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Chromium Picolinate and Rosiglitazone Improve Biochemical Derangement in a Rat Model of Insulin Resistance: Role of TNF-α and Leptin

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ABSTRACT
Objective: Incidence of Metabolic Syndrome (MS) is strongly associated with increased fructose consumption. This study aimed to elucidate the role of rosiglitazone, Chromium Picolinate (CP) and their combination on fructose-induced MS. **Materials and methods:** Four groups of rats (n = 10) were fed on Fructose-enriched Diet (FED) for 16 weeks. One served as FED-control while the remaining groups were treated with rosiglitazone (4 mg kg^{-1} day^{-1}), CP (80 μg kg^{-1} day^{-1}) or their combination during the last 6 weeks. A fifth group was fed on normal diet (normal group). At the end, blood samples were collected for estimation MS-related markers. **Results:** Histological examination of livers, kidneys and pancreases from all groups was done. Induction of MS was associated with increased weight gain and insulin resistance coupled with elevated levels of blood glucose, insulin, uric acid, urea, creatinine and lipids. FED also reduced plasma catalase and glutathione peroxidase activities parallel to increased serum leptin and TNF-α levels. This was coupled with marked histological changes in the livers, kidneys and pancreases. Treatment with rosiglitazone or CP attenuated most of the changes associated with MS. Besides, combination of both agents further improved disease markers and decreased hepatocytes fibrosis. **Conclusion:** The present results reveal the benefits of co-supplementation of rosiglitazone and CP in MS.

Key words: Fructose, rosiglitazone, chromium picolinate, leptin, tumor necrosis factor, insulin resistance

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INTRODUCTION
The Metabolic Syndrome (MS) is a constellation of risk factors for cardiovascular disease and type-2 diabetes that consists of abdominal obesity, dyslipidemia, hyperuricemia, β-cell dysfunction, insulin resistance and hyperglycemia[1]. One of the most important causes that contribute to the growing worldwide prevalence of MS, obesity and type-2 diabetes is the change of dietary habits principally due to the increased intake of simple sugars, mainly fructose, commonly used in the food industry and sugar-sweetened drinks[2].

Trivalent chromium (Cr III) is an essential nutrient required for normal carbohydrate and lipid metabolism[3]. Symptoms of chromium deficiency have been observed in humans with elevated blood glucose, insulin, triglycerides (TG) and cholesterol[4]. Chromium Picolinate (CP) as an organic chromium compound has been reported to have a higher bioavailability[5] and may also have beneficial effects on alleviation of the symptoms associated with dysfunctions of carbohydrate and lipid metabolism, such as hyperglycemia, dyslipidemia and type-2 diabetes mellitus[6]. As a result, CP has become a popular chromium supplement. The present work was therefore carried out to further investigate the potential role of CP in fructose-induced insulin resistance syndrome and to compare this effect to a standard well known insulin sensitizer, rosiglitazone (Rosi).

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MATERIALS AND METHODS
Drugs and chemicals: Rosi and CP were kindly provided as finely dispersible powder by Memphis Pharmaceutical Company (Egypt) and MEPACO Pharmaceutical Company (Egypt), respectively. They were suspended in 1% Tween 80 shortly before administration to animals. The concentrations of the drugs were adjusted so that each 100 g animal's body received orally 1 mL of either suspension containing the required dose. Fructose was purchased from Elnasr Pharma, Egypt. Mineral and vitamin mixtures were obtained from Sigma-Aldrich, USA. All other chemicals were of the highest grade commercially available.

Animals: Male Wistar albino rats, 120-140 g b.wt., were purchased from the National Cancer Institute, Cairo, Egypt and left to accommodate in the animal facility of

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the Faculty of Pharmacy, Cairo University, for 1 week before being subjected to experimentation. All animals were allowed free access to diet and tap water. The study was conducted in accordance with ethical procedures and policies approved by the Ethics Committee of Faculty of Pharmacy, Cairo University.

Induction of insulin-resistance syndrome: Insulin-resistance was induced by feeding rats a Fructose-enriched Diet (FED) for 10 weeks according to the method described by Bezerra *et al.*[7]. FED was composed of fructose (660 g kg^{-1}), soya protein (200 g kg^{-1}), sheep fat (60 g kg^{-1}), cellulose (30 g kg^{-1}), L-lysine (10 g kg^{-1}), choline chloride (10 g kg^{-1}), DL-methionine (10 g kg^{-1}), mineral mixture (10 g kg^{-1}) and vitamin mixture (10 g kg^{-1}). Diet was freshly prepared every 3-4 days and stored at 2-8°C till used.

Experimental design: Rats were provided with a FED for 10 weeks. Blood samples were collected randomly after 4, 6, 8 and 10 weeks from the initiation of the FED. Serum levels of Fasting Blood Glucose (FBG), TG and total cholesterol were estimated to ensure the induction of insulin-resistance syndrome. FED-fed rats were randomly allocated into 4 groups (8-10 rats each). One group served as FED-fed control group, while the other 3 groups were treated orally with Rosi (4 mg kg^{-1} day^{-1};[8], CP (80 μg kg^{-1} day^{-1};[9] or their combination, respectively for 6 weeks. Animals were maintained on the FED during the treatment period. A group of animals consisting of 12 rats was run concurrently and maintained on standard rat chow diet and assigned as the normal-control group. Body weight was recorded once weekly.

