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Protective Effect of Curcuma Longa Against Iron Overload on Pancreatic B-cell Function and Sorbitol Metabolism

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Abstract: Background: Iron that is not specifically chaperoned through its essential functional pathways is damaging to biological systems, in major part by catalyzing the production of reactive oxygen species (ROS). So, excess deposition of iron in the parenchymal tissues of several organs results in cell injury and functional insufficiency. Curcuminoids from Curcuma longa are naturally occurring phytochemical possesses diverse pharmacologic effects iron chelating activities and antioxidant effect. Materials and methods: Thirty male albino rats were equally divided into 3 groups (10 rats for each). The rats were kept on standard ration free from iron as control (grp 1), received 2.0 gm of ferrous sulfate/kg/day B wt in the (grp 2) and simultaneously given 2.0 gm /kg/day B wt of ferrous sulfate and 25 mg/kg/day of curcumin for 8 weeks (grp 3). Blood samples were collected and the serum was separated for biochemical study. The pancreas were collected and specimens from the pancreas tissue samples were homogenized for determination of aldose reductase, sorbitol and sorbitol dehydrogenase, while other specimens from the pancreas fixed in 10% buffered neutral formalin solution and then investigated histopathologically. Results: There are a significant changes in the biochemical parameters in rats received the iron. The serum iron, glucose, insulin resistance, ferritin, oxidized glutathione (GSSG) and malondialdehyde (MDA) as lipid peroxidation product also aldose reductase, sorbitol of pancreatic tissues in group received ferrous sulfate are significantly increased while, reduced glutathione (GSH), insulin, ß-cell function and GSH/GSSG ratio were significantly decreased but sorbitol dehydrogenase not affected. Meanwhile, these changes were improved in the treated rats group which received the curcumin. The histopathological findings were coincided with our biochemical findings in both iron overload and treated group. Partial replacement of some pancreatic acini with fibrosis tissue infiltrated with lymphocytes. Interstitial tissue had blood vessels with endotheliosis, hyalinized vascular wall with perivascular edema and haemorrhage, in the rats received the iron. Conclusion: It could be concluded that the curcumin is effective in prevention the toxic effects of iron-overload that can potentially cause oxidative stress with the formation ROS of hydroxyl radicals and pancreatic β-cell damage.

Key words: Curcuma longa; iron; pancreas; ROS; phytochemical; iron chelating.

INTRODUCTION

The importance of iron in pathophysiology of disease is derived from the ease with which iron is reversibly oxidized and reduced. This property while essential for its metabolic functions makes iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species free radicals (Suzen and Buyukbingol, 2003).

Reactive oxygen species (ROS) have been implicated in a wide range of biological functions, but they can be both essential and highly toxic to cellular homoeostasis (Halliwell *et al.,* 1992). Under normal conditions, potentially toxic ROS are primarily generated by mitochondrial respiratory metabolism and are efficiently neutralized by cellular antioxidant defense mechanisms. However, several conditions are known to disturb the balance between the production of ROS and cellular defense, resulting in cellular destruction and dysfunction. An imbalance between pro- and anti-oxidant factors plays an important role in many processes, including an excess of iron concentrations (McCullough and Bartfy, 2007) and diabetes mellitus (Hockenbery *et al.,* 1993; Dypbukt *et al.,* 1994; Ratan *et al.,* 1994; Sandstrom and Buttke, 1993).

Excess deposition of iron in the parenchymal tissues of several organs (e.g. liver, heart, pancreas, joints, endocrine glands) results in cell injury and functional insufficiency (Bacon and Briton, 1989; Kaczorowska-Hac *et al.,* 2007). Mammals have evolved mechanism to maintain systemic iron within optimal range that foster

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erythroid development and function while satisfying other body needs (Wrighting and Andrews, 2008).

Reduction of glucose by the enzyme aldose reductase (AR) leads to the formation of sorbitol, which, in some tissues, is further oxidized to fructose upon sorbitol dehydrogenase-catalyzed oxidation (Suzen and Buyukbingol, 2003). Sorbitol is a tissue poison and its accumulation increases osmotic pressure and may damage the tissues by causing them to swell (Harding, 1999) and epithelial cells apoptosis (Murata *et al.,* 2001). Aldose reductase of the polyol metabolic pathway, apart from its role as a sorbitol producer and detoxifier of toxic aldehydes, osmoregulator in many organs has been implicated in the etiology of long-term diabetic complications (Gabbay, K H., 1975; Gui *et al.,* 1995).

