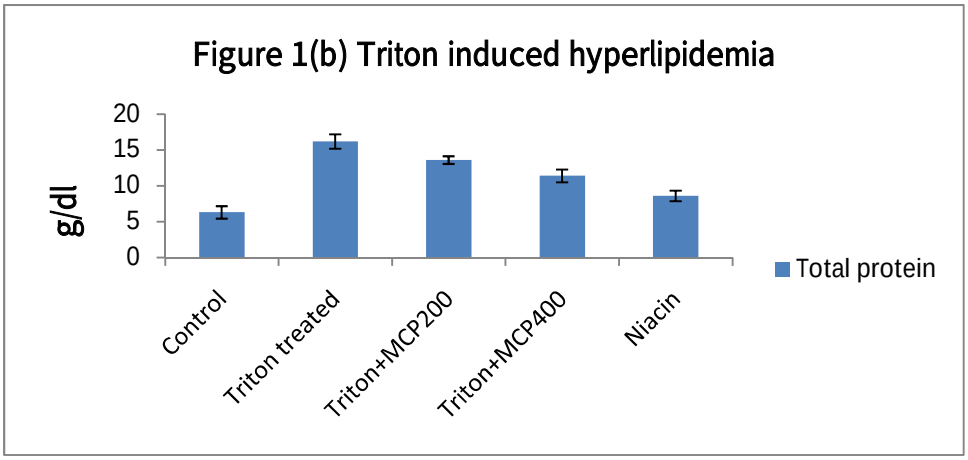
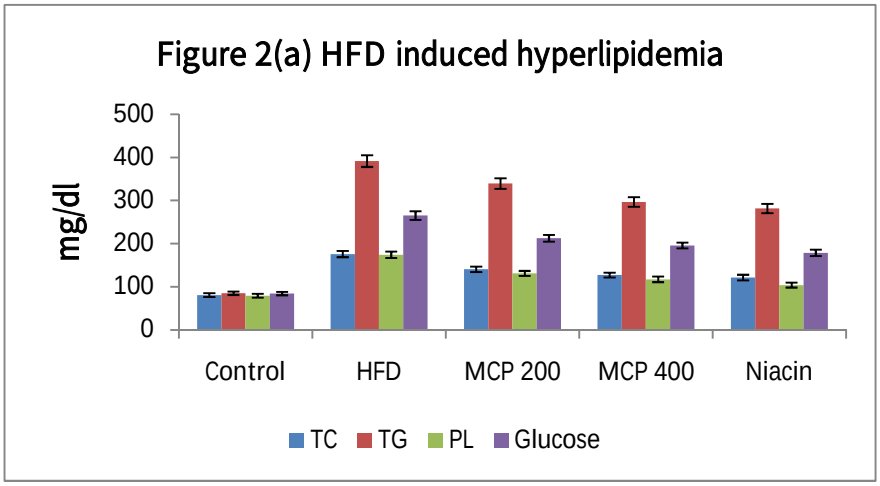


Table-2 HFD induced hyperlipidemia.

Group	TC (mg/dl)	TG (mg/dl)	PL (mg/dl)	Glucose (mg/dl)	FFA (mg/dl)	Total protein (mg/dl)
Control	81.0±4.3	85.3±3.9	79.5±4.5	84.4±3.8	1.9±0.02	4.8±0.39
HFD	176.1^{***}±7.3	391.2^{***}±13.4	174.3^{***}±7.5	265.2^{***}±9.7	4.95^{***}±0.23	13.8^{***}±0.58
MCP 200	140.7^{*}±6.3	339.5^{**}±12.2	131.2^{*}±5.6	212.7^{**}±7.8	4.2^{*}±0.42	10.9^{*}±0.64
MCP 400	127.3^{**}±5.3	296.8^{***}±11.1	117.4^{**}±6.4	195.6^{***}±6.6	3.9^{**}±0.37	9.2^{***}±0.39
Niacin	121.7^{**}±6.3	281.5^{***}±10.9	104.2^{***}±5.7	178.8^{***}±7.4	3.8^{**}±0.21	8.9^{***}±0.45

Effect of MCP on HFD induced hyperlipidemia. Control group was compared with HFD fed; MCP treated with HFD rats. $p < 0.05$ was considered statistically significant. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).