By the end of the treatment period, waist circumference was measured as an indicator for central obesity. Animals were then fasted for 12 h and blood samples were withdrawn from the retro-orbital plexus for the estimation of the levels of FBG, insulin, lipids, leptin, tumor necrosis factor-alpha (TNF-α), creatinine, uric acid and urea. In addition, homeostasis model assessment of insulin resistance (HOMA-IR score) as an indicator of insulin resistance and LDL-cholesterol (LDL-C) were calculated. Plasma activities of catalase and glutathione peroxidase (GSH-Px) enzymes were also estimated as oxidative stress biomarkers. After sacrification, samples of liver, kidney and pancreas from each group were preserved in 10% formalin prepared in saline and kept for histopathological examination.

Determination of the selected parameters

Fasting serum glucose level: FBG level (mg dL^{-1}) was determined colorimetrically using a test reagent kit (EMAPOL, Poland). Enzymatic oxidation of glucose forms hydrogen peroxide (H_2O_2) which, reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a red violet quinoneimine that can be measured colorimetrically at 500 nm.

Serum insulin level: Following a solid phase two-site enzyme immunoassay, serum insulin level (μIU mL^{-1}) was determined using a test reagent kit (DRG Instruments GmbH, Germany). The bound conjugate was detected by reaction with tetramethylbenzidine (TMB). The reaction was stopped by adding an acid to give a colorimetric end point that was read spectrophotometrically at 450 nm.

Insulin resistance index: Insulin resistance was calculated from FBG and insulin levels as described[10]:

$$\text{Insulin resistance (HOMA-IR) score} = \frac{\text{serum glucose (mmol L}^{-1})\times\text{Serum insulin (}\mu\text{IU mL}^{-1})}{22.5}$$

Serum leptin level: Leptin level (ng mL^{-1}) was estimated using a test reagent kit (DRG Instruments GmbH, Germany). The assay is a solid phase Enzyme-linked Immunosorbent Assay (ELISA) based on the sandwich principle. The bound conjugate was detected by reaction with TMB. The reaction was then stopped by adding acid to give a colorimetric end point that was read spectrophotometrically at 450 nm.

Serum TNF-α level: TNF-α level (pg mL^{-1}) was determined using a test reagent kit (ID labs, Canada). The assay depends on direct sandwich technique in which a monoclonal antibody specific for rat TNF-α had been pre-coated into the microplate. TMB-substrate solution was added to react with formed conjugate to yield a blue color that turned yellow when the stop solution was added. The wells were then measured at 450 nm.

Serum total cholesterol level: Total cholesterol level (mg dL^{-1}) was estimated using a test reagent kit (Biodiagnostics, Egypt). Hydrolysis followed by oxidation of cholesterol yield H_2O_2 as a byproduct. The latter, gives a colored quinoneimine derivative measured at 500 nm upon reaction with 4-aminoantipyrine in the presence of phenol and peroxidase.

Serum HDL-cholesterol level: Using phosphotungistic acid and magnesium ions as precipitating agent, HDL-cholesterol (HDL-C) level was determined using a test reagent kit (Biodiagnostics, Egypt). HDL-C present in the supernatant is then subjected to hydrolysis followed by oxidation to yield H_2O_2 which gives a colored quinoneimine derivative measured at 500 nm upon reaction with 4-aminoantipyrine in the presence of phenol and peroxidase.

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Serum LDL-cholesterol level: The level of LDL-C in serum samples was calculated using the formula[11]:

$$LDL-C\,(mg\,dL^{-1}) = Totalcholesterol - (HDL-C + \frac{TG}{5})$$

Serum triglycerides level: Estimation of TG level (mg dL^{-1}) was done using a test reagent kit (EMAPOL, Poland). Hydrolysis of TG with lipoprotein lipase form glycerol, which is converted under the influence of glycerol kinase to glycerol-3-phosphate. The latter is oxidized to yield H_2O_2 which, reacts with 4-aminoantipyrine and 4-chlorophenol in the presence of peroxidase enzyme to give a red colored quinoneimine product that can be measured colorimetrically at 546 nm.

Serum uric acid level: Uric acid level (mg dL^{-1}) was determined using a test reagent kit (Biodiagnostics, Egypt). Uric acid is hydrolyzed by uricase enzyme yielding allantoin and H_2O_2. The latter reacts with 3,5-dichloro-2-hydroxybenzenesulphonate and 4-aminoanyipyrine in the presence of peroxidase to give a rose-red colored quinoneimine can be measured colorimetrically at 510 nm.

Serum urea level: Using the urease-Berthelot reaction, urea level (mg dL^{-1}) was estimated by a test reagent kit (Biodiagnostics, Egypt). Urea is hydrolyzed by urease enzyme giving ammonium ions which are then measured by the Berthelot reaction. The formed blue dye indophenols product can be measured colorimetrically at 550 nm.

Serum creatinine level: Creatinine level (mg dL^{-1}) was determined by colorimetric kinetic assay using a test reagent kit (Biodiagnostics, Egypt). Creatinine reacts with picric acid in an alkaline solution to form a yellow colored complex. The intensity of the formed yellow dye increases by time and can be measured colorimetrically at 495 nm.

