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EFFECT OF POLYHERBAL FORMULATION ON FRUCTOSE INDUCED METABOLIC SYNDROME IN RATS

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ABSTRACT

Increased fructose consumption is strongly associated with incidence of metabolic syndrome (MS).This study aimed to elucidate the role of aqueous extract of Polyherbal Formulation (PHF) on fructose-induced metabolic syndrome. MS was induced by administration of fructose as 10% solution in drinking water for 120 days. Rats were divided into five groups, one served as normal control and other four groups of rats (n =6) were administered fructose as 10% solution in drinking water for 120 days. One of them served as fructose fed control while the remaining three groups were treated with Atorvastatin (10mg/kg/day), metformin (70 mg/kg/day) and PHF (400 mg/kg/day). Estimation of markers related to MS was done for every 30 days and at the end of the study. Analysis of the data was performed by two way Analysis of Variance and subsequent analysis was performed using bonferroni multiple comparison test. The P values smaller than 0.05 were selected to indicate statistical significance between groups. Induction of MS was associated with increased body weight gain and elevated levels of blood glucose, Insulin, MDA, total triglycerides, total cholesterol and systolic blood pressure. It also reduced levels of serum HDL, reduced glutathione, catalase and superoxide dismutase. Histopathological studies of liver and kidney also supported the findings. Aqueous extract of PHF attenuated most of the changes associated with MS. The findings of this study proves the benefits of PHF in fructose-induced model of MS.

Key words: Polyherbal formulation, Insulin resistance, Oxidative stress, Anti-hyperlipidemic, Hypoglycemic.

INTRODUCTION

Fructose is used extensively in carbonated beverages, baked goods, canned fruits and dairy products (Hanover and White, 1993). The increasing consumption of fructose could play a potential role in the etiology of obesity and metabolic syndrome (Bray *et al*., 2004). MS is a cluster of pathologies compromising insulin resistance, hyperinsulinemia, hypertiglyceridemia, accelerated atherosclerosis and hypertension (Reaven, 1998 & Wajchenberg *et al*., 1994). In addition, it is associated with morbidities such as increased risk of developing cardiovascular disease, type 2 diabetes mellitus and renal disease (Lorenzo *et al*., 2003). In contemporary times, MS is becoming an alarming concern for the developing world. The prevalence of metabolic syndrome is increasing worldwide and is a growing threat to global health. People with metabolic syndrome are twice as likely to die from heart attack or stroke compared with people without MS and has a fivefold greater risk of developing type 2 diabetes (Alberti *et al*., 2006).

Due to lack of therapeutic intervention for metabolic syndrome, there is an increase interest in natural products to treat or prevent the metabolic syndrome. The use and acceptance of herbal medicine have increased in recent years (Megalli *et al*., 2005) and it is believed that approximately 80% of the world population is almost entirely dependent on herbal medicine (Srinivasan, 2005). Plant derived therapeutic agents may serve as effective agents for treatment or prevention of metabolic syndrome as they often contain diverse collections of therapeutically active compounds with multiple mechanisms of action that may potentiate

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each other activity with increased benefit (Rohith *et al*., 2014). For the effective management of MS, a combinational therapy which includes an anti-hyperglycemic agent, anti-hyperlipidemia agent, anti-hypertensive agent plus an anti-platelet agent would be useful (Deedwania *et al*., 2006). Hence, it would be apt to employ a Polyherbal formulation containing herbs with the above activities in the management of MS. So we have prepared a PHF which consists of the following plants- *Eclipta alba*, *Hemidesmus indicus, Nelumbo nucifera*, *Terminalia chebula*, and *Zingiber officinale*. The purpose of the study was to assess aqueous extract of PHF in the management of clinical symptoms of MS against Fructose induced insulin resistance model in experimental animals Rats.

MATERIALS AND METHODS

Animals

Wistar rats of either sex weighing about 120-150g, 8-10 weeks old procured from Raghavendra Enterprises, Bengaluru were used in the study. They were housed in animal room in Pharmacology department, under conventional laboratory conditions on a 12 hours light/dark cycle and constant temperature (22 ±1°C). Throughout the study food and water were supplied *ad libitum*. All experimental procedures were conducted in accordance with ethical procedures and policies approved by the Institutional Animal Ethical Committee (IAEC) of Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupathi and with permission from Committee for the purpose of Control and Supervision of Experiments on Animals (1016/a/06/CPCSEA/004/2011), Ministry of Social Justice and Empowerment, Government of India.

Composition and Preparation of PHF

1gm of the Polyherbal formulation in the present study is composed of the following five plants in the following proportions: *Eclipta alba* - 150mg (Ananthi *et al*., 2003), *Hemidesmus indicus* - 100mg (Navneet B. Gadge and Sunil S. Jalalpure, 2011), *Nelumbo nucifera* - 300mg (Huralikuppi JC *et al*., 1991), *Terminalia chebula* - *150mg* (Gandhipuram Periyasamy *et al*., 2006). and *Zingiber officinale* - 300mg (Bhandari *et al*., 1998). The above plant materials were procured from Sri Srinivasa Ayurveda Pharmacy (TTD, Tirupati) and local market, shade dried and coarsely powdered. The decoction of Polyherbal formulation was prepared by boiling it with distilled water for 30 min in the ratio of 1:9. The decoction was then filtered and wt/ml was determined randomly.

Chemicals and Instruments

Atorvastatin and Metformin were obtained as gift samples from Dr.Reddy's laboratories ltd., India. Fructose used was of analytical grade. Diagnostic kits used in this study were procured from Agappee diagnostics Ltd., India and Span Diagnostics Ltd., India. Auto analyzer (Mispa Excel), UV-Visible spectrophotometer (Analytical systems, model no: AUV 2060), Electronic balance (Shimadzu, model no: DS- 852 J), colorimeter (Inco, model no: CL- 157), Homogenizer (Ever shine, model no: 607), Cooling centrifuge (Remi, model no: KKLO- 9013), LE 5002 storage pressure meter (LETICA scientific instruments, SPAIN).

