ISSN: 0975-766X
Research Article
Available Online through
www.ijptonline.com

EVALUATION OF LIPID PEROXIDATION AND ANTIOXIDANTS ACTIVITY OF METFORMIN IN HIGH FRUCTOSE FED DIET INDUCED TYPE II DIABETIC RATS

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Received on 09-06-2010 ***Accepted on 28-06-2010***

ABSTRACT

The purpose of this study was to investigate the possible antioxidant effect of metformin in high fructose fed diet-induced diabetic rats. The increased level of lipidperoxidation and altered levels of enzymatic and non enzymatic antioxidants were seen in high fructose fed consumed animals. The administration of metformin significantly normalized the altered levels of lipid peroxidation and antioxidant status. In conclusion, this study indicates that the administration of metformin improves antioxidant status by reducing lipidperoxidation and enhancing the antioxidant enzymes activities in liver of diabetic rats.

Key Words: Anti-oxidant, Fructose, Lipidperoxidation, Metformin.

INTRODUCTION

Currently, there are 150 million diabetics world wide, and this number is likely to increases to 300 million or more by the year 2025 due to increases in sedentary lifestyles, consumption of energy-rich diets and obesity[1] .In modern medicine, there is still no satisfactory effective therapy available to cure diabetes[2] .Therefore, it has become necessary to search for an economically and therapeutically effective treatment, especially for usage in developing and under-developed countries. Many indigenous medicinal plants have been found to be useful to successfully manage diabetes[3] .A high fructose diet (>60% of total calories) fed rats provide a useful animal model of insulin resistance[4]. The biological factors involved increased the

consequences of an environment that promotes increased food intake and decreased physical activity. Multiple actrologies may result in similar degree of obesity[5].

The location of excess body fat may affect the amount of health risk association with over weight. For over weight women, weight loss may lead to significant improvement in physical health. Recently the relationship between oxidative stress and insulin action has attracted many researchers. It has been suggested that oxidative stress can impair insulin action[6] .In a previous report we have demonstrated increased erythrocyte lipid peroxidation in high fructose-fed rats[7]. Further, supplementation of α-tocopherol, an antioxidant, improved antioxidant potential and insulin action in high fructose-fed rats [8].

Metformin, an insulin sensitizer, has become the established treatment for type 2 diabetes mellitus. Proposed mechanisms for the anti-hyperglycemic effect include enhanced insulin-stimulated glucose uptake from the blood into the tissues, decreased glucose production in the liver by suppression of hepatic glyconeogenesis and decreased intestinal absorption of glucose. Faure et al., suggest that metformin has antioxidant activity in addition to its effect on insulin sensitivity as metformin improved the antioxidant defence in erythrocytes of normal rats[8]. Furthermore, the free radical scavenging properties to metformin could not be demonstrated under in vitro conditions either at low or high concentrations. The present study was carried out to investigate the effects of Metformin on fructose fed rats by evaluating oxidative stress in liver.

MATERIALS AND METHODS

Animals

Adult male albino rats of Wistar strain weighing 170-200 g were used for the study. The rats were housed in polypropylene cage and kept under standard laboratory conditions (temperature 25±2°C; natural light-dark cycle). The rats were provided with food and water *ad libitum*. The commercial rat feed contained 5%fat, 21 % protein,55% nitrogen free extract and 4% fibre (w/w) with adequate minerals and vitamin contents.

Chemicals

Fructose and Metformin were purchased from Sigma chemical Co. (St.Louis, MO, USA). The rest of the chemicals and biochemical's were obtained from local firms (India) and were of analytical grade.

Preparation of control and high fructose feed

The control and high fructose fed were prepared by the method of Nandhini *et al*[9].

Treatment Schedule

The animals were randominised into experimental and control groups and divided into 4 groups of six animals each. Animals in

Group-I Control animals received the control diet and tap water *ad libitum* for 4 weeks.

Group-II Fructose-fed animals received the fructose enriched diet and tap water *ad libitum* for 4 weeks.

Group-III Fructose-fed animals received the fructose diet and tap water *ad libitum* for 4 weeks. Metformin was given during the third and fourth weeks.

Group-IV Control animals received the control diet and tap water *ad libitum* for 4 weeks. Metformin was administered during the third and fourth weeks of the experimental period.

