Chrysin administration of glucocorticoid antagonism and carbohydrate metabolism in HFFD rats

Running title: Chrysin pretreatment glucocorticoid antagonism in HFFD rats

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ABSTRACT

Fructose feeding has been shown to induce insulin resistance in rats, associated with hyperinsulinemia, hyperglycemia, and hypertriglyceridemia. Male Wistar rats of body weight 160-180g were divided into four groups containing six rats each. Control animals received the control diet containing starch while fructose-fed animals received a fructose-enriched diet (greater than 60 percent of total calories). High fructose fed rats (HFFD) elevated the intracellular glucocorticoids (GC) content in both liver and adipose tissue and enhanced the glucocorticoid receptor nuclear translocation. The plasma GC level remained unchanged. The levels of glucose 6 phosphatase, fructose 1, 6-bisphosphatase, in were elevated, accompanied by increased plasma insulin and glucose levels and decreased hepatic glycogen content. Treatment with chrysin reduced plasma glucose 6 phosphatase, fructose 1,6-bisphosphatase, tissue GC levels and the expression of GC-targeted genes involved in lipid accumulation, and it improved insulin sensitivity. High homeostasis model assessment (HOMA) values indicated insulin resistance in HFFD rats, while the HOMA values in chrysin-treated HFFD rats were comparable to those of control rats. We conclude that administration of chrysin improves glucose metabolism and insulin resistance are mediated by enhanced GC in liver and adipose tissue and that GC antagonism in fructose-fed rats, possibly through improved insulin-sensitizing actions of the active constituents.

KEY WORDS: chrysin, HFFD rats, glucose metabolism, glucocorticoids, HOMA, hyperinsulinemia.

ABBREVIATIONS:

F-1, 6-P - fructose-1, 6-bisphosphatase; GCs - Glucocorticoid hormones; G-6-P- glucose-6- phosphatase; HFFD-High fructose fed diet; HOMA-IR - homeostatic model assessment.

INTRODUCTION

Rats fed a High Fructose fed diet (>60% of total calories) provide a useful model of insulin resistance [1]. The condition is associated with compensatory hyperinsulinemia and other metabolic abnormalities like hyperglycemia, hypertension, and dyslipidemia [2]. These changes are also found in patients with the multimetabolic syndrome or syndrome X [3]. Fructose-fed rats form a useful experimental model of insulin resistance. Glucocorticoid

hormones (GCs) orchestrate carbohydrate, lipid and protein metabolism and regulate energy homeostasis through genomic and nongenomic actions in cells. The genomic effects of GCs are mediated through ligand-dependent activation of the glucocorticoid receptor (GR), which is expressed in almost every cell type [4]. Activated GR acts as a transcription factor and targets the genes related to glucose and triglyceride (TG) metabolism [5]. Chronic excess of GCs is associated with insulin resistance, while reduction of GCs improves insulin sensitivity [6]. For example, the levels of circulating GCs are high in patients with insulin resistance, and increased GC production induces hypercortisolism mediated insulin resistance through activation of the GR [7]. Over expression of 11-β-hydroxysteroid dehydrogenase type 1 (11βHSD1), a ketoreductase that enhances tissue availability of GC results in a phenotype that resembles the characteristic features of the metabolic syndrome (MS) such as obesity, glucose intolerance and hypertriglyceridemia, whereas 11βHSD1 deficiency increases insulin action and is associated with decreased TG levels and enhanced fatty acid oxidation [8].

Plant polyphenolic compounds the flavanoids consist of number of classes, as flavanols, flavones and flavans. A naturally occurring flavones, Chrysin (5,7-dihydroxy flavones structure shown in Fig. 1) contained in flowers blue passion flower (Passiflora caerulea), Indian trumpet flower, as well as in edible of mushrooms [9], honey and propolis [10]. At the same time it possess antioxidant capacity, anti-inflammatory activity, anti-allergic, anti-cancer, antiestrogenic, anxiolytic [11], antihypertensive properties [12]. Chrysin having tyrosinase inhibitory activity, moderate aromatase inhibitory activity, and another important role are inhibits estradiol-induced DNA synthesis. C-iso-prenylated hydrophobic derivatives of chrysin are potential P-glycoprotein modulators in tumour cells [13]. The present study aimed to evaluate the effect of chrysin on glucocarticoids, carbohydrate metabolism and glycogen in the HFFD rats against the control and unsupplemented groups.