Plasma catalase activity: Catalase activity (IU L^{-1}) was estimated using a test reagent kit (Biodiagnostics, Egypt). Catalase reacts with a known amount of H_2O_2 to form H_2O and O_2. The remaining H_2O_2 afterwards reacts with 3,5-dichloro-2-hydroxybenzenesulphonic acid and 4-aminophenazone in the presence of peroxidase to form a rose-red colored quinoneimine that can be measured colorimetrically at 510 nm.

Plasma glutathione peroxidase activity: The activity of GSH-Px (IU mL^{-1}) was determined by a kinetic assay at 37°C using a test reagent kit (Cayman, USA). The oxidation of NADPH to $NADP^+$ in the presence of GSH-Px is accompanied by a decrease in absorbance at 340 nm providing an indirect spectrophotometric means for monitoring cellular GSH-Px activity.

Preparation of sections for histopathologic examination: Liver, pancreas and kidney samples of 3-4 rats of each group were isolated immediately after scarification. Kidneys were opened along the convex side to insure good fixation and then all samples were fixed in 10% formalin prepared in saline for at least 3 days. Afterwards, all the specimens were washed in tap water for half an hour and then dehydrated using ascending grades of alcohol (70, 80, 90% and finally absolute alcohol). Specimens were then cleared in xylene and impregnated in soft paraffin wax at 55°C and embedded in hard paraffin. Sections of 6 µm thickness were cut using a slide microtome then, stained with hematoxylin and eosin[12] for histopathological examination. Images were captured and processed using Adobe Photoshop version 8.

Statistical analysis: Data were expressed as Mean±SEM. Comparison between the mean values of different groups was carried out by using one way analysis of variance (ANOVA), followed by Tukey-Kramer *post hoc* test for multiple comparisons. In all data analysis, $p \leq 0.05$ was considered significant.

RESULTS

Effect of Rosi or CP and their combination on weight gain and central obesity

Body weight gain: During the experimental period, FED resulted in a gradual increase in body weight starting from the third week after initiation of the diet and continued thereafter. An average body weight of 364 g was reached by the end of the experiment compared to 272 g for the normal-control group (Fig. 1, 2).

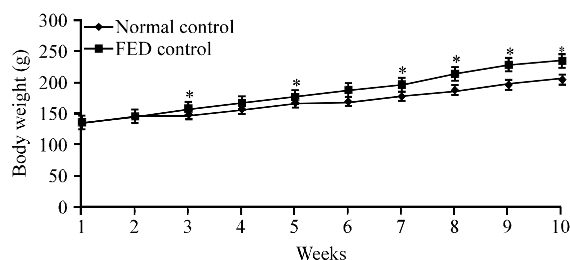

Fig. 1: Effect of fructose-enriched diet (FED) feeding on body weight of rats. Results are expressed as Means±SEM (n = 12). *Significantly different from normal-control group at $p \leq 0.05$

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Treatment of insulin resistant rats with Rosi, CP and their combination reduced the body weight gain by almost 6%, 19 and 15%, respectively (Fig. 2).

Central obesity: By the end of the experimental period FED-control rats showed an increase of waist circumference by ca. 19%, an effect which was not altered by the treatment with either Rosi or CP. However, combination of both drugs reduced the elevated waist circumference by almost 12% (Table 1).

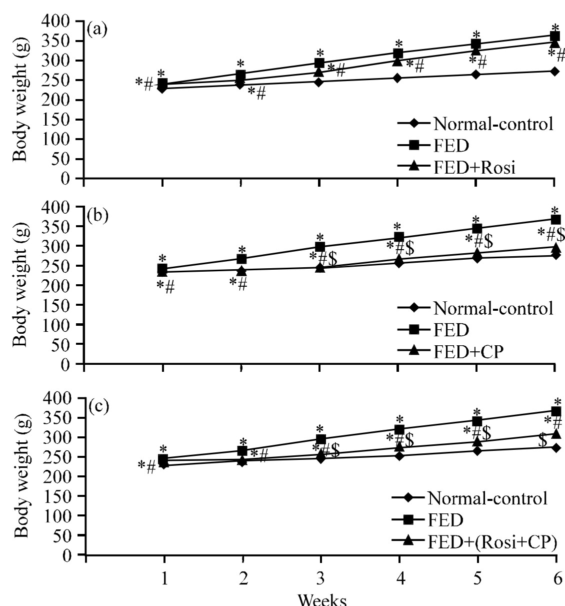

Fig. 2(a-c): Effects of oral treatment with (a) rosiglitazone (4 mg kg^{-1}), (b) chromium picolinate (80 μg kg^{-1}) and their (c) combination, on body weight gain of insulin-resistant rats. FED: Fructose-enriched diet, Rosi: Rosiglitazone; CP: Chromium picolinate. Results are expressed as Means±SEM (n = 12). *Significantly different from normal-control group at $p \leq 0.05$, #Significantly different from FED-control group at $p \leq 0.05$, $Significantly different from Rosi-treated group $p \leq 0.05$

Effect of Rosi or CP and their combination on biochemical determinations

FBG level: FBG level was nearly doubled in the serum of FED-control rats. Treatment with Rosi, CP and their combination significantly decreased FBG by ca. 20, 19 and 25%, respectively (Table 1).