Aldose reductase enzyme is involved in many pathological processes that have become major threats to human health (Oates, P J., 2008; Alexiou *et al.,* 2009). Oxidative stress and the polyol pathway have recently been found to be linked in pathological states (Suzen, S., 2006; 2007; Suzen *et al.,* 2007). It is possible to prevent the tissues damage via inhibition of the activity of aldose reductase (Harding JJ., 1999) and apoptosis resulting from the accumulation of sugar alcohols (Murata *et al.,* 2001). Reducing sugars are known to produce ROS mainly through the glycation reaction (Sakurai and Tsuchiya, 1988; Mullarkey *et al.,* 1990; Wolff and Dean, 1987). Under diabetic conditions, glucose is converted into fructose through the polyol pathway, leading to increased levels of the latter sugar (Kashiwagi *et al.,* 1992; Yorek *et al.,* 1993; Tilton *et al.,* 1995). Fructose has a stronger reducing capacity than glucose, and the glycation reaction is easily induced by fructose that play an important role in the diabetes induced deterioration in various organ systems (McPherson *et al.,* 1988; Suarez *et al.,* 1989) and oxidative modifications (e.g. lipid peroxidation) after exposure to reducing sugars and iron (Zunguin *et al.,* 2006).

Curcuminoids from Curcuma longa are naturally occurring phytochemical possesses diverse pharmacologic effects including antioxidant, anti-inflammatory, anticancer and iron chelating activities (Srichairatanakool *et al.,* 2007).

The aim of the present study was to determine the effect of curcuma longa against iron overload induced oxidative stress on insulin, insulin resistance, sorbitol metabolism and β cell function. The structural changes in pancreas were also investigated.

MATERIAL AND METHODS

1- Material:

Iron:

Ferrous sulfate was obtained from Sd FiNE-CHEM LiMiTED,(INDIA) and was given in the maximum dose 2gm/ kg/day (Shahidi and Naczk, 2003).

Curcumin:

Curcumin is yellow coloured phenolic pigment (Cooper *et al.,* 1994), obtained from powdered rhizome of *C. longa* Linn, (Family-Zingiberaceae) was obtained from Shanghai Seni Pharma- Tech Co., Ltd. (Shanghai-China-Mainland) and was given in the dose 25 mg/kg/day (Saravanan and Pari, 2005).

2-animals and Experimental Design:

Thirty adult male albino rats (200-220gm) were used in this study. They were obtained from the National Research Centre Cairo, Egypt.

They were kept under constant experimental conditions with free access to food and water. They were left for one week for accommodation before starting the study. They were monitored for body weight once a week and the doses were adjusted weekly according to body weight. Animals were randomly divided into three equal groups (n=10) as following: (Grp1) served as control group and was given normal diet throughout the period of study, (Grp2) Iron overloaded group, where rats received packed biscuits (50–60 g/day) enriched with ferrous sulphate (0.2%, w/w) daily for 8 weeks and (Grp3) Curcumin group was given curcumin concomitant with iron overload in diet throughout the period of study (8 weeks).

Methods:

At the end of experiment, blood was obtained from orbital sinus and centrifuged for 15 minutes at 3000 rpm and serum was collected and used for measurement of insulin by Ultra Sensitive Rat Insulin ELISA Kit (Michael *et al.,* 2000), glucose was determined spectrophotometrically as described by Trinder (Trinder, P., 1969), iron concentration by Andrews (Andrews, N.C., 1999), ferritin according to Cox *et al.,* (2002), reduced

glutathione (GSH) was assayed colourimetrically at 412 nm according to the method of Hu *et al.,* (2003) and oxidized glutathione (GSSG) was measured as described by Tietze (1969) and malondialdehyde (MDA) which represents TBARS according to the method of Yashioka *et al.,* (1979). Insulin resistance and beta cell function was calculated using HOMA model (Matthews *et al.,* 1985; Wallace *et al.,* 2004; Matthews, 2001; Muniyappa *et al.,* 2008). The specimens of pancreas were collected and homogenized. Aldose reductase activity of the freshly prepared supernatant was assayed spectrophotometrically by determining the decrease in NADPH concentration at 340 nm using a UV-1700 visible spectrophotometer (Shimatzu) (Cerelli, 1986) and sorbitol dehydrogenase was determined as described by Wolf *et al* (1973), Sorbitol was determined according to Bergmeyer (Bergmeyer, 1988).