MATERIALS AND METHODS

Animals

Healthy male adult albino rats (Wistar strain) 6-7 weeks old, weighing 160-180g was procured from “Sri Venkateswara Enterprises”, Bangalore, India. They were housed in a clean sterile polypropylene cages with proper aeration and lighting (12 ± 1 hr day / night rhythm) throughout the experimental period. During the course of the experiments, the temperature was maintained between 27ºC ± 2ºC. The animals were fed with commercially available pelleted rat feed (Gold-Mohur, M/S Hindustan Lever Ltd, Mumbai, India) during the acclimatization period and water *ad libitum*. The usage and handling of experimental rats was done according to the rules and regulations given by the Institutional Ethics Committee.

After one week of acclimatization the animals were divided into two batches. One batch was provided with a control diet containing starch as the source of carbohydrate and the other was fed a fructose-enriched diet for 45 days. They were fed either a control diet, containing 60% corn starch, 20% casein, 0.7% methionine, 5% groundnut oil, 10.6% wheat bran, 3.5% salt mixture and 0.2% vitamin mixture, or a high-fructose diet, which had the same composition as the control diet, except that corn starch was replaced with an equal amount of fructose. The total experimental duration was 45 days. Supplementation of chrysin (25mg kg^{-1} body weight) was given orally for the last 15 days of the experimental period. This dose selected based on our previous stud. The rats were divided into four groups and consisting of six rats each.

Experimental Design

Group I : Normal control rats.

Group II : Control rats treated with chrysin (25 mg kg^{-1} body weight) twice daily for a period of last 15 days of the experimental period.

Group III : High Fructose fed rats (>60% fructose for 45 days).

Group IV : High Fructose fed rats treated with chrysin (25 mg kg^{-1} body weight) twice daily for last 15 days of the experimental period.

Chemicals

Fructose, bovine serum albumin, glucose-6-phosphate, γ-glutamyl paranitroaniline, nicotinamide adenine dinucleotide (NAD^+, NADH), nicotinamide adenine dinucleotide phosphate ($NADP^+$, NADPH), reduced glutathione, oxidized glutathione, adenosine triphosphate (ATP), adenosine monophosphate (AMP) and 1,2,4-aminonapthol sulphonic acid were obtained from Sigma Chemical Company, ST. Louis, MO, USA.

All other chemicals and reagents used were of analytical grade with highest purity. They were obtained from Glaxo Laboratories, Mumbai, SD Fine Chemicals, Mumbai and Sisco Research Laboratories, Pvt. Ltd., India.

Collection of Samples

At the end of experimental period, the rats were fasted overnight and killed by cervical decapitation under mild ether anesthesia. Blood was collected in heparinised tubes to separate the plasma. Liver, kidney and adipose tissues were immediately dissected out, washed in ice-cold saline to remove the adhering blood, dried, weighed accurately, frozen and stored at −80°C until use.

Biochemical analysis

An aliquot of blood was used to estimate glucose by the method of Sasaki *et al*.[14] and fructose by that of Rao [15]. Plasma was separated by centrifugation, and plasma insulin was determined by the enzyme linked immunosorbent assay method (Magiwell kit, United Biotech, Mountain View, CA). The insulin values are expressed in μU/mL.

While the values of homeostatic model assessment (HOMA-IR) [16] and quantitative insulin sensitivity check index (QUICKI) [17] were arrived at from fasting glucose and insulin levels by calculation. The formulae for HOMA-IR and QUICKI are given below:

HOMA-IR = Fasting glucose(mg/dL) fasting insulin(μU /mL)/22.5

QUICKI = 1/ Log insulin(mU /L) X log glucose(mg/dL)

HbA1c was determined by the method of Nayak and Pattabiraman [18] as modified by Bannon [19]. Fructosamine was estimated by the procedure of Dolhofer and Wieland [20], while hemoglobin in whole blood and hemolysate was determined by the method of Drabkin and Austin [21].

Plasma and tissue corticosterone levels were determined by the fluorometric procedure of Silber et al [22]. The hepatic gluconeogenic enzymes glucose-6-phosphatase (glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) and fructose-1, 6-bisphosphatase (fructose-1, 6-bisphosphate 1 phosphohydrolase, EC 3.1.3.11) were assayed by the method of Koide and Oda [22] and Gancedo and Gancedo [23], respectively. Glycogen content in liver tissue was estimated by the method of Morales et al [24].

