Effect of caffeic acid and pioglitazone in an experimental model of metabolic syndrome

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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 pioglitazone (Pio), caffeic acid (CA) and their combination on fructose-induced MS. **Materials and methods:** Seven groups of rats (n=8) were used. Groups 1-3 were fed on normal diet and received 1% Tween 80 (normal control), Pio (2.7 mg/kg) and CA (20 mg/kg), respectively. Groups 4-7 were fed on fructose-enriched diet (FED) for 15 weeks. The 4th group served as positive control group, and the remaining groups received Pio, CA and their combination, respectively. Treatments started 10 weeks after the beginning of fructose feeding. At the end of the study, blood samples were collected for estimation of MS related markers. **Results:** 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 as well as activities of liver transaminases. FED also reduced glutathione peroxidase activity and total antioxidant capacity, increased nitric oxide and lipid peroxides contents parallel to increased serum levels of leptin and tumor necrosis factor-alpha. Treatment with Pio or CA attenuated most of the changes associated with MS. Besides, combination of both agents further improved disease markers. **Conclusion:** The present results reveal the benefits of co-administration of CA and Pio in MS.

***Index Terms*-** Caffeic acid, insulin resistance, metabolic syndrome, oxidative stress, pioglitazone.

I. INTRODUCTION

Metabolic syndrome (MS) is currently a major worldwide epidemic. It represents by definition a disorder related to imbalance of energy utilization and storage. Its features include abdominal obesity, hypertension, dyslipidemia, insulin resistance with elevated fasting blood glucose, and glucose intolerance as well as establishment of pro-thrombotic and pro-inflammatory states **(Grundy et al., 2004)**.

At present the MS is already affecting more than a quarter of the world's adult population. Its prevalence is further growing in both adults and children due to a life style characterized by high calorie nutrition combined with low physical activity **(Ford et al., 2010; Friend et al., 2013)**.

Epidemiological and biochemical studies strongly indicate that consumption of a fructose-enriched diet (FED) contributes to increased prevalence of the modern epidemic of obesity, the basis for MS **(Astrup and Finer, 2000)** and insulin resistance **(Stanhope and Havel, 2008)**.

Pioglitazone (Pio) is one of thiazolidinediones which are synthetic peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists that regulate several cellular functions decreasing insulin resistance **(González-Ortiz et al., 2007)**. Pioglitazone also exerts beneficial effects on the plasma lipid profile, leading to a lower risk of acute myocardial infarction, stroke, or heart failure **(Goldberg et al., 2005; Graham et al., 2010)**.

Natural products have proven historically to be a promising pool of structures for drug discovery and a significant research effort has recently been undertaken to explore the PPAR-γ - activating potential of a wide range of natural products originating from traditionally used medicinal plants or dietary sources **(Wang et al., 2014)**. Caffeic acid (CA) is a widespread phenolic acid that occurs naturally in many agricultural products such as fruits, vegetables, wine, olive oil, and coffee **(Mattila and Kumpulainen, 2002)**. Caffeic acid is a potent antioxidant with metal chelating properties **(Psotová et al., 2003)** as well as anti-inflammatory **(Chao et al., 2010)** and antidiabetic activities **(Gülçin, 2006)**.

Accordingly, the present study was designed to investigate the beneficial effect of Pio and CA treatments and their combination in fructose-fed rats and evaluate their effects on insulin resistance, lipid profiles and oxidative stress biomarkers and as well as liver and kidney function tests.

II. MATERIAL AND METHODS

Experimental animals

Fifty six adult male Wistar rats, weighing 120-150 g were used in the present study. Rats were housed in separated metal cages and maintained under the standard laboratory environmental conditions; temperature 25±2ºC and 12:12 h light/dark cycle with free access to food and water ad libitum. All rats were acclimatized for minimum period of one week prior to the beginning of study. All experimental protocols were approved by the ethics committee at the Faculty of Pharmacy, Cairo University.

Chemicals and drugs

Pioglitazone was kindly provided as a gift from Uni-Pharma Co (Egypt). Caffeic acid was purchased from Sigma Aldrich (USA). Each drug was suspended in 1% Tween 80 shortly before administration to animals. Fructose was purchased from El-Nasr Pharmaceutical and Chemical Industries (Cairo, Egypt). All other chemicals included were of the highest analytical grade available.

Induction of metabolic syndrome

MS was induced by feeding rats a FED for 10 weeks according to the method described by Bezerra et al. **(Bezerra et al., 2001)**.

Experimental design

After acclimatization to the laboratory conditions, the animals were randomly divided into seven groups (n=8) placed in individual cages and classified as follows:

Groups 1-3 were fed on normal diet and received 1% Tween 80 (normal control), Pio (2.7 mg/kg) and CA (20 mg/kg), respectively. Groups 4-7 were fed on FED for 15 weeks. The 4th group served as positive control group (FED-control), and the remaining groups received Pio, CA and their combination, respectively. Treatments started 10 weeks after the beginning of fructose feeding and continued for another 5 weeks.

Sampling

Blood sampling

By the end of the treatment period, animals were fasted for 12 h, weighed and blood samples were withdrawn from the retro-orbital venous plexus under light ether anesthesia for the separation of serum and plasma samples. Serum samples were used for the determination of the levels of glucose, insulin, lipids, leptin, tumor necrosis factor-alpha (TNF-α), creatinine, uric acid and urea as well as the activities of serum alanine transaminase (ALT) and aspartate aminotransferase (AST). Plasma samples were used to estimate the activity of glutathione peroxidase (GPx).