Pathological Specimens:

At the termination of each experiment, necropsy was performed and specimens from the pancreas were collected and fixed in 10% neutral buffered formalin solution. Five micron thick paraffin sections were prepared and stained with hematoxylin and eosin (HE) and then investigated histopathologically (Bancroft *et al.,* 1996). The pathologist who carried out such histopathologic findings had no prior knowledge of the different experimental groups.

Statistical Analysis:

All obtained data were represented as mean ±SE. Differences between the mean values were statistically analyzed by using one-way analysis of variance (ANOVA) utilizing computerized statistical program (SPSS). $P < 0.05$ *and* $P < 0.01$ were considered statistically significant.

RESULTS AND DISCUSSION

Biochemical Changes:

There was significant increase in blood glucose, ferritin and insulin resistance in iron overloaded rats compared with control group, while insulin and beta cell function was significantly decreased. Curcumin administration induced a significant decrease in blood glucose, ferritin levels and insulin resistance when compared with iron overloaded group. While curcumin administration caused a significant increase in insulin levels and beta cell function (Table 1).

In addition iron overload induced a significant rise in oxidized glutathione levels (GSSG) used a significant reduction in serum reduced glutathione and GSH/GSSG ratio compared with control group. While curcumin increased GSH and GSH/GSSG and will decrease GSSG levels significantly when compared with iron overloaded group (Table 2). Figure (1) demonstrated that serum MDA levels were elevated in iron overloaded rats compared with control group while curcumin decreased these levels. Figure (2) showed that iron overload significantly elevated iron levels. While curcumin was significantly reduced these levels.

There are a significant increase in Aldose reductase and sorbitol in rats received iron when compared with control group, While Sorbitol dehydrogenase are not affected, and curcumin administration induced a significant decrease in aldose reductase and sorbitol level when compared with iron overloaded group, While Sorbitol dehydrogenase are not affected (Table 3).

Table 1: Effect of iron overload and curcumin on blood glucose, Ferritin, insulin, insulin resistance and ß-cell function.

Parameter	Normal group	Iron overload Group	Iron+Curcumin Group
Gucose(mmol/l)	3.89±0.09	7.34±0.23*	4.7±0.05**
S. Ferritin (µg/l)	154.20± 2.04	225.41± 2.17*	180.30 ± 4.32**
Serum insulin (µU/ml)	11.4±0.21	8.54±0.31*	11.7±0.27**
Insulin Resistance	1.94±0.07	2.74±0.14*	2.07±0.05**
ß-cell function	944.5±187.4	44.5±2.3*	442.2±39.8**

Values are expressed as means±S.E.
*Significantly different from normal control at $P < 0.05$.
** Significantly different from iron overload group at $P < 0.05$.

Table 2: Effect of Curcumin on reduced glutathione(GSH), oxidized glutathione (GSSG) and their ratio (GSH/GSSG).

Parameter	Normal group	Iron overload Group	Iron+Curcumin Group
GSH (mg/l)	15.32±0.44	5.65*±0.302	12.04**±0.51
GSSG (mg/l)	0.22±0.02	0.89*±0.05	0.31**±0.02
GSH/GSSG	71.28±4.9	6.55*±0.48	40.40**±3.64

Values are expressed as means±S.E .
*Significantly different from normal control at $P < 0.05$.
**Significantly different from iron overload group at $P < 0.05$.

Table 3: Effect of iron overload and curcumin on pancreatic aldose reductase, sorbitol and sorbitol dehydrogenase.

