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**Research Article** 

## EFFECT OF POLYHERBAL FORMULATION ON METABOLIC DERANGEMENTS IN EXPERIMENTAL MODEL OF HIGH FRUCTOSE DIET INDUCED METABOLIC SYNDROME

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**ABSTRACT:** High fructose diet induces metabolic syndrome. Therefore an attempt has made to evaluate the effect of Polyherbal formulation in male *Wistar* rats by oral administration of 350 mg/Kg body weight (HF1) and 500 mg/Kg body weight (HF2). The clinical symptoms established in high fructose diet induced metabolic syndrome were ameliorated by Poly herbal formulation. Food, water intake was increased in high fructose diet fed rats. Body weight, abdominal waist and Body mass index were increased in only high fructose fed rats whereas reduced with poly herbal formulation (HF1 and HF2) along high fructose. Biochemical components like fasting blood glucose levels, triglycerides, total cholesterol, LDL, VLDL, SGOT, SGPT, Uric acid, Malondialdehyde (MDA) were increased in only high fructose diet for 7 weeks in association with Herbal formulation HF1, HF2 for last three weeks separately.HDL-C levels were found increased in rats even though supplied with polyherbal formulation. Histopathology results of pancreas and liverof only high fructose fed rats indicated the infiltration of inflammatory cells and fat accumulation which was ameliorated with HF1 and HF2.

Key words: High Fructose diet, Herbal formulation, Metabolic syndrome, Male Wistar rats.

## INTRODUCTION

High intake of refined carbohydrates (fructose), modern fast food and soft drinks are the causes of obesity, metabolic syndrome and cardiovascular disease (Bray *et al.*, 2004 and Gross *et al.*, 2004). Metabolic syndrome is a cluster of multiple pathologies involving central obesity, insulin resistance, dyslipidemia, hyperglycemia and hypertension (Reaven and Banting, 1988 and Zimmet *et al.*, 2005). As the metabolic syndrome become more prevalent among the individuals worldwide, there will be a predominant rise in prevalence of type 2 diabetes and cardiovascular disorders (Grundy, 2008 and Enas *et al.*, 2007). It is also associated with numerous factors like depression, reproductive disorders, dementia and steatosis (Kahn *et al.*, 2005). Dietary components have been hypothesized as major etiological factor in the metabolic syndrome. Increased intake of fructose is correlated with the increase in obesity and diabetes among individuals (Bray *et al.*, 2004 and Gross *et al.*, 2004). Animals fed with high fructose diet developed clinical characteristics associated with the metabolic syndrome (Moura *et al.*, 2009). The possibility that the excessive fructose intake increase the metabolic derangements like high triglyceride and very low density lipoprotein (VLDL) production through *denovo* lipogenisis (Jung *et al.*, 2010). These elevated intracellular and systemic lipids might cause oxidative stress and inflammation, in turn triggers insulin resistance leading to high blood glucose levels.

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Due to lack of therapeutic intervention for metabolic syndrome, there is an increase interest in natural products to treat or prevent the metabolic syndrome. 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 ofaction that may potentiate each other activity with increased benefit. The principal constituent curcumin (*Curcuma longa*) has antihyperglycemic effect in the high fat fed rats was reported by Moselh yet al., (2011).Kung et al.,(2012) demonstrated the hypoglycemic activity of *Gymnemasylvestre* extracts. *Emblicaofficinalis* extract (Ascorbic acid) and *Salaciaoblonga* extract (salacin) are potent antidiabetic, hypolipidemic and antioxidantagentsin Streptozotocin induced type 2 diabetes mellitus rats (Parminder et al., 2012; Bhagyajyothi et al., 2012). Inderjeet et al., (2012) have reported the effect of fruit extract of *Terminaliachebula* on metabolic components of metabolic syndrome, in rats. Though there are about 1200 plant extracts documented in ancient texts which produce salutary effects, investigations on effect of these extracts on metabolic syndrome were less studied. Hence present experiment has taken up to evaluate the efficacy of polyherbal formulation of 5 plant extracts from *Curcuma longa, Salaciareticulata, Gymnemasylvestre, Emblica officinalis, Terminaliachebula* and high fructose diet on the clinical symptoms of metabolic syndrome established in male *Wistar* rats.