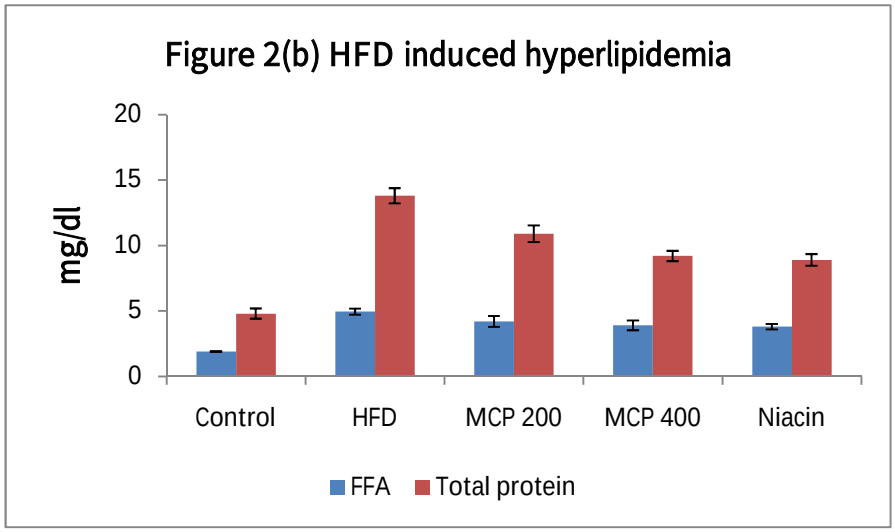
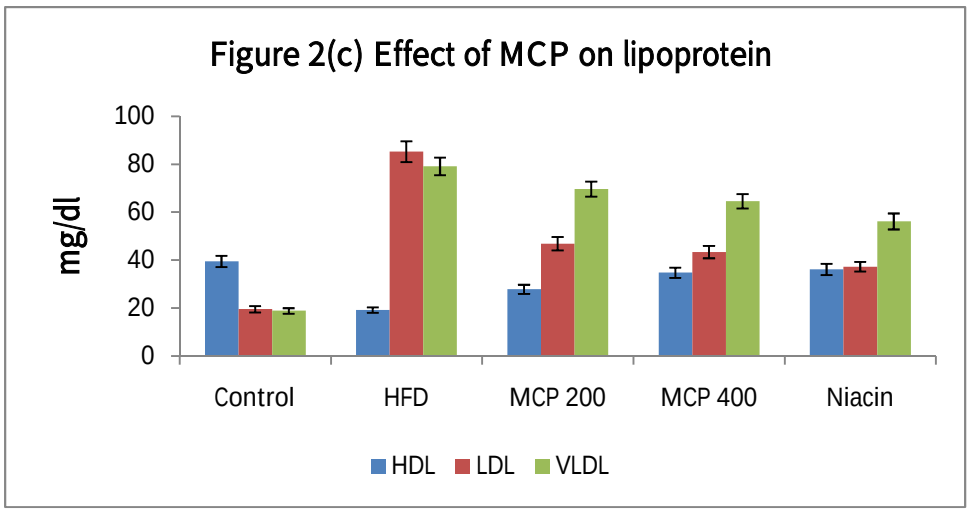


Table-3

Effect of MCP on lipoproteins

Group	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	AI
Control	39.5±2.34	19.6±1.34	18.9±1.15	1.05±0.02
HFD	19.2***±1.13	85.3***±4.34	79.1***±3.67	8.16***±0.56
MCP 200	27.9**±1.89	46.9**±2.78	69.7**±3.14	4.04**±0.45
MCP 400	34.8***±2.11	43.4***±2.56	64.6***±2.96	2.65***±0.34
Niacin	36.2***±2.34	37.3***±1.98	56.2***±3.33	2.36***±0.28

Effect of MCP on HFD induced hyperlipidemia. Control group was compared with HFD fed; MCP treated with HFD rats. $p < 0.05$ was considered statistically significant. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).