Insulin level: Insulin level was increased in the serum of FED-control rats by nearly 2-fold. Treatment with Rosi did not affect this increased level, but it was almost normalized by the treatment with either CP alone or its combination with Rosi (Table 1).

HOMA-IR score: FED-control rats showed nearly 4-fold rise in the HOMA-IR score. Treatment with Rosi, CP or their combination lowered the raised level by approximately 34, 60 and 59%, respectively (Table 1).

Leptin level: Insulin resistance syndrome was associated with 2-fold increase in serum leptin level. Treatment with the selected agents was accompanied by normalization of this increased level (Fig. 3).

TNF-α level: In FED-control rats TNF-α level was elevated by ca. 125%. Treatment with Rosi and CP significantly decreased the raised level by 77 and 71%, respectively. Combination of both drugs behaved in the same manner, reducing the raised TNF-α level even below that of the normal-control rats (Fig. 4).

Total cholesterol level: Insulin resistance syndrome was accompanied by increase in total cholesterol level by about 48%. Treatment with Rosi or CP did not affect that level. However, combination of both drugs reduced the elevated level by about 24% when compared to Rosi or CP alone (Fig. 5).

HDL-cholesterol level: FED-control rats showed a reduction in HDL-C level by about 57%. Treatment with Rosi did not affect the reduced level. On the other hand, CP and combination of both drugs significantly raised HDL-C level by ca. 12% and 35%, respectively (Fig. 5).

Table 1: Effect of 6-week daily treatment with rosiglitazone, chromium picolinate or their combination on central obesity, fasting blood glucose and insulin levels, as well as, HOMA-IR score of insulin resistant rats

		Fructose-fed			
Parameter	Normal-control	Control	Rosi (4 mg kg^{-1})	CP (80 μg kg^{-1})	Rosi+CP
Waist circumference (cm)	15.44±0.47	18.43±0.14*	18.04±0.29*	17.46±0.24*	16.25±0.48#$
FBG (mg dL^{-1})	67.13±4.07	130.70±2.78*	104.12±4.18*#	106.23±4.77*#	98.43±2.01*#
Insulin (μIU mL^{-1})	12.95±0.42	24.10±2.97*	21.71±2.49*	12.57±0.50#$	12.36±0.29#$
HOMA-IR score	2.15±0.13	7.96±1.14*	5.28±0.29*#	3.18±0.13#	3.26±0.18#

Rosi: Rosiglitazone, CP: Chromium picolinate, FBG: Fasting blood glucose, HOMA-IR: Homeostasis model assessment of insulin resistance. Results are expressed as Means±SEM (n = 8). *Significantly different from normal-control group at p = 0.05. #Significantly different from fructose-fed group at $p \leq 0.05$. $Significantly different from Rosi-treated group at $p \leq 0.05$

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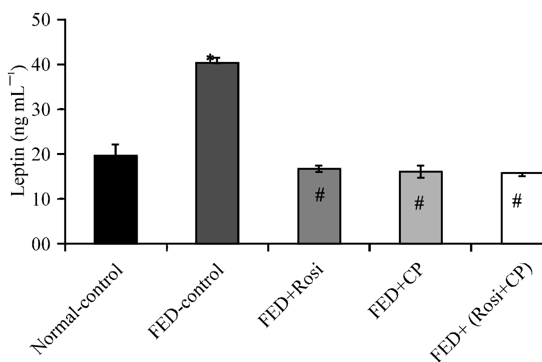

Fig. 3: Effect of oral treatment with rosiglitazone (4 mg kg^{-1}), chromium picolinate (80 µg kg^{-1}) or their combination on serum leptin level of insulin resistant rats. FED: Fructose-enriched diet; Rosi: Rosiglitazone; CP: Chromium picolinate. Results are expressed as Means±SEM (n = 12), *Significantly different from normal-control group at p≤0.05, #Significantly different from FED-control group at p≤0.05

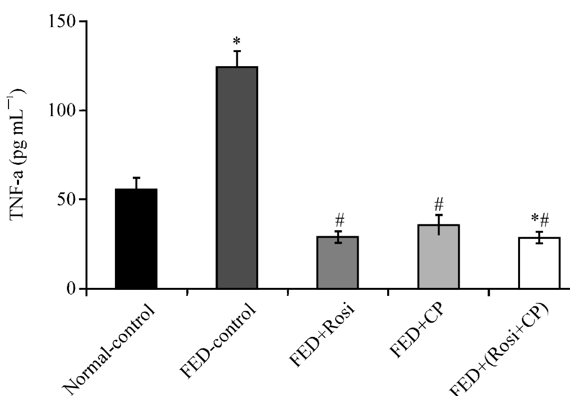

Fig. 4: Effect of oral treatment with rosiglitazone (4mg kg^{-1}), chromium picolinate (80 µg kg^{-}) or their combination on serum TNF-α level of FED-fed rats. FED: Fructose-enriched diet; Rosi: Rosiglitazone; CP: Chromium picolinate; TNF-α: tumor necrosis factor-alpha, Results are expressed as Means±SEM (n = 12), *Significantly different from normal-control group at p≤0.05, #Significantly different from FED-control group at p≤0.05

LDL-cholesterol: MS syndrome was associated with a 2-fold increase in serum LDL-C level. Treatment with the selected drugs did not show any significant decrease in this elevated level moreover, CP administration resulted in a further elevation in LDL-C level of insulin resistant rats by about 46% (Fig. 5).