## MATERIALS AND METHODS

## Plant material

Standard herbal extracts of *Curcuma longa* (Rhizomes), *Salacia reticulate* (Root), *Gymnemasylvestre* (leaves), *Emblica officinalis* (fruits), *Terminaliachebula* (fruits) are obtained in dry powdery form from Chemiloids Pvt. Ltd. Vijayawada (Reference no: SR/KN/CL/1/2003). The dried powdered material (300 g) was extracted with ethanol (95%) for 72 hr in a Soxhlet apparatus. The extract was made solvent free by distillation under reduced pressure. The resulting ethanol extract was then used for further studies.

### Animal model

Male *Wistar* rats of 6 weeks old were obtained from Sanzyme Ltd. (Earlier known as Uni Sankyo Ltd.) each weighing 150-170g .All the rats were fed with standard chow diet for 1 week and housed in ventilated cages at  $23 \pm 2^{\circ}C$  temperature, 12-hr light and dark cycle in order to adapt to laboratory conditions.Institutional Animal Ethics Committee (IAEC) had approved the experimental protocol. Animals were maintained in accordance with regulations as per CPCSEA guidelines, Department of Animal Welfare and Government of India.

### Chemicals

Thiobarabituricacid, Trichloroacetic acid and other reagents were purchased from Qualikems Fine Chem Pvt. Ltd. 1,1,3,3-tetra ethoxy propane was purchased from Sigma-Aldrich. The diet was purchased from NIN and modified by adding sufficient water and Bengal gram powder to make paste form for ease feeding.

### Acute toxicity studies for dosage fixation

Were performed as per Organisation of Economic Cooperation and Development (OECD) 423 guidelines (Acute toxic class method). 5 animals were randomly selected and kept in cage 5 days prior to dosing. The animals kept overnight fasting; aqueous extract of herbal formulation (2000 mg/kg) administered orally using oral gavage. The animals were observed carefully for first 30 minutes followed by observation for every 4 hrs in 24 hrs for signs of toxicity, physical changes, and respiratory changes neurological and behavioural pattern and continued for 14 days for any lethality and death.

### Animal grouping

Selected animals were fed with high fructose diet for 4 weeks to develop metabolic syndrome. To study the effect of polyhedral formulation rats were divided into four treatments of six animals each as T1(Control)- Receives Chow dietfor 7 weeks,T2(HFD)- Receives modified high fructose diet (65% Fructose) for 7 weeks,T3(HF-1)-Receives modified high fructose diet(65% Fructose) for 7 weeks and poly herbal formulation of 350 mg/kg body weight in saline for 3 weeks and T4(HF-2) - Receives modified high fructose diet(65% Fructose) for 7 weeks and poly herbal formulations are freshly prepared before administering to rats.

### **Biochemical estimation**

Rats were fasted overnight and the blood was withdrawn by retro orbital puncture under light ether anesthesiabefore treatment and after treatment. The serum was seperated by centrifugation of collected blood at 3000rpm, 4°C for 15 min. Available sera was examined for uric acid content by an enzymatic spectro- photometric (ES) method. Glucose level was determined using an autoanalyzer (Turbochem 100, CPC diagnostics). Triglyceride, total cholesterol were determined by enzymatic colorimetric method of Werner *et al.*,(1981) and Allain *et al.*(1974) using auto analyzer (Turbochem 100, CPC diagnostics). LDL and HDL cholesterol was calculated according to Friedewald *et al.*, (1972) and Lopes *et al.*, (1977).

Serum VLDL Cholesterol:- VLDL(mg/dl) = Total cholesterol - (HDL + LDL)

The levels of SGOT and SGPT were estimated by standard procedures of Thomas (1988), Moss and Henderson (1999). MDA (Malondialdehyde) levels were recorded according to Wade and Rij (1988). The changes in the body weight (By using balance) and BMI (Body mass index) were measured twice a week whereas feeding and water intake was observed daily throughout the experiment. Atherogenic index and Systolic blood pressure (Non-Invasive blood pressure technique) was measured at the end of experiment to determine the cardiovascular risks. Oral Glucose tolerance test (OGTT) was performed at the end of the experiment in all the four group animals by oral administration of 2g/kg body weight.

#### Histopathological studies

At the end of experiment, the rats were sacrificed in ether by cervical dislocation for isolation of fat pads (epididymal fat pad, mesentric fat pad and retroperitoneal fat pad), Muscle (Gastrocnemius muscle) and organs (heart, liver, kidney, spleen, testis and lungs). The isolated fat pads and organs were blotted, dried and then weighed. The liver and pancreas were placed in formalin. They were later sectioned using microtome (6µm sections), degraded in alcohol and embedded in paraffin section. The tissue sections were stained with haematoxylin and eosin to evaluate the pathological manifestations.