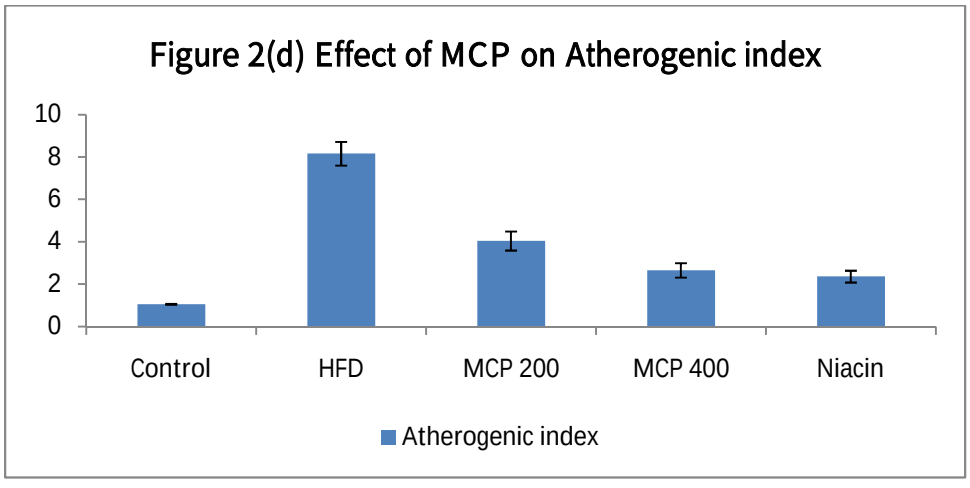


Table-4 Glucose tolerance test

Group	0 min	30 min	60 min	90 min	120 min
Control	92±4.25	142±5.67	147±6.77	136±4.75	89±5.78
HFD	105*±4.98	192**±7.45	189**±6.94	165**±7.54	161***±9.34
MCP 200	91*±3.67	138**±4.88	143**±5.32	132**±4.27	88***±5.38
MCP 400	90**±3.34	137**±4.76	144**±4.96	130**±4.89	89***±3.99
Niacin	93*±4.11	139**±4.56	145**±3.90	131**±5.34	91***±4.35

Control group was compared with HFD fed; MCP treated with HFD rats. $p < 0.05$ was considered statistically significant. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