Biochemical Estimations

After the experimental period the animals were fasted overnight and sacrificed by cervical decapitation. Liver were dissected out from all the animals, washed in ice-cold saline and kept in ice-cold container for various biochemical estimations. Thiobarbituric acid reactive substances (TBARS) by Fraga *et al*[10]; Lipid hydroperoxide (Beuge and Aust[11]);Reduced glutathione(Ellman[12]); Super oxide dismutase (Kakkar *et al*[13]); Catalase (Sinha[14]); Glutathine peroxidase (Rotruck *et al*[15]);Vitamin-E (Baker *et al*[16]) and Vitamin-C (Omaye *et al*[17]).

Statistical analysis

Values are mean ± S.D for six rats in each group and statistical significant differences between mean value were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparison, values of $P<0.05$ was considered to be significant. Statical package for social studies (SPSS) 7.5 versions was used for the statistical analysis.

RESULTS

Table 1 shows the levels of TBARS and lipid hydroperoxides in liver of control and experimental animals in each group. The increased levels of liver TBARS and lipidperoxidation were seen in fructose-fed rats (Group-II) compared to control rats (Group-I).The administration of metformin were significantly lower the altered levels of TBARS and lipid hydroperoxides in fructose-fed rats (Group-III). No significant changes were seen in control and Metformin alone rats (Group- I &IV).

Table 2 shows the activities of enzymatic antioxidants (Superoxide dismutase (SOD), Catalse (CAT) and Glutathione peroxidase (GPx)) in liver of control and experimental animals in each group. The activities of the SOD, CAT and GPx in liver were significantly decreased in fructose-fed rats (Group-II) when compared to the control rats (Group I). The administration of Metformin were significantly improved the enzymatic antioxidant status to fructose-fed rats (Group-III). No significant changes were seen in control and Metformin alone administrated rats (Group- I &IV).

Table 3 shows the levels of the non-enzymatic antioxidants in (GSH, Vitamin-C and Vitamin-E) in liver of control and experimental animals in each group. The levels of non enzymatic antioxidant were significantly decreased in fructose-fed rats (Group II) when compared to control animals (Group I). The animals treated with metformin showed a significant improvement of altered levels of enzymatic antioxidants when compared with untreated fructose-fed rats (Group-II). No significant changes were seen in control and Metformin alone administrated rats (Group- I &IV).

Table-1.The levels of TBARS and lipid hydroperoxides in liver of control and experimental animals.

Parameters	Control	Fructose-fed animals	Fructose-fed animals + Metformin	Metformin
TBARS (n mol/mg tissue)	0.13±0.01[a]	0.19±0.01[b]	0.12±0.03[c]	0.14±0.01[a]
Lipid hydroperoxide (n mol/mg tissue)	1.11±0.03[a]	1.22±0.04 [b]	0.14±0.04[c]	1.06±0.01 [a]

Values shown are mean ± SD (n=6).Statistical significant (ANOVA followed by Tukey's test): a compared with control, b compared with Fructose,* significant at $p<0.05$.

Table-2. The activities of enzymatic antioxidants in liver of control and experimental animals.

Parameters	Control	Fructose-fed animals	Fructose-fed animals + Metformin	Metformin
SOD (U/mg protein)	230.57± 21.20[a]	114.62±10.48[b]	183.20±8.43 [c]	229.69±29.20[a]
CAT (µ mol of H_2O_2/min/mg protein)	257.36±21.72 [a]	152.76±15.2[b]	196.94±18.26[c]	259.47±25.27[a]
GPx (µg/min/mg protein)	0.20±0.02 [a]	0.15±0.05[b]	0.82±0.08[c]	0.21±0.02[a]

Values shown are mean ± SD (n=6).Statistical significant (ANOVA followed by Tukey's test): a compared with control, b compared with Fructose,* significant at $p<0.05$.

Table-3. Levels of non-enzymatic antioxidants in liver of control and experimental animals.

Parameters	Control	Fructose-fed animals	Fructose-fed animals + Metformin	Metformin
GSH (mg/g tissue)	20.80±2.31[a]	14.30±1.43[b]	18.20±1.01[c]	21.20±2.78[a]
Vitamin-C (mg/g tissue)	1.40±0.04[a]	0.50±0.02[b]	0.90±0.09[c]	1.41±0.40[a]
Vitamin-E (mg/g tissue)	1.33±0.01[a]	3.35±0.3[b]	2.31±0.21[c]	1.34±0.12[a]

Values shown are mean ± SD (n=6).Statistical significant (ANOVA followed by Tukey's test): a compared with control, b compared with Fructose,* significant at $p<0.05$.