Acute toxicity study

Acute toxicity study was performed for the aqueous extract of PHF as per OECD guideline-423. The animals were fasted overnight and then divided into five groups each containing three animals. Group 1-5 were given 5mg/kg, 50mg/kg, 300mg/kg and 2000 mg/kg bodyweight aqueous extract of PHF and were observed for any behavioral, neurological and autonomical changes for a period of 24 hours and were kept under observation for 14 days.

Selection of dose

Based upon the results of the acute toxicity study, the dose of the extract was taken as 400 mg/kg body weight which was one fifth of highest dose (2000 mg/kg) administered in the acute toxicity study (Singh *et al*., 2012).

Oral glucose tolerance test (OGTT)

OGTT was performed to determine the hypoglycemic activity of the aqueous extract of PHF. Rats were fasted to overnight and divided into five groups with 3 animals in each. Group -1 received distill water to serve as normal, group-2 animals were treated with Metformin (0.5 mg/kg, p.o.) to serve as standard. Group - 3, 4 and 5 were treated with Polyherbal formulation in three different doses (200, 400 and 1000 mg/kg, p.o). All the groups were treated with respective drugs 30 min prior to the glucose load (2.5 g/kg p.o). Blood samples were collected at 0, 30, 60, 90 and 120 min after glucose loading. Serum was separated and blood glucose levels were measured using standard analytical procedure.

Experimental plan

After acclimatization period of one week rats were divided into 5 groups (n=6) as follows:

Group 1: This group received regular diet and water *ad libitum*. This group didn't receive any medication and served as normal control.

Group 2: This group received regular diet and water *ad libitum* and fructose was administered as 10% solution in drinking water for 4 months.

Group 3: This group received regular diet and water *ad libitum* and fructose was administered as 10% solution in

drinking water for 4 months followed by treatment with atorvastatin (10 mg/kg/day p.o.).

Group 4: This group received regular diet and water *ad libitum* and fructose was administered as 10% solution in drinking water for 4 months followed by treatment with metformin (70 mg/kg/day p.o.).

Group 5: This group received regular diet and water *ad libitum* and fructose was administered as 10% solution in drinking water for 4 months followed by treatment with PHF (400 mg/kg/day p.o.).

For every 30 days, change in body weight, waist circumference and body mass index were noted and blood samples were collected from retro orbital plexus of each animal for serum analysis. Blood glucose, triglycerides, total cholesterol and HDL-Cholesterol were measured for every 30 days during the study and at the end of the study. VLDL and LDL were calculated as per Friedewalds equation (mg/dl). VLDL = TG/5.

LDL = TC - HDL - VLDL

Plasma insulin levels, HOMA values and systolic blood pressure were measured at the end of the study. Animals were sacrificed at the end of the study followed by estimation of anti-oxidant enzymes and lipid peroxidation in liver, kidneys and liver, pancreas were excised, stored in 10% formalin solution for histopathological evaluation. SOD activity was estimated using the method of Misra and Fridovichi L (1976). Catalase was assayed at 240 nm by monitoring the disappearance of H_2O_2 described by Aebi (1984). Reduced glutathione was determined by the method as described by Moron *et al*. (1979). The lipid peroxidation level was assessed spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method, as illustrated by Slater and Sawyer (1971).

Statistical analysis

All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one way and two way ANOVA followed by the bonferroni multiple comparison test using computer based fitting program (Prism, Graph pad.). The Statistical significant was set at $P < 0.05$.

RESULTS

Acute toxicity study

In the acute toxicity study, it was observed that none of the doses (i.e. 5, 50 300, 2000 mg/kg) of PHF produced any lethality among the tested animals when administered as a single dose. The animals did not show any gross behavioral changes except for an increase in urination.

Oral glucose tolerance test (OGTT)

After oral glucose challenge, at 0 min the plasma glucose profiles were similar among all the groups. At 90 and 120 min a significant decrease in the blood glucose levels were observed in Metformin and PHF treated groups, when compared to the control group ($p<0.05$). This decrease in blood glucose levels was within the normal range indicating hypoglycemic action of PHF (Table 1).

Body weight waist circumference and body mass index (BMI)

Fructose significantly increased bodyweight, waist circumference and body mass index (BMI) as compared with normal control group but these parameters were significantly decreased in the atorvastatin, metformin and PHF administered groups compared to the fructose control group (Table 2,3,4).

Serum glucose, insulin levels Homeostatic model assessment (HOMA) values

Throughout the study there was a significant increase in the serum glucose levels in the fructose fed rats. Metformin, PHF interrupted the rise in glucose level whereas atorvastatin group showed a rise in serum glucose levels. Insulin levels and calculated HOMA values were increased in the fructose fed rats. Treatment with metformin and PHF showed a decrease in plasma insulin, calculated HOMA values when compared to the fructose control group whereas atorvastatin treated groups showed an increase in plasma insulin levels, HOMA values (Table 5, Figure 1&2).

Lipid profile

Administration of 10 % fructose solution resulted in increase in serum levels of cholesterol, triglycerides, VLDL, LDL and reduction in HDL levels. A significant reversal in serum levels of cholesterol, triglycerides, VLDL, LDL and HDL levels was noticed in the animals treated with atorvastatin, metformin and PHF when compared with the fructose group (Tables 6, 7, 8, 9, and 10).

LDL/HDL ratio and Atherogenic index

A substantial increase in the atherogenic index and LDL/HDL ratio was observed in fructose control group. While a significant drop was observed in the LDL/HDL ratio and atherogenic index in animals treated with atorvastatin, metformin and PHF (Tables 11, 12).