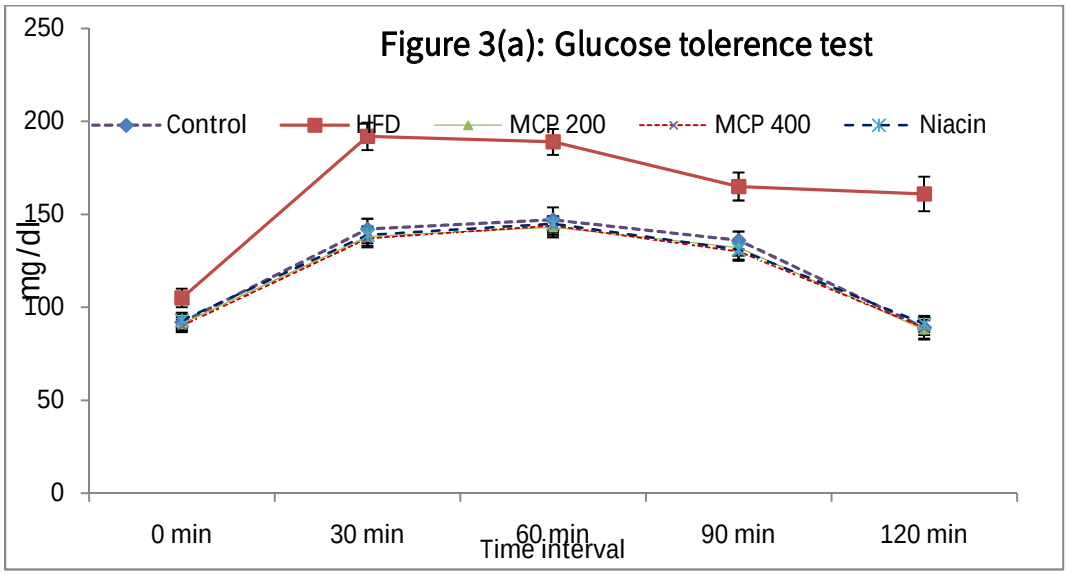
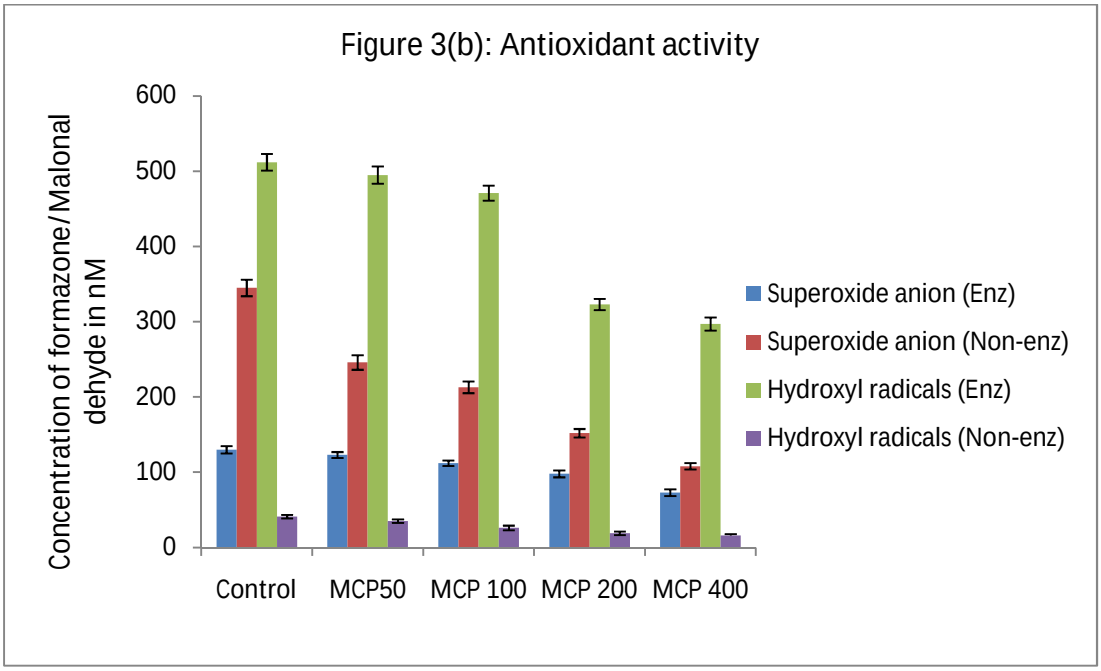


Table-5

Antioxidant activity (Concentration of formazone/malonal dehyde in nM)

Group	Superoxide anion (Enz)	Superoxide anion (Non-enz)	Hydroxyl radicals (Enz)	Hydroxyl radicals (Non-enz)
Control	130±4.89	345±10.98	512±10.98	41±2.35
MCP50	123*±3.96	246***±9.77	495**±11.45	35*±2.31
MCP 100	112**±3.56	213***±7.83	471**±9.85	26**±3.11
MCP 200	98***±4.65	152***±5.63	323***±7.57	19***±2.26
MCP 400	73***±4.57	108***±4.34	297***±8.67	16***±1.98

Effect of MCP on superoxide anion and hydroxyl free radicals generation. Experimental group compared with reference group. $p < 0.05$ was considered statistically significant. $p < 0.05$ was considered statistically significant. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).



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