### Statistical Analysis

Values were expressed as mean  $\pm$  standard error. The results were statistically analyzed for significant differences using one way ANOVA followed by Dunnets post-test to compare control group with other groups. Analysis of results and plotting of graphs were carried out using Graph Pad Prism (version 5.01 for windows). All the data presented were the average values of five replications.

### **RESULTS AND DISCUSSION**

Present study shows that high fructose diet develops metabolic syndrome in the Male Wistar rats. Abdullah et al.,(2011) have reported that high fructose diet fed to rats have developed metabolic syndrome and altered excretory pattern. Acute toxic studies reported that animals showed good tolerance to test dose of 2000mg/kg body weight of herbal formulation. There were no signs of toxicity and mortality observed. The behavior pattern was normal and there was no sign of neurological abnormalities. So the extract was found to be safe for long term administration in rats(Data not presented). The food intake and water intake was initially reduced during first week in T2, T3 and T4 group rats but increased significantly in the later period of experiment when compared to normal diet fed rats (data not shown). Mansour et al., 2013 have reported that high fructose fed rats treated with suitable formulation reduces the excess intake of food and water in early days. In comparison with the control(T1) the body weight and abdominal waist were significantly (p < 0.001) increased in the animals receiving only high fructose diet (T2) whereas those got decreased by 20.65%,2% in HF1(T3) and 25.41%,7% in HF2(T4) respectively (Table 1). High fructose diet increases the body weight of rats whereas it reduces the body weight if Emblica extract has given in addition (Hyun Young Kim et al., 2010). The glucose levels increased significantly in HFD fed control rats (T2) by 7<sup>th</sup> week. Increase in body weight can be correlated with the increase in glycolysis and in turn increase in glucose levels(Takakoyokozawa et al., 2008). Results shows that increase in plasma glucose levels was significantly attenuated (P<0.001) by herbal formulation. In T3 group rats glucose level were reduced by 31.39% and in T4 groupby 34.65% with herbal formulation (Table 1). Figure 1 explains the oral glucose tolerance test of all the four group animals at 0, 30, 60, 90, 120 min respectively after administration of 2g/kg body weight of animal. The results from the study clearly indicate that herbal formulation significantly reduced the glucose level after 90 min of administration in T3 and T4 groups when compared to T2 group. Administration of Gravinol reduces the serum glucose levels in metabolically altered rats which were fed with high fructose diet (Takakoyokozawa et al., 2008).

Table 1 explains that there was a significant increase in total cholesterol (P<0.001), triglycerides (P<0.001) and VLDL-C (P<0.001) with decrease in HDL-C (P<0.001) in high fructose fed rats control (T2) when compared to normal diet fed rats(T1-Control). This state of dyslipidemia was attenuated significantly by treating the groups T3 and T4 with HF1(Herbal formulation 1) and HF2(Herbal formulation 2). HF1 and HF2 decreased the elevated triglyceride levels by 47.84% (T3) and 51%(T4) and conversely increased the HDL-C levels by 41.05%(T3) and 45.03%(T4). Serum LDL-C, Serum VLDL-C and total cholesterol was significantly (P<0.001) decreased by both the formulations in T3 and T4 group animals.Present results regarding cholesterol,triglycerides, HDL,LDL,VLDL are in close correlation with the reports of Sunil *et al.*, (2012) worked on Quercetin impact on diet induced metabolic syndrome.

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The atherogenic index was also decreased in both the groups (T3 and T4) treated with formulation HF1 and HF2 (Table 1). Figure 2 depicts that blood pressure was significantly (P<0.001) increased in the high fructose diet fed rats (T2) by 39.4% when compared with normal diet fed rats (T1). In comparison with the T2 group animals, HF2 decreased thesystolic blood pressure of T4 group animals by 9.02% whereas HF1 did not show any significant decrease in T3 group animals (Figure 2). Bezerra *et al.*, (2000) have reported that insulin resistance is related to hypertension in animals which fed with high fructose diet.