	Normal group	Iron overload	Iron+Curcumin
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Parameter		Group	Group
Aldose reductase (U/mg tissue)	0.7±0.041	1.8±0.013 *	0.75±0.032 **
Sorbitol g/mg tissue	0.45±0.036	1.14±0.051 *	0.54±0.005 **
Sorbitol dehydrogenase U/mg tissue	0.58±0.033	0.53±0.009	0.62±0.021

Values are expressed as means±S.E.
*Significantly different from normal control at $P < 0.01$.
**Significantly different from iron overload group at $P < 0.01$.

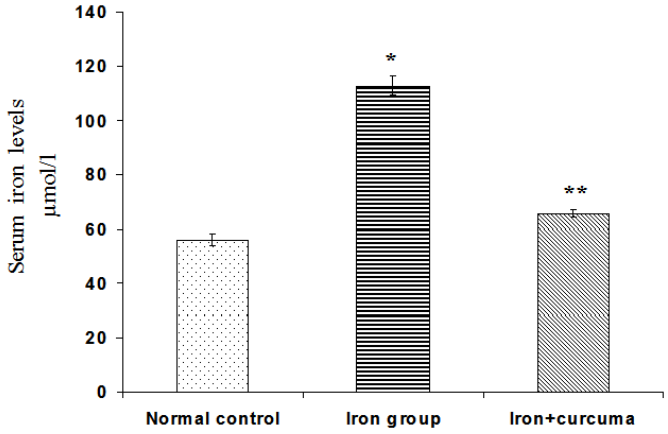

Fig. 1: Effect of curcumin on serum iron levels.
*Significantly different from normal control at $P < 0.05$.
**Significantly different from iron overload group at $P < 0.05$.

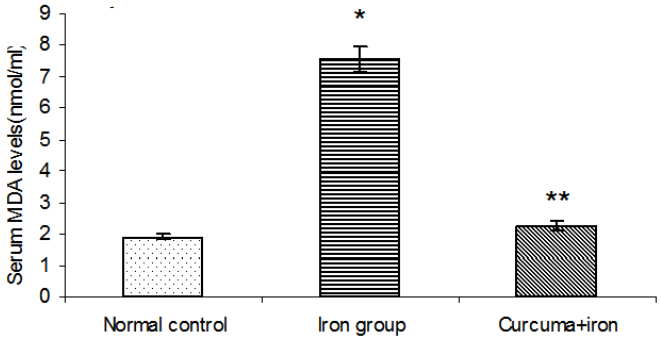

Fig. 2: Effect of curcumin on serum MDA levels in iron overloaded rats.
*Significantly different from normal control at $P < 0.05$.
**Significantly different from iron overload group at $P < 0.05$.

Histopathological Results:

Histopathological of pancreas showing both exocrine and endocrine structure appeared normal (fig. 3).

While, pancreas of iron overload showed partial replacement of some pancreatic acini with fibrosis tissue infiltrated with lymphocytes. Interstitial tissue had blood vessels with endotheliosis, hyalinized vascular wall with perivascular edema and haemorrhage (fig. 4). Retention of some secretion within the pancreatic duct. The latter was lined by flattened epithelium with periductular fibrosis. But curcumin treated group showing normal structure (fig. 5).

Discussion:

Hereditary haemochromatosis have decreased insulin secretory capacity with a compensatory increase in insulin sensitivity. Insulin secretory capacity improves after normalisation of iron stores in subjects with hereditary haemochromatosis. Glucose tolerance status improves incompletely and the tissue iron levels are an important determinant of insulin secretion and insulin action (Abraham *et al.*, 2006).

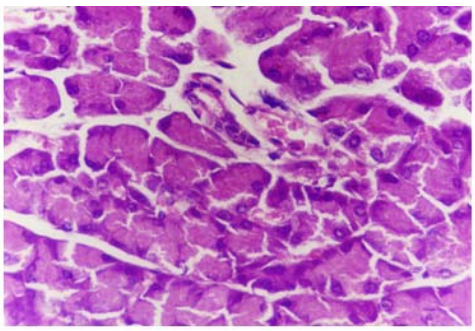

Fig. 3: Normal group Pancrease with normal pancreatic tissues HE x 300.