RESULTS

Fructose-fed rats showed significantly higher levels of glucose, fructose, fructosamine, insulin, HbA1c, and glycated protein, as compared with control rats. On administration of chrysin the levels were decreased as compared with untreated fructose-fed rats and were near normal. HOMA values were high in fructose-fed rats, suggesting insulin insensitivity. chrysin administration decreased the values, indicating improved insulin sensitivity (Table 1).

Corticosterone levels in plasma, liver and adipose tissue

Plasma corticosterone levels were not significantly different between the control and the HFFD groups. Chrysin treatment to control animals increased the plasma corticosterone levels and in the HFFD animals as compared to HFFD rats. However, the increase in plasma corticosterone level with chrysin treatment was not significantly different when compared with the respective untreated groups. Corticosterone levels in liver and adipose tissue were higher significantly in HFFD animals as compared to control rats (figure 2). The levels were reduced in liver and adipose tissue in the HFFD+chrysin group as compared to the HFFD group.

Hepatic enzymes and glycogen content

The activities of hepatic gluconeogenic enzymes glucose-6- phosphatase and fructose-1, 6-bisphosphatase were higher in HFFD rats as compared to control mice (figure 3). HFFD animals administered chrysin registered decreases in the activities of these enzymes as compared to the HFFD group. Hepatic glycogen content was decreased in the HFFD- group as compared to the control group. The level of glycogen was higher in the HFFD + chrysin group as compared to the HFFD groups (figure 4-5). With chrysin treatment, the hepatic enzyme levels and the glycogen content of control animals remained unaltered.

DISCUSSION

Insulin resistance in fructose-fed rat model has been attributed to a low level of insulin-stimulated glucose oxidation due to modifications in the post-receptor cascade of insulin action [26]. High levels of dietary fructose and severe hyperglycemia may have interactive effects, which contribute to the progression and development of pathology. The increase in blood glucose level is also associated with hyperinsulinemia in fructose-fed rats suggests impaired insulin action. This is also supported by high HOMA values. Increased levels of HbA1c, fructosamine, and glycated protein in fructose-treated rats are sequelae of hyperglycemia. During hyperglycemia, glucose can react non enzymatically with neighboring proteins through the Maillard reaction. Among the other proteins, hemoglobin is particularly susceptible to glycation. Moreover, it has been shown that plasma proteins can also undergo glycation [27]. Fructosamine is formed in blood through nonenzymatic reaction between glucose and albumin. The fructosamine assay provides a short-term index (7–10 days) of glycemic control, while HbA1c reflects the average blood glucose concentration during the previous 4–8 weeks.

High-fructose feeding resulted in hyperinsulinemia, metabolic dyslipidemia and GC excess. Administration of chrysin attenuated the effects of fructose by reducing the GC action and by controlling GC-mediated lipid abnormalities. Intracellular GC activation is regulated by a triad consisting of G-6-P, F-1,6-P. Glucose-6- phosphatase (G-6-P) is a multi component enzyme that is tightly related with the endoplasmic reticular membrane and catalyzes the dephosphorylation of G-6-P, the terminal step of glycogenolysis and gluconeogenesis [28]. G-6-P is a key enzyme of glucose homeostasis since it catalyzes the ultimate reaction of

both glycogenolysis and gluconeogenesis. Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as G-6-P, fructose-1, 6-bisphosphatase (F-1, 6-P), phosphor enolpyruvate carboxykinase, and pyruvate carboxykinase [29]. F-1, 6-P is one of the key enzymes of gluconeogenic pathway. It is present in liver and kidney but absent from heart, muscle, and smooth muscle. In this study, the increased activities of G-6-P and F-1, 6-P in liver and kidney of fructose fed rats may be due to insulin deficiency. In *A. aspera* fructose rats, the activities of these two enzymes were significantly $P < 0.05$ reduced, which is responsible for the improved glycemic control. This may be due to chrysin role of alteration in the rate of gluconeogenic key regulatory enzymes have been effectively regulated and thus controlled the gluconeogenic pathway.