Systolic blood pressure

In normal control group no significant variation in systolic blood pressure was found. A weighty increase in the systolic blood pressure was observed in fructose treated group at the end of the study. PHF, atorvastatin and metformin treated groups showed significant decrease in systolic blood pressure compared to fructose control group at the completion of the study (Table 13).

Lipid peroxidation (LPO), Superoxide dismutase (SOD), Catalase (CAT) and Reduced glutathione (GSH)

Oxidative stress was significantly increased in liver, kidney of fructose administered group as evidenced by increased levels of SOD, CAT and GSH and decreased levels of LPO. PHF, atorvastatin and metformin treated groups had shown significant increase in levels of endogenous antioxidants (SOD, CAT, and GSH) and significant decrease in LPO (Tables 14, 15, 16, 17).

Histopathology of pancreas and liver

The livers of fructose alone administered animals showed severe necrosis, infiltration of inflammatory cells and fatty changes. This was reversed and liver was restored to normal architecture in rats treated with metformin and PHF while atorvastatin treated rats showed mild hepatic damage (Plate 1). An extensive pancreatic damage was observed in fructose control group when compared to the normal group. Metformin and PHF treated groups showed a shielding effect by decreasing the extent of necrosis when compared to fructose control group however atorvastatin treated group showed mild pancreatic damage (Plate 2).

Table 1. Effect of PHF on Oral Glucose Tolerance Test (OGTT)

Groups	Blood glucose (mg/dl)			
	0 min	30 min	90 min	150 min
Normal Control	54.14 ±3.06	109.76 ± 11.21	91.97 ±4.44	71.26 ± 3.61
Standard Control	55.60 ± 4.79	111.13 ± 14.04[a]	62.87 ± 4.20 [a]	51.57 ± 2.63 [a]
PHF (200mg/kg)	53.32 ± 4.27	110.84 ± 8.05 [a]	78.38 ± 2.65 [a]	65.3 ± 3.12 [a]
PHF (400mg/kg)	58.36 ± 3.20	104.14 ± 9.54 [a]	76.85 ± 6.57 [a]	60.19 ± 4.80 [a]
PHF (1000mg/kg)	59.62 ± 4.50	121.56 ± 4.40 [a]	67.38 ± 5.48 [a]	56.3 ± 6.52 [a]

Values were expressed as mean ± SEM; N = 3; [a] = $p < 0.05$, when compared to the control.

Table 2. Effect of PHF on body weight (gm) in different groups of animals

Groups	Body weight (gm)				
	0^{th} day	30^{th} day	60^{th} day	90^{th} day	120^{th} day
Normal control	143 ± 1.1	162 ± 0.65	181 ± 0.41	201 ± 0.41	221 ± 0.48
Fructose control	133 ± 0.85[a]	181 ± 0.59[a]	223 ± 1.0[a]	271 ± 0.48[a]	321 ± 0.71[a]
Atorvastatin	141 ± 0.48[b]	172 ± 0.65[b]	185 ± 0.48[b]	170 ± 0.85[b]	173 ± 0.63[b]
Metformin	132 ± 0.65	168 ± 0.65[b]	204 ± 0.65[b]	195 ± 0.65[b]	215 ± 0.65[b]
PHF	142 ± 0.82[b]	175 ± 0.58[b]	187 ± 0.65[b]	192 ± 0.61[b]	224 ± 0.65[b]

Values were expressed as mean ± SEM; N = 6; [a] = [p] < 0.05, when compared to the normal;
[b]= [p] < 0.05, when compared to the control.

Table 3. Effect of PHF on waist circumference (cm) in different groups of animals

Groups	Waist circumference (cm)				
	0^{th} day	30^{th} day	60^{th} day	90^{th} day	120^{th} day
Normal control	12 ± 0.25	13 ± 0.075	13 ± 0.041	14 ± 0.041	14 ± 0.48
Fructose control	12 ± 0.065	13 ± 0.048	15 ± 0.065[a]	16 ± 0.041[a]	17 ± 0.085[a]
Atorvastatin	11 ± 0.041[b]	12 ± 0.065[b]	13 ± 0.043[b]	13 ± 0.041[b]	13 ± 0.048[b]
Metformin	13 ± 0.041[b]	13 ± 0.065	14 ± 0.085[b]	14 ± 0.025[b]	15 ± 0.041[b]
PHF	11 ± 0.63[b]	12 ± 0.041[b]	14 ± 0.048[b]	13 ± 0.017[b]	14 ± 0.065[b]

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 4. Effect of PHF on Body mass index (BMI) (gm/cm^2) in different groups of animals

Groups	Body mass index (gm/cm^2)				
	0^{th} day	30^{th} day	60^{th} day	90^{th} day	120^{th} day
Normal control	0.41 ± 0.0050	0.45 ± 0.0025	0.47 ± 0.0041	0.50 ± 0.0029	0.54 ± 0.0041
Fructose control	0.46+ 0.0025	0.49 ± 0.0025[a]	0.54 ± 0.0029[a]	0.65 ± 0.0041[a]	0.80 ± 0.0065[a]
Atorvastatin	0.45 ± 0.0022	0.47 ± 0.0041	0.53 ± 0.0029	0.51 ± 0.0041[b]	0.59 ± 0.0041[b]
Metformin	0.46 ± 0.0029	0.48 ± 0.0029	0.52 ± 0.0029	0.53 ± 0.078[b]	0.65 ± 0.0029[b]
PHF	0.47 ± 0.065	0.48 ± 0.095	0.51 ± 0.018[b]	0.51 ± 0.018[b]	0.55 ± 0.0048[b]

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control.