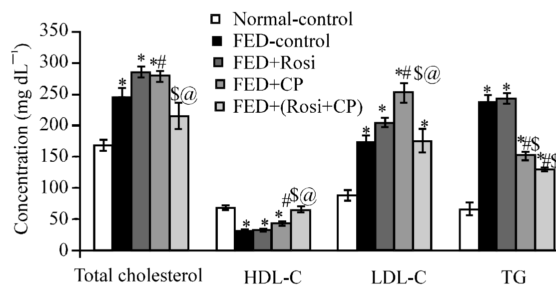

Fig. 5: Effect of oral treatment with rosiglitazone (4 mg kg^{-1}), chromium picolinate (80 µg kg^{-1}) or their combination on serum lipid profile of FED-fed rats. FED: Fructose-enriched diet, Rosi: Rosiglitazone, CP: Chromium picolinate, HDL-C: HDL-cholesterol: LDL-C: LDL-cholesterol, TG: triglycerides, Results are expressed as Mean±SEM (n = 12), *Significantly different from normal-control group at p =0.05, #Significantly different from FED-control group at p≤0.05, $Significantly different from Rosi-treated group at p≤0.05, @Significantly different form CP-treated group at p≤0.05

TG level: TG level was increased in the serum of FED-control rats by almost 4-fold, an effect that was not significantly affected by the treatment with Rosi. Treatment with CP or combination of both drugs decreased the raised level by almost 38 and 47%, respectively (Fig. 5).

Uric acid level: Insulin resistance syndrome resulted in an increase in uric acid level by nearly 69%. Treatment with Rosi, CP and their combination lowered the raised level by ca. 41, 55 and 64%, respectively (Table 2).

Urea level: FED-control rats showed an elevation of urea level of about 3-folds, an effect that was not significantly affected by the treatment with any of the test agents (Table 2).

Creatinine level: Creatinine level was nearly doubled in the serum of insulin resistance rats. Treatment with Rosi, CP and their combination had no effect on the increased creatinine level (Table 2).

Catalase activity: MS was accompanied by nearly a 3-fold reduction in plasma catalase activity. Administration of Rosi and CP non-significantly elevated the reduced activity by about 33 and 44%, respectively. However, combination of both drugs showed a marked increase in catalase activity by ca. 76% (Fig. 6).

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Table 2: Effect of 6-week daily treatment with rosiglitazone, chromium picolinate or their combination on serum creatinine, urea and uric acid levels of insulin resistant rats

		Fructose-fed			
	Normal-control	Control	Rosi (4 mg kg^{-1})	CP (80 µg kg^{-1})	Rosi+CP
Uric acid (mg dL^{-1})	2.22±0.13	7.05±0.31*	4.16±0.52*#	3.19±0.29#	2.56±0.12#$
Urea (mg dL^{-1})	6.94±1.04	17.73±1.17*	16.43±1.06*	14.73±0.59*	15.42±0.51*
Creatinine (mg dL^{-1})	0.31±0.042	0.71±0.044*	0.73±0.073*	0.71±0.083*	0.68±0.069*

Rosi: Rosiglitazone, CP: Chromium picolinate, Results are expressed as Means±SEM (n = 8), *Significantly different from normal-control group at $p \leq 0.05$. #Significantly different from fructose-fed group at $p \leq 0.05$. $Significantly different from Rosi-treated group at $p \leq 0.05$

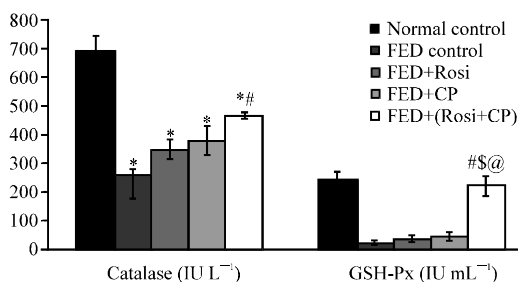

Fig. 6: Effect of oral treatment with rosiglitazone (4 mg kg^{-1}), chromium picolinate (80 µg kg^{-1}) or their combination on plasma catalase and GSH-Px activities of FED-fed rats. FED: fructose-enriched diet; Rosi: rosiglitazone; CP: chromium picolinate; GSH-Px: glutathione peroxidase, Results are expressed as Mean±SEM (n = 12), *Significantly different from normal-control group at $p \leq 0.05$, #Significantly different from FED-control group at $p \leq 0.05$, $Significantly different from Rosi-treated group at $p \leq 0.05$, @Significantly different form CP-treated group at $p \leq 0.05$

Glutathione peroxidase activity: Maintaining rats on a FED for 16 weeks resulted in 9-times decrease in GSH-Px activity. Neither Rosi nor CP administration showed any significant change in the reduced enzyme activity. On the other hand, their combination almost normalized the GSH-Px activity (Fig. 6).