The changes in the enzyme activities and glycogen content in fructose fed rats are indicative in the liver at gluconeogenic state. The activities of the regulatory enzymes likes glucokinase, G-6-P, glycogen phosphorylase and hexokinase were altered during chronic fructose feeding leading to hepatic insulin resistance. Furthermore, fructose feeding has been shown to lead to a decrease in the ability of insulin to suppress activation of hepatic G-6-P activity. Phosphoglucomutase and G-6-P exhibited a common pattern. Both enzymes appeared to require a minimum supply of protein (about 20%). In fact, if carbohydrate or fat was substituted for up to 80% of the calories contributed by protein, the same levels of activity as in the livers of rats fed the 100% protein diet were observed. The specific activities remained approximately the same under the various dietary conditions, this being especially clear in the case of phosphoglucomutase. L-type pyruvate kinase (EC 2.7.1.40) is a key enzyme in the glycolytic pathway whose activity fluctuates according to the dietary status in the liver. Reduction in the hepatic glycogen concentration has been reported in this model [30]. Fasting liver and soleus muscle glycogen were markedly reduced in fructose fed rats compared with control. Hyperglycemia per can increase hepatic glycogen synthesis and contribute to the direct pathway to total glycogen synthesis in rats. From the results of this study, it was clearly understood that on administration of the chrysin to the rats fed with the HFFD, the alteration in the rate of gluconeogenic key regulatory enzymes like glucokinase, G-6-P, glycogen phosphorylase and hexokinase have been effectively regulated and thus controlled the gluconeogenic pathway. It also looked at the levels of sugars and amino sugars bound to proteins.

Plasma corticosterone is bound to the corticosteroid binding globulin, also known as transcortin [31] (32). The plasma corticosterone level measured in this study represents the concentration of total corticosterone (free and bound) in circulation, and it remained unaffected by high-fructose-diet feeding. The level was increased by chrysin-treated groups, a level not significantly different from that of untreated groups. The level of corticosterone in plasma is regulated by the hypothalamo-pituitary-adrenal (HPA) axis involving corticotropin-releasing hormone (CRH) of the hypothalamus and the adrenocorticotrophic hormone (ACTH) of the pituitary gland [32]. Yang et al suggested that chronic treatment with chrysin in db/db mice dose-dependently increased the serum corticosterone and ACTH levels through dysregulation of the HPA axis at the level of ACTH, causing a rise in the synthesis and release of plasma corticosterone levels [33]. In the present study, the fructose treatment and chrysin did not alter GC levels in plasma but increased the levels in tissues.

CONCLUSION

In our study provides evidence for the contribution of GC elevation during insulin resistance induced by high intake of simple sugar. The modulation of GC signalling by chrysin could be effective for treating diet-induced insulin resistance.

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Table 1: Effect of chrysin on fasting glucose and insulin in plasma and HOMA and QUICKI values of experimental animals at the end of the study period.

	Control	**Control+25 mg chysin**	**HFFD rats**	**HFFD rats+25 mg chrysin**
Glucose, mg/dL	86.68±7.34 [a]	85.77±7.23 [a]	198.37±14.73	94.62±7.48
Insulin, µU/mL	19.85±1.8 [a]	18.99±1.6[a]	42.67±3.5[b]	21.02±1.6[c]
Fructose (mg/dL)	0.45±0.02 [a]	0.44±0.02[a]	0.74±0.06[b]	0.49±0.03[c]
Fructosamine (mmol/dL)	0.82±0.07 [a]	0.81±0.07[a]	1.44±0.09[b]	0.85±0.03[c]
Glycated protein (μM)	2.63±0.09 [a]	2.61±0.09[a]	4.96±0.15[b]	2.65±0.08[c]
Hemoglobin (g/dL)	14.05±0. 72[a]	14.16.±0.71[a]	09.47±0.34 [b]	13.96±0.52[c]
HOMA	3.93±0. 02[a]	3.86.±0.01[a]	7.63±0.04 [b]	4.08±0.02[c]
QUICKI	0.32±0. 02[a]	0.33±0.01[a]	0.20±0.02[b]	0.31±10[c]
Plasma co	189.83±6.4	189.73±6.5	208.4±7.4	194.8±5.4

Each value is mean ± S.D. for six rats in each group.

Values not sharing a common superscripts (a, b and c) differ significantly at $P < 0.05$ (DMRT).