Table 5. Effect of PHF on blood glucose (mg/dl) in different groups of animals

Groups	Blood glucose (mg/dl)				
	0th day	30th day	60th day	90th day	120th day
Normal control	96 ± 0.41	92 ± 1.3	99 ± 0.48	102 ± 0.41	107 ± 0.65
Fructose control	96± 1.7	141 ± 0.48[a]	160 ± 0.41[a]	180 ± 0.48[a]	210 ± 1.0[a]
Atorvastatin	90 ± 0.91	95 ± 0.48[b]	134 ± 0.61[b]	149 ± 2.3[b]	178 ± 0.63[b]
Metformin	94 ± 0.71[b]	97 ± 0.48[b]	101 ± 0.48[b]	81 ± 0.41[b]	94 ± 1.8[b]
PHF	102 ± 0.41	107 ± 0.8[b]	104 ± 0.71[b]	99 ± 0.25[b]	112 ± 0.62[b]

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 6. Effect of PHF on Triglycerides (mg/dl) in different groups of animals

Groups	Triglycerides (mg/dl)				
	0th day	30th day	60th day	90th day	120th day
Normal control	91 ± 0.29	97 ± 0.48	102 ± 0.48	110 ± 0.48	107 ± 1.4
Fructose control	91± 0.49	106 ± 0.41[a]	112 ± 0.48[a]	137 ± 0.85[a]	158 ± 0.65[a]
Atorvastatin	96 ± 0.41	106 ± 0.85	95 ± 0.41[b]	101 ± 0.41[b]	96 ± 1.8[b]
Metformin	96 ± 0.48	111 ±	117 ± 0.64[b]	128 ± 0.65[b]	122 ± 0.48[b]
PHF	100 ± 1.5	112 ± 0.63[b]	102 ± 0.25[b]	111 ± 0.65[b]	108 ± 0.58[b]

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b]= $p < 0.05$, when compared to the control

Table 7. Effect of PHF on Total Cholesterol (mg/dl) in different groups of animals

Groups	Total cholesterol (mg/dl)				
	0th day	30th day	60th day	90th day	120th day
Normal control	76 ± 0.24	82 ± 0.29	91 ± 0.29	99 ± 1.1	105 ± 2.8
Fructose control	77± 0.48	125 ± 0.48[a]	140 ± 0.41[a]	153 ± 2.4[a]	165 ± 0.48[a]
Atorvastatin	80 ± 0.41	91 ± 0.29[b]	108 ± 0.29[b]	78 ± 1.1[b]	88 ± 0.48[b]
Metformin	75 ± 0.48	95 ± 0.41[b]	105 ± 0.29[b]	96 ± 2.2[b]	127 ± 0.65[b]
PHF	78 ± 0.48	87 ± 0.48[b]	102 ± 0.25[b]	99 ± 0.29[b]	107 ± 0.48[b]

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 8. Effect of PHF on HDL – Cholesterol (mg/dl) in different groups of animals

Groups	HDL – cholesterol (mg/dl)				
	0th day	30th day	60th day	90th day	120th day
Normal control	48 ± 0.65	46 ± 1.3	47 ± 0.41	51 ± 1.1	47 ± 0.71
Fructose control	45 ± 0.55	39 ± 0.65[a]	32 ± 0.41[a]	24 ± 0.85[a]	16 ± 0.65[a]
Atorvastatin	48 ± 0.75	50 ± 0.65[b]	47 ± 0.48[b]	54 ± 0.65[b]	55 ± 2.1[b]
Metformin	47 ± 0.65	46 ± 2.2[b]	46 ± 1.8[b]	49 ± 1.3[b]	47 ± 0.48[b]
PHF	47 ± 0.41	49 ± 0.65[b]	45 ± 1.3[b]	42 ± 0.71[b]	51 ± 0.65[b]

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 9. Effect of PHF on LDL – Cholesterol (mg/dl) in different groups of animals

Groups	LDL – Cholesterol (mg/dl)				
	0th day	30th day	60th day	90th day	120th day
Normal control	8.5 ± 0.048	20 ± 0.065	24 ± 1.3	26 ± 0.25	32 ± 0.29
Fructose control	14 ± 0.069	67 ± 0.048[a]	87 ± 0.33[a]	108 ± 0.48[a]	117 ± 0.48[a]
Atorvastatin	11 ± 0.079	19 ± 0.071[b]	30 ± 0.048[b]	21 ± 0.048[b]	20 ± 0.041[b]
Metformin	13 ± 0.076	26 ± 0.041[b]	35 ± 0.025[b]	39.3 ± 0.075[b]	49 ± 0.065[b]
PHF	8.6 ± 0.095	16 ± 0.041[b]	19 ± 0.34[b]	26 ± 0.24[b]	28 ± 0.9[b]

Values were expressed as mean ± SEM; N =6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 10. Effect of PHF on VLDL – Cholesterol (mg/dl) in different groups of animals

Groups	VLDL – Cholesterol (mg/dl)				
	0th day	30th day	60th day	90th day	120th day
Normal control	19 ± 0.087	20 ± 0.045	20 ± 0.047	22 ± 0.41	21 ± 0.25
Fructose control	20 ± 0.085	21 ± 0.085[a]	22 ± 0.13[a]	28 ± 0.065[a]	32 ± 0.065[a]
Atorvastatin	20 ± 0.048	21 ± 0.071	19 ± 0.087[b]	20 ± 0.085[b]	18.5 ± 0.096[b]
Metformin	19 ± 0.15	22 ± 0.095	20 ± 0.041[b]	22 ± 0.065[b]	26 ± 0.13[b]
PHF	22 ± 0.075[b]	22 ± 0.11	20 ± 0.041[b]	22 ± 0.048[b]	20 ± 0.091[b]

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 11. Effect of PHF on LDL/HDL ratio in different groups of animals