Parameters	Control diet(T1)		HFD(T2)		HF-1(T3)		HF-2(T4)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
FinalBW (g)	208.3	7.37	262.5###	8.33	208.3***	5.27	195.83***	7.08
Abdominal waist(cm)	16.03	0.22	17.5	0.54	15.7	0.13	14.98	0.46
BMI $(g/cm^2)$	0.56	0.02	0.60	0.02	0.52	0.01	0.43	0.05
Fasting glucose levels (mg/dL)	78.16	1.99	122.66###	3.80	84.16***	3.40	80.16***	1.37
Triglycerides (mg/dL)	88.98	3.06	179.51###	3.3	93.65***	2.57	87.93***	3.22
Total cholesterol (mg/dL)	85.01	2.58	144.91###	3.54	80.1***	1.88	83.23***	1.78
HDL-C (mg/dL)	46.11	2.58	28.91###	0.87	40.53***	0.51	41.93***	2.03
LDL-C (mg/dL)	21.10	2.82	80.09###	3.63	21.19***	2.18	23.71***	2.31
VLDL – C(mg/dL)	17.79	0.61	35.90###	0.66	18.39***	0.61	17.58***	0.64
Atherogenic index	0.28	0.01	0.79	0.02	0.36	0.01	0.32	0.01
SGOT (IU/L)	23	1.07	42.08###	0.96	30.67***	1.65	26.67***	1.84
SGPT (IU/L)	46.71	1.99	76.95###	2.37	55.71***	3.62	49.01***	2.83

Table 1: Physiological and metabolic variables of experimental animals

Values expressed in the table are mean values of 6 animals in each group of five replications..BW- Body weight; BMI- body mass index; HDL-C- High density lipoprotein cholesterol; LDL-C- Low density lipoprotein cholesterol; VLDL-C – Very low density lipoprotein cholesterol; serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT ).Mean value was significantly different from that of normal diet fed rats.

<sup>#</sup>P < 0.05, <sup>##</sup>P < 0.01, <sup>###</sup>P < 0.001; Mean value significant from that of high fructose diet fed control rats-\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

Table 2: Absolute	e weights of	f organs,	muscle	and	fat	pads
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Organ	Control diet (T1)	HFD (T2)	HF-1 (T3)	HF-2 (T4)	
	(weighting)	(weight in g)	(weight in g)	(weight in g)	
Heart	$0.68 \pm 0.01$	$1.35 \pm 0.02^{\#\#\#}$	$1.28 \pm 0.03$	$1.20 \pm 0.05*$	
Lungs	$0.9 \pm 0.02$	$1.17 \pm 0.05^{\#\#}$	$1.69 \pm 0.009 ***$	$1.80 \pm 0.02 ***$	
Liver	$7.78 \pm 0.09$	$9.16 \pm 0.11^{\#\#}$	8.51 ±0.053***	8.18 ±0.07***	
Kidney	$1.82 \pm 0.02$	$1.94 \pm 0.01^{\#}$	$1.83 \pm 0.03*$	1.81 ±0.02**	
Testis	$1.88 \pm 0.01$	$2.58 \pm 0.02^{\# \# \#}$	$2.32 \pm 0.04 ***$	2.25± 0.01***	
Spleen	0.61±0.006	$0.73 {\pm} 0.007^{\# \# \#}$	$0.67 \pm 0.01*$	0.54 ±0.02***	
Muscle(gastrocnemius, soleus and plantaris)	1.17 ±0.01	4.95 ±0.06 <sup>###</sup>	3.79 ±0.02***	3.44 ±0.09***	
Mesenteric fat pad	$1.17 \pm 0.01$	$1.4 \pm 0.01^{\#\#}$	$1.28 \pm 0.02*$	1.14± 0.03***	
Epididymal fat pad	$1.26 \pm 0.05$	$2.38 \pm 0.04^{\#\#\#}$	2.17 ±0.02**	1.74 ±0.02***	
Retroperitoneal fat pad	$0.75 \pm 0.06$	$3.73 \pm 0.03^{\# \# \#}$	2.74 ±0.04***	2.21 ±0.05***	

The values expressed in the table are Mean  $\pm$  Standard error of 6 animals of each group.Mean value was significantly different from that of normal diet fed rats.  ${}^{\#}P < 0.05$ ,  ${}^{\#\#}P < 0.01$ ; Mean value significant from that of high fructose diet fed control rats-  ${}^{*}P < 0.05$ ,  ${}^{**}P < 0.01$ ,  ${}^{***}P < 0.001$ .