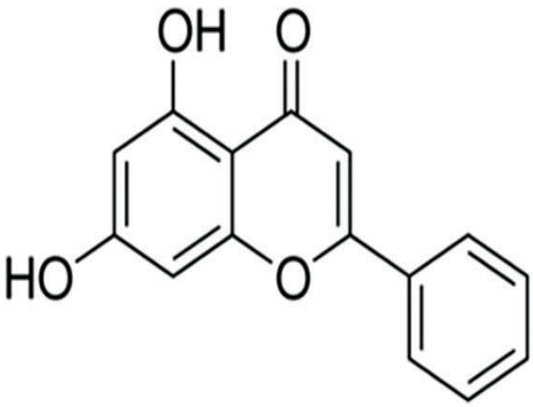

Figure 1: Chemical structure of chrysin (5,7 dihydroxyflavone)

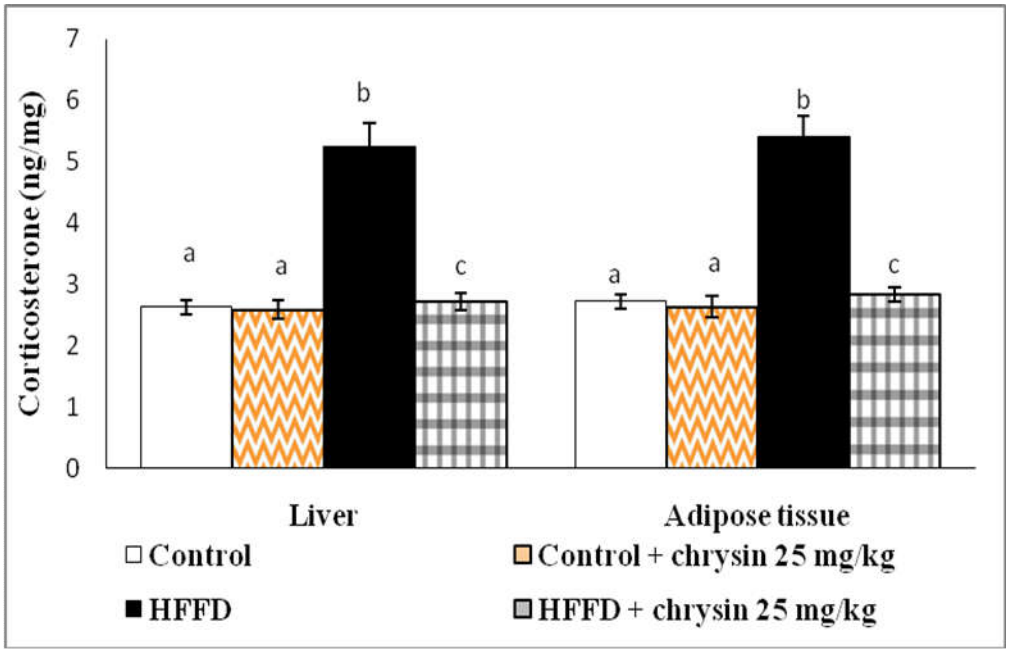

Figure 2. Effect of chrysin in liver and adipose tissue corticosterone levels experimental animals

Columns are mean ± SD of six rats from each group.

Columns not sharing a common superscripts (a, b and c) differ significantly at $P < 0.05$ (DMRT).

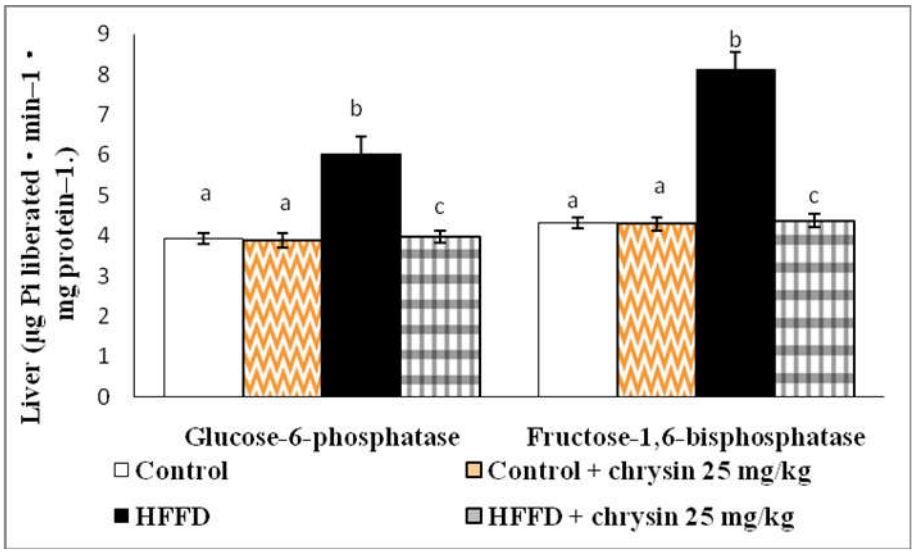

Figure 3. Effect of chrysin in Glucose-6-phosphatase, Fructose-1,6-biphosphatase levels in liver experimental animals

Columns are mean ± SD of six rats from each group.