Groups	LDL/HDL ratio				
	0th day	30th day	60th day	90th day	120th day
Normal control	0.27 ± 0.005	0.48 ± 0.0053	0.56 ± 0.014	0.56 ± 0.0014	0.71 ± 0.0065
Fructose control	0.29 ± 0.0018	1.8 ± 0.0061[a]	3.0 ± 0.0030[a]	4.9 ± 0.046[a]	8.21 ± 0.0041[a]
Atorvastatin	0.26 ± 0.0028	0.38 ± 0.0039[b]	0.85 ± 0.015[b]	0.27 ± 0.0078[b]	0.34 ± 0.0041[b]
Metformin	0.29 ± 0.0018	0.55 ± 0.0079[b]	0.93 ± 0.020[b]	0.18 ± 0.0026[b]	0.62 ± 0.0068[b]
PHF	0.26 ± 0.018	0.35 ± 0.011[b]	0.40 ± 0.0025[b]	0.92 ± 0.0054[b]	0.62 ± 0.0054[b]

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 12. Effect PHF on Atherogenic index (A.I.) in different groups of animals

Groups	Atherogenic index (A.I.)				
	0th day	30th day	60th day	90th day	120th day
Normal control	0.56 ± 0.0079	0.95 ± 0.00116	0.96 ± 0.015	1.27 ± 0.0068	1.77 ± 0.065
Fructose control	0.72 ± 0.0012	2.23 ± 0.0017[a]	3.52 ± 0.0030[a]	6.20 ± 0.013[a]	10.42± 0.043[a]
Atorvastatin	0.67 ± 0.0057	0.82 ± 0.0041[b]	1.23 ± 0.016[b]	1.14 ± 0.0011[b]	0.72 ± 0.0071[b]
Metformin	0.64 ± 0.0086	0.96 ± 0.0068[b]	1.476 ± 0.0032[b]	0.73 ± 0.0054[b]	1.0 ± 0.0048[b]
PHF	0.74 ± 0.0087	0.76 ± 0.056[b]	0.82 ± 0.0047[b]	1.45 ± 0.0023[b]	1.01 ± 0.064[b]

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 13. Effect of PHF on Systolic Blood pressure in different groups of animals

Groups	Systolic B.P. (mm of Hg) (120 Day)
Normal control	98 ± 3.755
Fructose control	142.5[a] ± 2.135
Atorvastatin	125.8[b] ± 5.357
Metformin	119.2[b] ± 3.308
PHF	111[b] ± 2.302

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 14. Effect of PHF on Superoxide dismutase in different groups of animals

Group	SOD(U/mg protein)	
	Liver	Kidney
Normal control	8 ± 0.803	10.44 ± 0.8189
Fructose control	4.394[a] ± 0.5114	5.684[a] ± 0.5915
Atorvastatin	7.644[b] ± 0.8871	9.684[b] ± 0.4858
Metformin	7.658[b] ± 0.7251	10.58[b] ± 0.5968
PHF	9.808[b] ± 0.8279	11.33[b] ± 0.7398

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 15: Effect of PHF on Catalase in different groups of animals

Group	CAT (uM H_2O_2consumed/mg protein)	
	Liver	Kidney
Normal control	10.68 ± 0.5358	11.21 ± 0.8978
Fructose control	6.136 [a] ± 0.4472	5.106 [a] ± 0.4806
Atorvastatin	10.84 [b] ± 0.7989	10.87 [b] ± 0.5743
Metformin	10.99 [b] ± 0.5024	10.98 [b] ± 0.7882
PHF	10.79 [b] ± 0.6334	10.0 4 [b] ± 0.5328

Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 16. Effect of PHF on reduced glutathione in different groups of animals

Group	Reduced glutathione(ug of GSH/mg protein)	
	Liver	Kidney
Normal control	9.854 ± 1.002	10.62 ± 0.7486
Fructose control	5.35 [a] ± 0.6426	5.978 [a] ± 1.001
Atorvastatin	10.05 [b] ± 0.4901	11.17 [b] ± 0.916
Metformin	10.52 [b] ± 0.6366	10.79 [b] ± 0.6432
PHF	10.58 [b] ± 0.689	10.3 [b] ± 0.8089

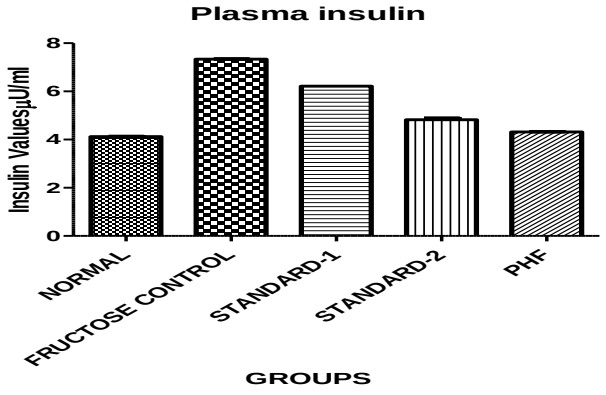
Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Table 17. Effect of PHF on Lipid peroxidation in different groups of animals

Group	Lipid peroxidation (nM of MDA/mg protein)	
	Liver	Kidney
Normal control	0.448 ± 0.06721	0.4888± 0.05928
Fructose control	1.288 [a] ± 0.1411	1.091 [a] ± 0.09316
Atorvastatin	0.4532 [b] ± 0.07929	0.4852 [b] ± 0.05576
Metformin	0.4384 [b] ± 0.0457	0.4152 [b] ± 0.1386
PHF	0.514 [b] ± 0.1125	0.591 [b] ± 0.05875