Effect of Rosi or CP and their combination on histopathological changes

Liver tissue: FED-control rats showed marked dilatation and congestion in the central and portal veins with severe fibrosis extending from the portal area in between hepatocytes distorting the normal architecture of liver tissue. The hepatocytes showed marked fatty changes and hydropic degeneration in diffuse manner, an effect that was reversed by the treatment with Rosi. Treatment of insulin resistant rats with CP showed marked improvement in the general structure of liver tissue. However, fatty changes in the focal area specially surrounding the portal region were still observed. Combination of both agents showed significant decrease in fibrosis. The hepatocytes around the central vein appeared normal however; hydropic degeneration was still detected in the periphery of the hepatic lobules (Fig. 7).

Kidney tissue: Histopathological examination of renal sections of FED-control rats showed focal hemorrhage with congestion in the cortical blood vessels and degeneration in the lining endothelium of the tubules. Proliferation in lining endothelium of the golmerular tuft and focal inflammatory cell infiltration in between the tubules was also noticed. Treatment with Rosi, CP or their combination markedly reduced focal hemorrhage and inflammatory cell infiltration. However, congestion in the glomerular tuft was still observed (Fig. 7).

Pancreas tissue: Pancreatic sections of insulin resistant rats showed marked hypertrophy in the islets of Langerhan's in diffuse manner. Fibrosis within the lobules of the pancreas with thickening of the blood vessel walls was observed. Islets of Langerhan's showed slight increase in the amount of connective tissue in between the cells. Treatment with Rosi restored the normal structure of the pancreas, however, neither CP nor the combination of both drugs showed any significant change in the pancreatic tissue of insulin resistant rats (Fig. 7).

DISCUSSION

In the present study fructose-induced insulin resistance syndrome was used to explore the potential therapeutic effects of Rosi, CP and their combination on some biochemical derangements induced by FED. Rosi is a highly potent and selective agonist for peroxisome prolifertor activated receptor-γ (PPAR-γ)[13]. Its primary action is the improvement of insulin sensitivity in muscles and adipose tissue, as well as, inhibition of hepatic gluconeogenesis.

CP may also have beneficial effects on alleviation of the symptoms associated with dysfunctions of

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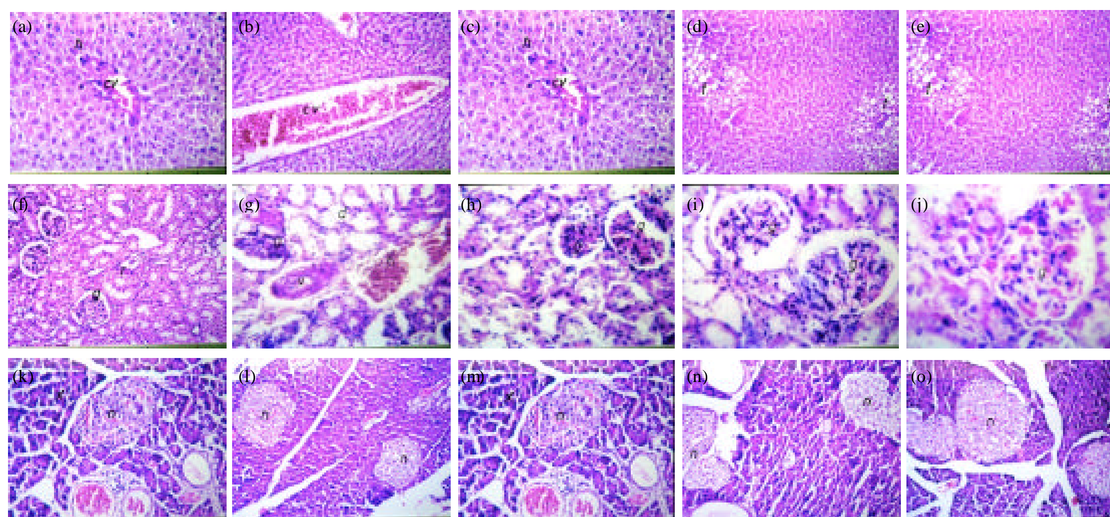

Fig. 7(a-o): Effect of oral treatment with rosiglitazone (4 mg kg^{-1}), chromium picolinate (80 μg kg^{-1}) or their combination on structure of different organs isolated from insulin resistant rats. Tissues are stained with hematoxylin and eosin (magnification×200). FED: Fructose-enriched diet, Rosi: Rosiglitazone, CP: chromium picolinate, (a) Normal hepatic tissue showing polyhedral hepatocytes with eosinophilic cytoplasm and large rounded vesicular nuclei, (b) FED hepatic tissue with marked dilatation and congestion in central and portal veins with severe fibrosis, fatty changes and hydropic degeneration, (c) Rosi-treated FED rats with normal hepatic structure, (d) CP-treated FED rats showing general hepatic tissue improvement, fatty changes in focal area around portal region still observed, (e) (Rosi+CP)-treated FED rats showing normal hepatocyte appearance with hydropic degeneration still detected in periphery of hepatic lobule, (f) Normal renal tissue showing glomerulus that is located between many tubules (g) FED renal tissue with focal hemorrhage, congestion in cortical blood vessels and proliferation in lining epithelium (h) Rosi-treated FED rats with marked improvement in renal tissue, congestion in glomerular tuft still observed (i) CP-treated FED rats with significant improvement in structure but vacuolization in endothelial cell still detected (j) (Rosi+CP)-treated FED rats showing congestion in glomerular tuft and focal hemorrhage at corticomedullary junction, (k) Normal pancreatic structure showing serous acini located in lobules, which contains islets of Langerhan's, (l) FED pancreatic tissue with diffuse manner hypertrophy in islets of Langerhan's with fibrosis in the lobules and thickening in blood vessel walls, (m) Rosi-treated FED rats with normal pancreatic structure, (n-o) CP- and (Rosi+CP)-treated rats, respectively showing no significant changes in fructose-induced pancreatic damage

carbohydrate and lipid metabolism, such as hyperglycemia, dyslipidemia and type-2 diabetes mellitus[6].