Columns not sharing a common superscripts (a, b and c) differ significantly at $P < 0.05$ (DMRT).

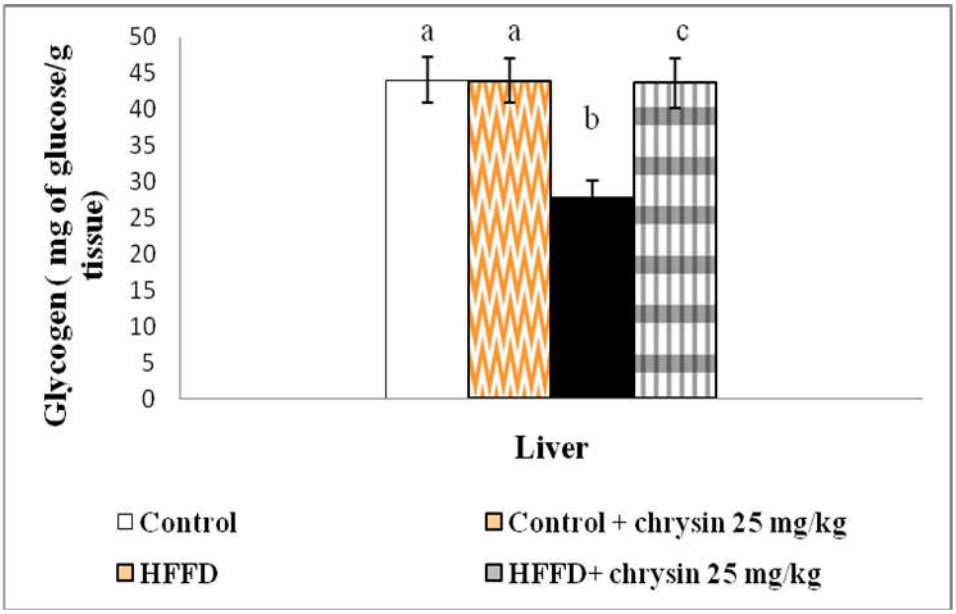

Figure 4. Effect of chrysin in Glycogen level in liver experimental animals

Columns are mean ± SD of six rats from each group.

Columns not sharing a common superscripts (a, b and c) differ significantly at $P < 0.05$ (DMRT).

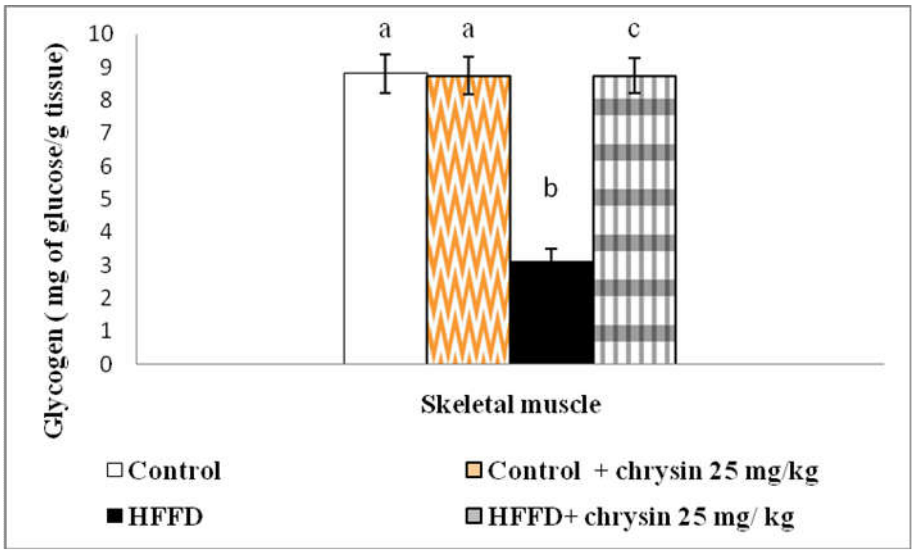

Figure 5. Effect of chrysin in Glycogen level in liver experimental animals

Columns are mean ± SD of six rats from each group.

Columns not sharing a common superscripts (a, b and c) differ significantly at $P < 0.05$ (DMRT).