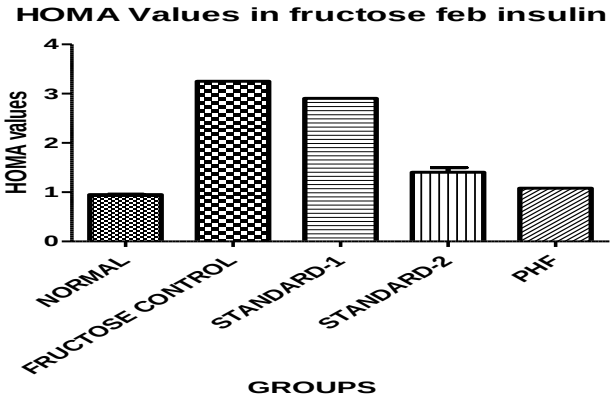
Values were expressed as mean ± SEM; N = 6; [a] = $p < 0.05$, when compared to the normal;
[b] = $p < 0.05$, when compared to the control

Figure 1. Effect of PHF on Plasma Insulin (µu/ml) in different groups of animals



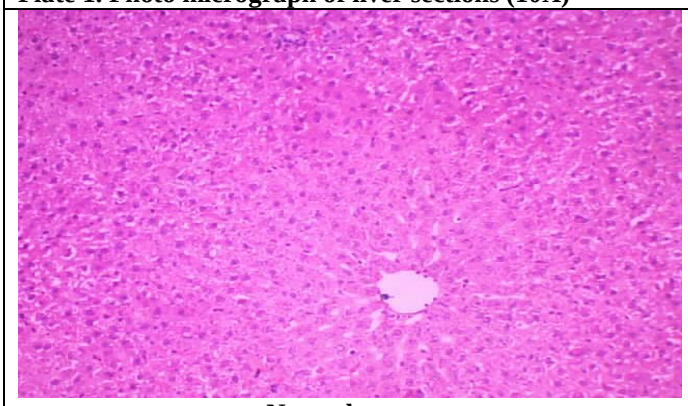
Values were expressed as mean ± SEM. N = 6.
[a] = $p < 0.05$, when compared to the normal
[b] = $p < 0.05$, when compared to the control

Figure 2. Effect of PHF on HOMA values in different groups of animals

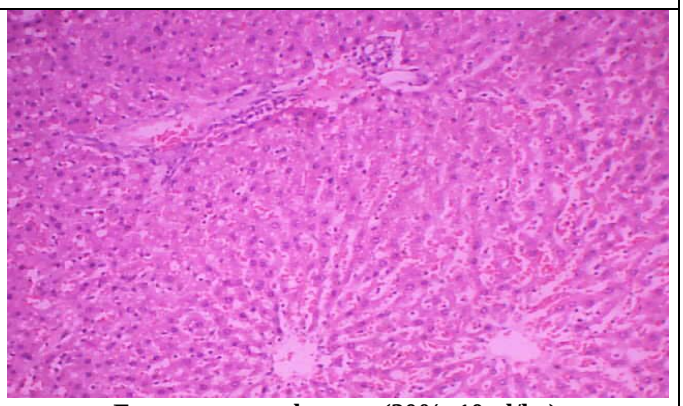


Values were expressed as mean ± SEM. N = 6.
[a] = $p < 0.05$, when compared to the normal
[b] = $p < 0.05$, when compared to the control

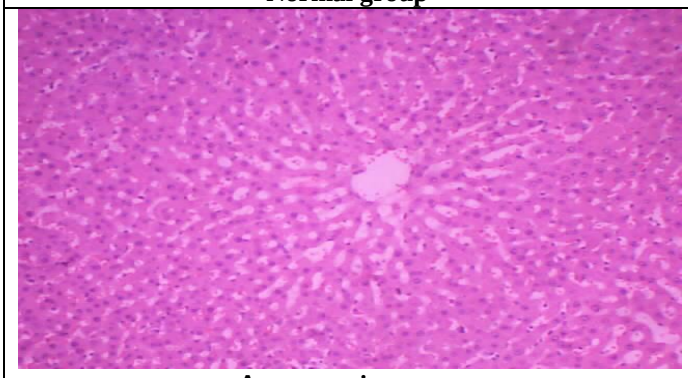
Plate 1. Photo micrograph of liver sections (10X)



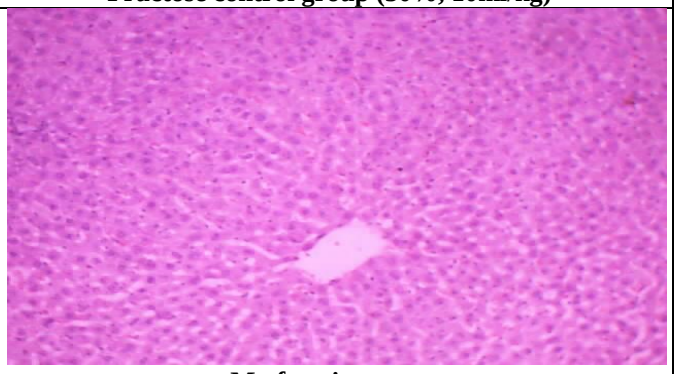
Normal group



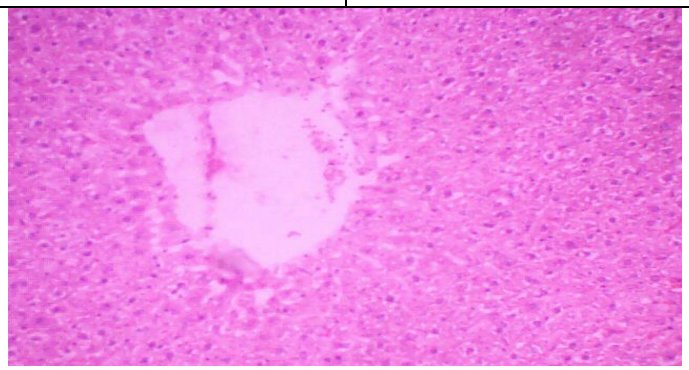
Fructose control group (30%, 10ml/kg)