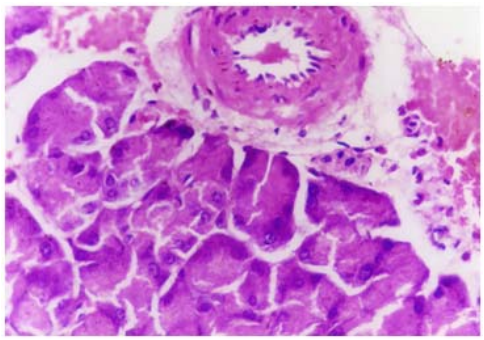

Fig. 4: Iron overload groupPancrease with endotheliosis of blood vessel with perivascular edema and haemorhage HE x 300.

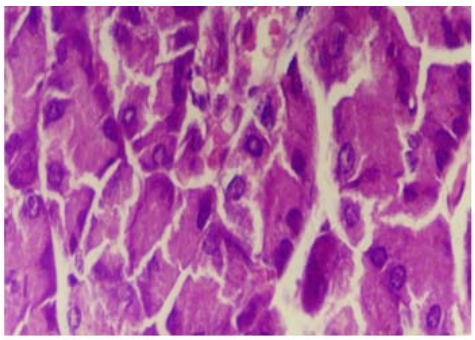

Fig. 5: Iron+Curcumin group Pancrease showing normal parenchymal tissues HE x 300.

The present study demonstrated that an increase in blood glucose level in group that received iron and this results in agreement with Rajpathak with his colleugus (Rajpathak *et al.,* 2006) those suggests that higher iron intake is associated with increased risk of type 2 diabetes. These results accompanied by beta-cells iron deposits-induced decreased insulin secretion in the present study. Beta-cells iron deposits-induced decreased insulin secretory capacity might be of primary importance to trigger diabetes in hemochromatosic patients (Ramey *et al.,* 2007). Iron induce insulin resistance of glucose transport in adipocytes through a mechanism independent of fatty acids and this explain the association between body iron stores and risk of type 2 diabetes mellitus (Green *et al.,* 2006). Heavy iron overload may cause fibrosis of parenchymal organs and the toxicity of iron is believed to involve increased oxidative stress, with iron-catalyzed production of reactive oxygen species causing oxidative damage to lipids, proteins, and nucleic acids (Bonkovsky *et al.,* 2003).

This study showed an increase in serum ferritin values and it has been associated with an increase insulin resistance (IR), this in confirm with other studies. Elevated values of serum ferritin (SF) that resulting in hepatic iron overload has been associated with the insulin resistance syndrome (IRS), defined by the presence of one or more of the following criteria: increased body mass index (BMI), diabetes, hyperlipidemia or hypertension. Further studies are required to investigate the pathophysiological mechanism and consequences

of increased SF levels in patients with IRS (Wrede *et al.,* 2006). There is a positive correlation between iron stores and insulin-resistance (IR) (Le Guenno *et al.,* 2007). A relationship between hepatic iron overload and insulin resistance, and a role for both iron overload and insulin resistance in hepatocellular damage (Piperno *et al.,* 2004). High ferritin levels may be a simple marker of insulin resistance (Vantyghem *et al.,* 2005). Iron overload in offspring of type 2 diabetics is present along with insulin resistance (Psyrogiannis *et al.,* 2003).

The excess of iron intake results in beta-cell oxidant stress and decreased insulin secretory capacity secondary to beta-cell apoptosis and desensitization of glucose-induced insulin secretion (Cooksey *et al.,* 2004). The toxic effects of iron overload including cellular apoptosis or necrosis in heart, spleen, and pancreas and, when coupled with the findings on lipid peroxidation, suggests that oxidative stress is involved in the pathogenesis of the lesions (Whittaker *et al.,* 1996).

The present study reports that iron overload induced oxidative stress involves the GSH depletion while GSSG and lipid peroxidation will increased in accordance with previous reports, where iron-overload can potentiate various forms of liver injury (Iancu and Shiloh, 2005) with oxidative stress through the formation of hydroxyl radicals (Buss *et al.,* 2002) and lipid peroxidation (Knutson *et al.,* 2000; Ozyurt *et al.,* 2006). In particular, the generation of reactive oxygen species (ROS) can result in reversible and irreversible cell and tissue damage (She *et al.,* 2002; Bouwstra *et al.,* 2008).