The results of the present study revealed that maintaining rats on FED for 16 weeks was associated with multiple disorders including increased weight gain, hyperglycemia, dyslipidemia, hyperinsulinemia, hyperleptinemia, abdominal obesity, hyperuricemia, oxidative stress and insulin resistance.

Treatment of FED-fed rats with Rosi reduced insulin resistance and hyperglycemia. Moreover, it normalized the elevated leptin level. These changes are in accordance with those of Toruner *et al.*[14], who reported that Rosi was able to reduce plasma leptin concentration. Lusting *et al.*[15] found that reduction of insulinemia improves insulin resistance and leptin sensitivity; thus promoting weight loss. Results of the present study showed that indeed Rosi decreased weight gain of FED-fed rats.

In spite of the fact that Rosi reduced weight gain of rats kept on a FED, it maintained visceral adiposity measured as abdominal circumference. This finding is quite consistent with that of Seda *et al.*[16] who reported that treatment of insulin resistance rats with Rosi promotes

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visceral adiposity despite of improving insulin resistance and lipid profile. This was supported in the present study by the finding that Rosi treatment reversed the histopathological changes of the liver and pancreatic tissues induced by FED.

Similarly, treatment of insulin resistant rats with CP reduced insulinemia, hyperglycemia, insulin resistance and leptin level. In addition, it significantly decreased body weight gain and resulted in non-significant reduction in central obesity. Cefalu *et al.*[17] found that CP supplementation to JCR-LA-corpulent rat, a model of MS animals, enhances insulin sensitivity, plasma glucose reduction and improves lipid profile. Combination of Rosi and CP behaved in the same manner and resulted in further reduction of insulinemia, hyperglycemia, insulin resistance and leptin level. Furthermore, it markedly reduced body weight gain and central obesity of FED-fed rats.

Chromium can modulate the activity of insulin by increasing the insulin-sensitive cell receptors or binding capacity and enhancing intracellular insulin signalling activity[5]. The hypoglycemic effect of trivalent chromium was reported under insulin-deficient conditions and exhibited significant antidiabetic potential in STZ-induced diabetes in rats[18].

Disturbed lipid metabolism is considered as another characteristic feature of MS. The current study shows that supplying rats with an FED elevated the levels of TG, LDL-C and total cholesterol in serum. These results are in harmony with those of other investigators[19,20]. On the other hand, HDL-C level was markedly reduced in the serum of FED-fed rats. This finding was in accordance with that of Ohmori *et al.*[21] who reported a decrease in serum HDL-C after 4-weeks of fructose feeding.

The results of the present study revealed that treatment of FED-fed rats with Rosi did not result in any significant improvement in FED-induced dyslipidemia. These changes are consistent with those of Goldberg *et al.*[22] who reported that treatment of patients with type-2 diabetes and dyslipidemia with Rosi not pioglitazone was associated with a significant increase in serum levels of TG, total cholesterol, LDL-C and HDL-C. Similarly, treatment of FED-fed rats with chromium picolinate did not show any significant change in total cholesterol, LDL-C and HDL-C levels. However, it markedly reduced the increased TG level. This is agreement of the finding of Sahin *et al.*[9] who found that treatment of STZ-diabetic rats with chromium picolinate markedly reduced TG level.

On the other hand, combined treatment of insulin-resistant rats with Rosi and chromium picolinate resulted in marked improvement of dyslipidemia as manifested by reduction in total cholesterol and TG levels as well as increased HDL-C level. An effect that might be due to the augmented action of both agents in such prespective.

In contradistinction to the individual effects of Rosi and CP, combination of them obviously improved dyslipidemia as manifested by decreasing total cholesterol and TG levels with elevation of the reduced HDL-C level which almost reached the normal control values. An effect that might be due to the augmented action of the combined drugs on FBG, leptin and insulin levels as well as insulin resistance induced by FED. In addition, the marked decrease in weight gain and abdominal obesity may contribute to the beneficial effect of the combination of Rosi and CP on lipid profile of insulin resistant rats.

The results of the present study also revealed that supplying rats with an FED elevated serum uric acid level. In FED-fed rats, increased plasma uric acid levels have been reported by several authors[23,24]. Unlike other simple sugars, fructose has the unique ability to increase uric acid production. The first step in the metabolism of fructose is the phosphorylation to fructose-1-P via fructokinase, an enzyme which utilizes adenosine monophosphate (ATP) as a phosphate donor. The accumulation of fructose-1-P depletes hepatic ATP and generates adenosine diphosphate (ADP). Metabolism of ADP stimulates adenosine monophosphate (AMP) deaminase and increases the degradation of nucleotides to form uric acid[25]. Hyperuricemia is thus commonly observed in MS Vuorinen-Markkola[26].