Atorvastatin group

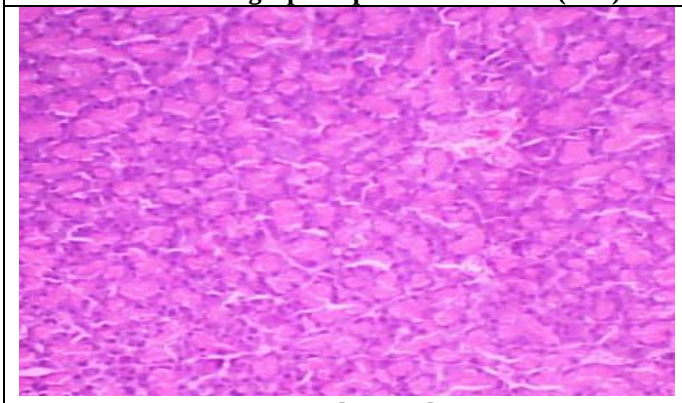


Metformin group

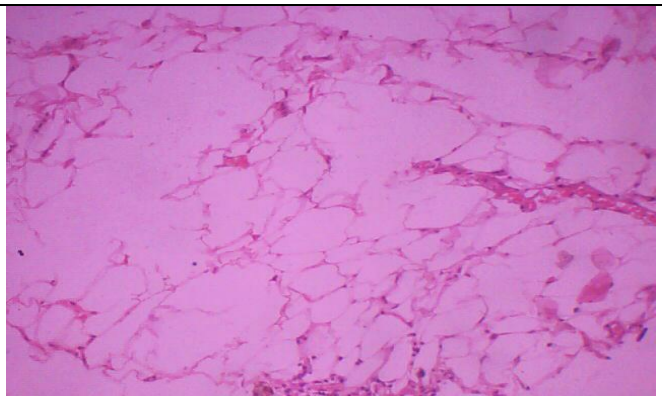


PHF group

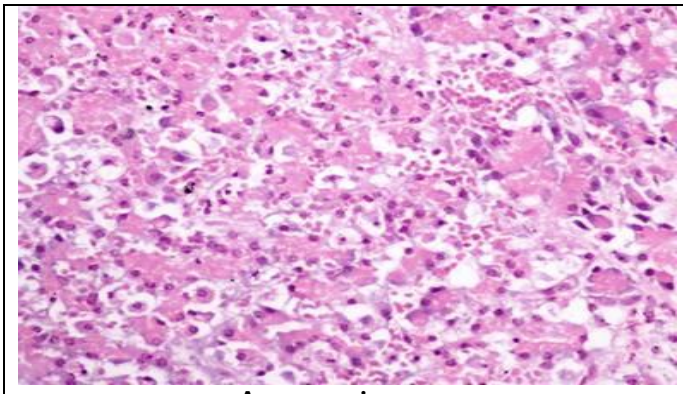
Plate 2. Photo micrograph of pancreas sections (10X)



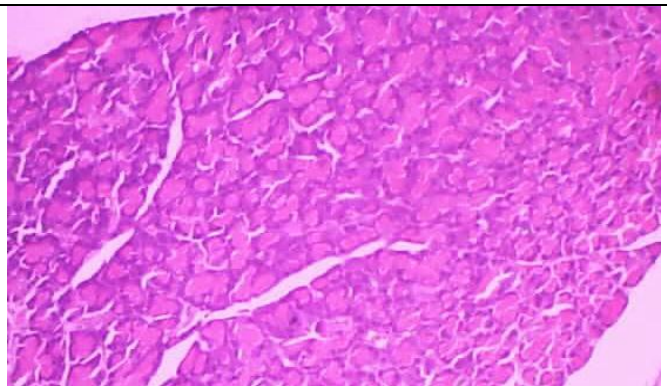
Normal control



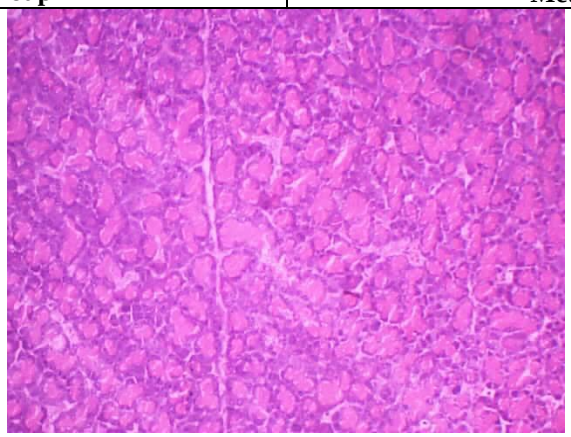
Fructose control



Atorvastatin group



Metformin group



PHF group

DISCUSSION

The Metabolic syndrome is a constellation of interrelated abnormalities namely obesity, insulin resistance, dyslipidemia, hyperglycemia and hypertension that increases the risk of type-II diabetes mellitus and cardiovascular diseases. This is a common metabolic disorder which increases in prevalence as the percentage population suffering from obesity increases (Vega *et al*., 2001). In spite of the availability of various anti-hyperlipidemic agents and anti-diabetic agents, which are generally combined in the management of MS, it is still becoming very tedious to manage MS as they suffer from high incidence of adverse effects. Thus there is still considerable interest in synthesis and evaluation of novel agents in the management of MS (Christos *et al*., 2006). Hence, for a number of reasons complimentary medicine has grown in popularity in recent years in the management of MS. Thus employing polyherbal formulations with numerous activities would be useful in the management of components of MS (Yamini *et al*., 2009).

In the present study, a polyherbal formulation was prepared by combining five herbs namely *Eclipta alba*, *Hemidesmus indicus*, *Nelumbo nucifera*, *Terminalia chebula*, and *Zingiber officinale*. Aqueous extract of PHF was prepared and evaluated for its hypolipidemic, hypoglycemic, anti-hypertensive and anti-oxidant effects against fructose induced insulin resistance model in rats.