Also, this study reports that iron overload was increase aldose reductase activity in accordance with Barisani with his colleugus (Barisani *et al.,* 2000) while sorbitol dehydrogenase are not affected and this lead to accumulation of sorbitol inside the cell with its complications and this in confirm with others (Singla and Dhawan, 2010) whose reported that accumulation of intracellular sorbitol due to increased aldose reductase activity has been implicated in the development of various secondary complications of diabetes.

Curcumin was stabilize lysosomal membrane and cause uncoupling of oxidative phosphorylation besides having strong oxygen radical scavenging activity, which was responsible for its antiinflammatory property (Kohli *et al.,* 2005).

The present study reports lowering the values of lipid peroxidation and oxidized glutathione and these results in agreement with others. Dietary curcumin lowers lipid peroxidation by enhancing the activities of antioxidant enzymes (Reddy and Lokesh, 1994; Arun and Nalini, 2002). Curcumin appear to be beneficial in preventing diabetes-induced oxidative stress in rats (Arun and Nalini, 2002; Suryanarayana *et al.,* 2007). Dietary curcumin decrease blood glucose level in this study as described by Arun & Nalini and Honda (Arun and Nalini, 2002; Honda *et al.,* 2006). Also the group that received curcumin will increase insulin secretion in accordance with previous studies (Abraham *et al.,* 2006; Arun and Nalini, 2002) normalisation of iron stores levels are normalize insulin secretion and insulin action. Curcumin stimulates the islet cells, regulates the abnormal metabolic process, and enhances the secretion of insulin, and thereby controls the diabetic condition (Lang *et al.,* 2003).

Phenolics in the turmeric meal determine the inhibitory effect of phenolic compounds on iron absorption (Tuntipopipat *et al.,* 2006). So, curcumin treated group lowering glucose level, increase insulin secretion and inhibiting insulin resistance (IR). Also, it is has elevated level of GSH and inhibit aldose reductase activity but sorbitol dehydrogenase are not affected in accordance with Arun and Nalini (2002) which may have been due to the decreased influx of glucose into the polyol pathway leading to an increased NADPH/NADP ratio and elevated activity of the potent antioxdiant enzyme. Lim and his colleugus Lim *et al.,* (2001) indicated that a potential inhibitor of aldose reductase synthesized a series of flavonoid derivatives and examined their effect on sorbitol accumulation in different rat tissues. Moreover, the activity of SDH (sorbitol dehydrogenase), which catalyzes the conversion of sorbitol to fructose, was lowered significantly on treatment with turmeric or curcumin (Arun and Nalini, 2002).

In present study pancreas of iron overload showed partial replacement of some pancreatic acini with fibrosis tissue infiltrated with lymphocytes. Interstitial tissue had blood vessels with endotheliosis, hyalinized vascular wall with perivascular edema and haemorrhage in agrrement with others (Ramey *et al.,* 2007). Diabetes mellitus is found with high frequency in patients with both primary and secondary hemochromatosis. In these conditions; the pancreas shows fibrosis, iron overload of acini interstitium and islet β cells (Ramey *et al.,* 2007). Several liver diseases can be associated with liver iron overload (Abergel *et al.,* 2006).

Recently a link has established between increased dietary iron intake particularly eating red meat and increased iron stores and the development of diabetes. A causative link with iron overload is suggested by the improvement of insulin sensitivity and insulin secretion with frequent blood donation and decreased iron stores (Jiang *et al.,* 2004; Fernandez-Real *et al.,* 2005). The insulin resistance syndrome (IRS) is a condition of increasing incidence in western countries (Ford *et al.,* 2002). The metabolic syndrome is closely linked to insulin resistance and numerous studies indicate a link to hepatic iron overload. Increased serum ferritin,

reflecting hepatic iron overload due to hemochromatosis or blood transfusions, is often associated with insulin resistance (Dandona *et al.,* 1983; Piperno, 1998).

Finally, The present study could be concluded that the curcumin is effective in chelate the effect of iron overload in the male rats that can potentially cause oxidative stress that affect on insulin, insulin resistance, sorbitol metabolism and β cell function and cause pancreatic damage. Also, curcumin is inhibit aldose reductase, so decrease sorbitol level. More research work is needed in order to explore its new areas of therapeutic applications.

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