DISCUSSION

The present study was examines lipidperoxidation, and the status of enzymatic and non enzymatic antioxidants in diabetic induced rats. The lipid peroxidation was analyzed by TBARS and lipid hydroperoxides in liver of control and experimental animals. The thiobarbituric acid (TBA) test is a very non-specific technique; it can offer an empirical window on the complex process of lipid peroxidation and is widely used as a marker[18]. Faure *et al* reported that rats fed with high fructose showed an increased lipid peroxidation, as indicated by the higher concentrations of plasma thiobarbitutric acid reactive substances (TBARS) and blood disulfide glutathione (GSSG) and the Cu-Zn-SOD activity[8]. Further they have reported that high fructose diet in rats leads to insulin resistance and a defect in free radical defense system. Rats fed with high fructose diet had higher TBARS in heart tissues, which had an increased susceptibility to peroxidative changes. It is also well known that the increased influx of calcium into the cell with a decreased efflux into the serum/plasma and increased level of plasma iron are believed to be stronger enough to induce the rate of lipid peroxidation.

Metormin therapy to fructose-fed rats lowered the levels of TBARS and lipid hydroperoxides, indicating decreases in lipid peroxidation. It is of interest to note that metformin improved red cell antioxidant activities in fructose-fed rats[19]. Impaired regeneration of NADPH could result in an increased oxidative state of the cell. Further, heightened catabolism of fructose would result in energy depletion in cells, making them more susceptible to peroxidation. In addition to this, hyperglycaemia, hypertriglyceridaemia, hyperinsulinaemia produced by fructose feeding can be related to increased lipid peroxidation levels found in these rats[20,21].

Reactive oxygen species can themselves reduce the activity of antioxidant enzyme, as to play a key role in antioxidant defense mechanisms, particularly during hyperglycemia and in states of insulin

resistance. Cu-Zn SOD is inactivated by the generation of specific lysine residues[22].The expression of the insulin resistance receptor gene requires certain transcription factors whose activity is modulated by GSH [23].Vitamin-E also has a beneficial effect on insulin action, as its supplementation could restore the GSH concentration in fructose-fed rats and improve the physical state of plasma membrane and insulin action in non-insulin dependent diabetes mellitus patients [24]. Fructose feeding also produces hyperglycemia and hypertriglyceridemia, which greatly contribute the increased rate of lipid peroxidation through the enhanced generation of free radicals[25]. As a result oxidative stress mediated free radicals causes increased exhaustion/inactivation of enzymatic antioxidants such as SOD and CAT.

Faure *et al* suggested that metformin has antioxidant activity in addition to its effect on insulin sensitivity as metformin improved the antioxidant defense in erythrocytes of normal rats[8]. In our study concluded that metformin treatment had no effect on the antioxidant parameters in liver of rats fed the control diet containing starch. Thus, the effects of metformin can possibly be attributed its well-known insulin sensitizing actions. Decreased activity of SOD and CAT are suggestive of reduced scavenging potential in insulin-resistant rats. Antioxidant enzymes are reported to be inactivated by O_2^- and ^-OH [26]. A decrease in the level of SOD in high fructose-fed rats may be due to the involvement of O_2^-.Based on this finding, we suggest that stimulation of antioxidant defenses and attenuation of lipid peroxidation are attributable to the metabolic control offered by metformin. The beneficial effects of metformin on these parameters could be significant considering the therapeutic potential of the drug.

CONCLUSION

In this study demonstrated the antioxidant and lipid peroxidation effects of metformin against high fructose-fed induced diabetic rats. The metformin significantly enhanced or maintained the activities of antioxidant enzymes (SOD, CAT, GPx, GSH, Vit-E and Vit-C) in the liver of the rats with fructose-fed induced diabetes. Furthermore, metformin inhibited lipid peroxidation (TBARS and Lipid hydroperoxide) in

the diabetic rats. Thus the present study documented that the metformin could exert a beneficial action against diabetic induced rats.

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