Acute toxicity study of PHF was carried out as per the OECD- 423 guidelines at a doses of 5, 50, 300 and 2000 mg/kg body weight. No mortality was observed in animals administered with PHF at all doses during the observation period of 14 days. In the oral glucose tolerance test, at 90 & 120 mins, a significant decrease in blood glucose levels were observed in PHF treated rats when compared with control rats. From the OGTT data, it was clear that administration of PHF at different dose levels effectively decreased the serum glucose levels without causing a hypoglycemic state.

The present study was designed with a normal and a fructose control group and two standard drug groups, atorvastatin (1 mg/kg p.o.) and metformin (70 mg/kg p.o.) were included as there is no single drug in market which can decrease both blood glucose and hyperlipidemia. PHF was administered to another group of rats at a dose of 400mg/kg body weight. All the groups except normal control were administered with fructose daily at a dose of 10ml/kg bodyweight p.o. for 120 days.

Results of the present study revealed that maintenance of rats on fructose (10%) for 120 days was associated with increased weight gain, hyperglycemia, dyslipidemia, hyperinsulinaemia, hypertension and

oxidative stress. These findings are quite consistent with that of Stanhope & Havel who indicated that fructose rich diet induced the development of pathophysiological characteristics associated with metabolic syndrome. The high fructose flux leads to enhanced accumulation of hepatic triglycerides and resulting in impairment of glucose and lipid metabolism (Wiernsperger *et al*., 2010). Results of the present study also exposed that supplying rats with a fructose for 120 days increased body weight, waist circumference, body mass index (BMI) and levels of total triglycerides, total cholesterol, LDL and VLDL in serum. These findings are quite consistent with Xolalpa-Molina who reported that studies in humans and animal models suggest that dietary components such as high fat and fructose can affect fatty infiltration and lipid peroxidation. These results are in harmony with those of other investigators (Ackerman *et al*., 2005 & Swanson *et al*., 1992).

In the present study, rats administered with PHF showed decrease in body weight, waist circumference, body mass index (BMI) and reduction in lipid levels, atherogenic index with an increase in serum HDL levels. These activities of PHF may be due to *H. indicus* present in the PHF which, prevents hyperlipidemia in albino rats by increasing the liver LDL receptor activity and decreasing hepatic triglyceride synthesis. The phytosterol, including sitosterol present might inhibit cholesterol absorption. Ginger oleo-resin present in *Z. officinale* reduces serum and hepatic cholesterol and increases fecal cholesterol excretion (Thomson *et al*., 2002). Ether extract of *T. chebula* have been proved to be possessing anti-oxidant activity (Sarmistha Saha *et al.,* 2015). This may help to reduce oxidative stress which can be a possible reason for oxidation of LDL.

The decrease in blood glucose levels in the PHF treated group indicates the hypoglycemic potential of the formulation probably by enhancing the peripheral utilization of glucose, correcting the impaired hepatic glycolysis and inhibiting gluconeogenesis which may be due to the presence of various alkaloids in *E.alba* (Ananthi *et al*., 2003), asparginin in *N. nucifera* (Huralikuppi JC *et al*., 1991), tannins and alkaloids in *T. chebula* (Gandhipuram Periyasamy *et al*., 2006,) and gingerol in *Z. officinale* (Westerterp-Plantenga *et al*., 2006) which were already reported to possess anti-diabetic activity.

Our study showed that long term fructose administration was associated with an increase in oxidative stress. Delbosc *et al*., found that fructose increased oxidative stress evidenced by decreased levels of SOD, catalase, GSH and is associated with MS in rodents. The enhanced lipid peroxidation in fructose fed rats could be associated with high circulating glucose levels which enhances free radical production from glucose autoxoidation and protein glycation (Nandhini *et al*., 2005). The PHF administered rats showed a substantial increase in antioxidant status which is credited to anti-oxidant abilities of *Eclipta alba, Zingiber officinale, Hemidesmus indica and Nelumbo nucifera* present in the formulation which were already proved to have potent antioxidant activity.

Dyslipidemias may also lead to emergence of insulin resistance as indicated by higher insulin levels and HOMA values in fructose control animals. The PHF treated rats showed improvement in insulin sensitivity indicated by decreased insulin levels and HOMA values. From the present study, though exact mechanisms of PHF against insulin resistance cannot be proposed but the hypoglycemic and anti-oxidant activity shown by the PHF, might have enhanced insulin sensitivity.

Atherogenesis and micro vascular complications due to fructose intake leads to increased systolic blood pressure as observed in fructose control group (Salvetti *et al*., 1993). The significant reduction in systolic blood pressure in PHF treated animals may be due to hypolipidemic, hypoglycemic and antioxidant properties of PHF.

Histological studies of liver and pancreas of fructose control group showed abundant fatty globules and necrosis, disorganized cell structure respectively. The administration of PHF in rats resulted in restoration of normal cell architectury in pancreas and decreased fatty globules in liver.

Rats treated with atorvastatin had shown reduction in lipid values, systolic blood pressure and oxidative stress but there was increase in serum glucose and insulin levels which was due to damage to pancreas as apparent from histopathological examination and there was a weighty decrease in body weight of the animals. So engagement of atorvastatin in the management of MS may not be apt as it leads to hyperglycemia with insulin resistance. Metformin administered group had shown decrease in blood glucose levels along with improvement in insulin sensitivity but the hypolipidemic effect was less when compared to PHF.

CONCLUSION

Result of this study shows that oral administration of PHF lowers serum glucose, insulin triglyceride, total cholesterol, LDL, VLDL, oxidative stress with an increase in HDL concentrations in fructose-fed rats. Moreover PHF is free from any adverse effects as observed in case of allopathic medications. If these results are extrapolated to humans then PHF might prove useful in the treatment and/or prevention of metabolic syndrome.

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