Treatment of insulin-resistant rats with Rosi significantly lowered serum uric acid level. Amelioration of insulin resistance by insulin sensitizers was shown to decrease serum uric acid level[27] CP treatment also decreased the elevated serum uric acid level in fructose-fed rats an effect that might be secondary to the reduction in insulin resistance and serum insulin concentration. Facchini *et al.*[28] considered hyperuricemia in MS to be the consequence of elevated serum insulin levels, which have been shown to stimulate renal reabsorption of uric acid. Consistent with this observation is the finding that thiazolidinediones, which improve insulin sensitivity and lower insulin levels, also reduce the level of serum uric acid in diabetic patients[27]. A typical consequence for the complementary effect of both Rosi and CP was observed in the current study thus, the combined agents succeeded to normalize the elevated serum uric acid level.

Furthermore, the findings of the present study demonstrated that MS syndrome in rats was associated with an elevation in serum urea and creatinine levels which reflects impairment in glomerular filtration rate. This was further supported by histological examination of kidney tissues which revealed hemorrhage, congestion

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and inflammatory cell infiltration of the glomerular tuft. Treatment of insulin-resistant rats with Rosi, CP and their combination failed to provoke any significant change in the elevated levels of serum urea and creatinine however, they partly improved hemorrhage and inflammatory cell infiltration of the glomerular tuft although congestion in the tuft was still observed. A fact that may explain the reported ineffectiveness of Rosi, CP or their combination on the serum levels of urea and creatinine.

Low-grade inflammation is now recognized as a common feature of the metabolic abnormalities observed in obesity[29]. TNF-α is increased in obesity and has been extensively characterized for its role in insulin resistance[30]. Fructose feeding in rats has been shown to increase hydrogen peroxide generation and inflammatory markers[31]. Increased plasma concentrations of TNF-α have been observed following fructose feeding in mice[32]. Furthermore, TNF-α mRNA was increased in hepatic tissues in fructose-fed mice (Belmadani[33]). These findings are in harmony with the results of the present study which showed that maintaining rats on an FED for 16 weeks definitely increased serum TNF-α level.

The results of the present study demonstrated that treating MS rats with Rosi, CP or their combination markedly decreased serum TNF-α level. These findings are quite consistent with that of Lee *et al.*[34] who showed that treating Otsuka Long-Evans Tokushima Fatty Rats with Rosi reduced serum inflammatory cytokines including TNF-α. Moreover, Jain[35] found that CP supplementation lowers the risk of vascular inflammation of streptozotocin-diabetic rats by reducing TNF-α level.

The results of the present study revealed that maintaining rats on an FED for 16 weeks reduced the activities of the antioxidant enzymes; catalase and GSH-Px. Delbosc *et al.*[36] found that fructose feeding increases oxidative stress and is associated with MS in rodents. Enhanced lipid peroxidation in fructose-fed rats could be associated with high circulating glucose levels, which enhance free radical production from glucose autoxidation and protein glycation. Prolonged exposure of rats to hyperglycemic condition reduces the activities of superoxide dismutase and other antioxidant enzymes[37].

Treating insulin resistant rats with Rosi slightly raised the reduced plasma catalase and GSH-Px activities. Yilmaz *et al.*[38] reported the antioxidant properties of the drug and found that Rosi treatment reduced malondialdehyde (MDA) level, a valuable indicator of lipid peroxidation in subjects with MS. The antioxidant effect of Rosi is not mediated by PPAR-γ but strictly depends on its ability to activate AMP-activated protein kinase which in turn, prevents the activity of NADPH-oxidase[39], a major source for production of ROS after exposure to hyperglycemia[40]. In addition Sener *et al.*[41] reported that treatment with Rosi decreased MDA contents of liver in rats by inhibiting neutrophil infiltration and the subsequent activation of inflammatory mediators that induce lipid peroxidation. This finding was supported in the present study by the fact that Rosi treatment reversed the FED-induced histopathological changes in the livers of insulin resistant rats.

Likewise, CP treatment was associated with a mild elevation in the activities of catalase and GSH-Px enzymes. Esen Gursel and Tekeli[42] found that supplying rats with high chromium (Cr) diet elevated the activities of antioxidant enzymes such as, catalase, superoxide dismutase and GSH-Px. Other studies suggested that Cr also improves cellular antioxidant capacity in rats[43,44]. Therefore, restoring Cr status in individuals with type 2 diabetes mellitus may counteract the deleterious effects of oxidative stress and help prevent complications associated with MS[4,5]. These data could explain the favorable effects of Rosi and CP in this perspective.

In conclusion, the findings of the current study prove the benefit of the co-supplementation of Rosi and CP in fructose-induced model of insulin resistance. Rosi and CP, in combination, offer further improvements to markers of disease risk, including lipid profile, hyperglycemia hyperinsulinemia, hyperleptinemia, increased oxidative stress and the level of circulating cytokines, which were apparent for either alone. These findings are sufficiently encouraging to support clinical trial of this promising drug combination.

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