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The serum MDA levels were significantly (P < 0.001) increased in high fructose diet control rats(T2) when compared to normal control rats(T1). However the administration of HF1 and HF2 significantly (p < 0.001) lowered serum MDA in T3 and T4 group rats (Fig 3). According to Chu et al., 2011 high fructose diet increases lipid peroxidation which in turn increases Serum MDA levels. Decrease in TBA levels decreases Serum MDA levels in high fructose fed rats (Kim *et al.*, 2010). Hyperuricemia was found increased significantly (p < 0.001) by two folds in HFD fed control rats(T2) when compared to normal control diet fed rats(T1), whereas it got decreased (P < 0.001) in T3 and T4 group rats treated with HF1 and HF2 (Fig 4). Abdulla et al., 2011 while working on altered excretory system in high fructose diet fed rats reported hyperuricemia is due to lack of negative feedback mechanism of Fructokinase results in accumulation of phosphorylated substrate, depletion of hepatic ATP which in turn increases degradation of nucleotides to uric acid. Table 1 explains that high fructose diet (T2) induced significant (p<0.001) elevation of SGPT and SGOT levels. In comparison with T2 group rats, HF2 decreased the SGPT and SGOT levels by 36.7%, 35.1% in T3 and T4 groups whereas HF1 decreased the levels by 27.2%, 36% respectively. Normalisation of liver functioning in high fructose fed rats by herbal formulations reduces the SGPT and SGOT levels (Daniel et al., 2013). Table 2 explains that high fructose diet significantly increases the weight of organs like liver, testis, kidney, spleen and lungs in T2 group rats. Italso increased the weight of gastrocnemius muscle and fat pads (epididymal, mesentric and retroperitoneal fat pads). In comparison with only HFD fed rats of group T2, weight of organs, muscle and fat pads was significantly suppressed by treatment with HF2 than HF1 in T4 and T3 groups. Present findings are in close collaboration with the studies of Yokozawa et al., 2008. Figure 5, 6 represents high fructose diet induced distorted effects on the histology and architecture of liver and pancreas.



Fig 1: Effect of formulation on Oral Glucose Tolerance Test on the last day of treatment



Fig 2: Effect of formulation on systolic blood pressure

Values are means for 6 rats and vertical bars are representation of standard error mean. Mean value was significantly different from that of normal diet fed rats.  ${}^{\#}P < 0.05$ ,  ${}^{\#\#}P < 0.01$ ; Mean value significant from that of high fructose diet fed control rats-  ${}^{*}P < 0.05$ ,  ${}^{**}P < 0.01$ ,  ${}^{***}P < 0.001$ .

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The HFD fed control rat (T2) liver exhibited many pathological manifestations when compared to normal diet fed rat liver (T1) (Fig 5b). The HFD fed rat exhibited severe necrosis, infiltration of inflammatory cells, hepatocoids and fatty change (5b). This was reversed and liver was restored to normal architecture when treated with HF2 in T4 group animals (Fig 5d).Impact of HF1 on the histology of liver of T3 Group rats was less prominent with little collection of inflammatory cells and mild fatty change (Fig 5c).Infiltration of inflammatory cells was observed in pancreas of HFD fed control rats (T2)(Fig 6b)when compared with normal diet fed control rats (T1)(Fig 6a).The beta cells are hypertrophied in high fructose fed rats which were normalised with HF2 in T4 (Fig 6d)group rather than with HF1(Fig 6c) in T3.



Fig: 3 Effect of formulation on serum MDA levels:

Values are means for 6 rats and vertical bars are representation of standard error mean. Mean value was significantly different from that of normal diet fed rats.  ${}^{\#}P < 0.05$ ,  ${}^{\#\#}P < 0.01$ ,  ${}^{\#\#\#}P < 0.001$ ; Mean value significant from that of high fructose diet fed control rats-  ${}^{*}P < 0.05$ ,  ${}^{**}P < 0.01$ ,  ${}^{***}P < 0.001$ .



Fig: 4 Effect of formulation on serum uric acid levels

Values are means for 6 rats and vertical bars are representation of standard error mean. Mean value was significantly different from that of normal diet fed rats.  ${}^{\#}P < 0.05$ ,  ${}^{\#\#}P < 0.01$ ; Mean value significant from that of high fructose diet fed control rats-  ${}^{*}P < 0.05$ ,  ${}^{**}P < 0.01$ ,  ${}^{***}P < 0.001$ .

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5a- Normal liver





5c- HF-1



### Fig 5: Histopathology of liver

The figures a, b, c, d are Normal control, Only high fructose fed control, High frustose fed+HF-1 (350 mg/kg) and High fructose fed+HF- 2 (500mg/kg) respectively, representing the histological sections of the liver tissue.



6a: Normal pancreas



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6d - HF-2

Fig 6: Histopathology of pancreas

The figures a, b, c, d are Normal control, only high fructose fed, High fructose fed+HF1Test1 (350 mg/kg) and High fructose fed+HF2(500mg/kg) respectively, representing the histological sections of the pancreas tissue

Thus in conclusion, the findings of the current study prove the benefit of the supplementation of herbal formulation in fructose enriched diet model of metabolic syndrome.

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