In addition, LDL-cholesterol (LDL-C) as well as indicators of insulin resistance, insulin sensitivity and β-cell function were calculated.

Homogenates sampling

Following blood samples collection, animals were anesthetized then sacrificed by decapitation. Livers and kidneys were rapidly excised and weighed. Then parts of the livers were used to prepare 10% homogenates. The prepared homogenates were used for the estimation of oxidative stress biomarkers including total antioxidant capacity (TAC) as well as the contents of lipid peroxides measured as malondialdehyde (MDA) and total nitrate/nitrite as an index of nitric oxide (NO).

Determination of the chosen parameters

Determination of body weight gain, liver and kidney indices

Body weight gain (%) was calculated using the following formula: Body weight gain (%) = Final body weight (after treatment) –Initial body weight (before treatment) / Initial body weight × 100.

Liver and kidney indices were calculated using the following formula: Organ index = Organ weight / Final body weight × 100.

Determination of serum fasting blood glucose (FBG), fasting insulin and leptin levels

FBG was measured using glucose diagnostic kit according to manufacturer's instructions based on the principle described by **Trinder (1969)**. Insulin and leptin in samples were estimated using enzyme linked immunosorbent assay (ELISA) diagnostic kits according to the method described by **Olsson and Carlsson (2005)**.

Determination of insulin resistance, insulin sensitivity and β-cell function

Insulin resistance and β -cell function were done using homeostasis model assessment (HOMA) **(Mattewes et al., 1985)**. Insulin sensitivity was calculated using quantitative insulin sensitivity check index (QISCKI) **(Katz et al., 2000)**.

Determination of serum total cholesterol, high density lipoprotein-cholesterol (HDL-C), triglyceride (TG) and LDL-C

Total cholesterol and HDL-C levels were determined using reagent kits obtained from BioChain, USA according to the methods described by **Richmond (1973)** and **Burstein et al. (1970)**, respectively. TG was determined according to the method described by **Schettler (1980)** using reagent kit obtained from Cayman Chemical Co., (USA). LDL-C was calculated according to the formula described by **Friedewald et al. (1972)**. LDL - C = Total cholesterol - HDL - (TG/5).

Determination of serum TNF-α level and ALT and AST activities

Serum TNF-α was estimated using solid phase two-site enzyme immunoassay diagnostic kit according to the method described by **Vilcek and Lee (1991)**. ALT and AST activities were determined using reagent kits obtained from Teco diagnostics (USA) according to the method of **Reitman and Frankel (1957)**.

Determination of serum urea, creatinine and uric acid levels

Urea and creatinine levels were determined using reagent kits obtained from BioAssay (USA) according to the methods described by **Fawcett and Scott (1960)** and **Bartels et al. (1972)**, respectively. Uric acid was determined according to the method of **Barham and Trinder (1972)** using reagent kit obtained from Bio Scientific Co. (USA).

Determination of oxidative stress biomarkers

Determination of plasma GPx activity and liver NO content were done using reagent kits obtained from Sigma-Aldrich (USA) according to the methods described by **Paglia and Valentine (1967)** and **Miranda et al. (2001)**, respectively. The method described by **Koracevic et al. (2001)** was used for the assessment of liver TAC content using kit obtained from Cayman (USA). MDA content of the liver was determined according to the method of **Fee and Teitelbaum (1972)** using reagent kit obtained from Cell Biolabs Inc. (USA).

Statistical Analysis

Data were expressed as means ± standard error (SE). Statistical analysis was performed using Graphpad prism software program, version 5.01 (GraphPad Softwae, Inc., San Diego, USA).

One-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test was used for

comparison of means of different groups. The level of statistical significance was set at $p < 0.05$.

III. RESULTS

Effects of Pio, CA and their combination on body weight gain as well as liver and kidney indices.

As shown in Table 1, feeding rats with FED for 15 weeks showed significant increases in body weight and liver index as compared to normal-control (NC) group. Treatment of insulin resistant rats with the combination of Pio and CA significantly decreased body weight gain by 28.86% as compared to FED-C group. Moreover, treatment of FED-fed rats with CA significantly reduced kidney index by 43.23% when compared to FED-C group (Table 1).

Effects of Pio, CA and their combination on parameters related to insulin level and insulin resistance.

As shown in Table 2, feeding rats with FED for 15 weeks showed a significant increase in insulin resistance as well as serum glucose and insulin levels as compared to NC-group. On the other hand, FED decreased insulin sensitivity and β-cell function significantly as compared to NC group. Treatment of FED-fed rats with Pio or CA alone or in combination significantly decreased serum insulin levels by 42.08%, 39.10% and 29.12%, respectively as well as FBG levels by 47.79%, 45.74% and 35.28%, successfully when compared to FED control (FED-C) group. Moreover, treatment of FED-fed rats with Pio or CA alone or in combination significantly decreased insulin resistance by 69.63%, 66.70%% and 53.79%, respectively and increased insulin sensitivity by 15.68%, 14.46% and 9.62%, respectively when compared to FED-C group. Also, results showed that treatment of FED-fed rats with Pio or CA alone significantly enhanced β-cell function by 97.79% and 85.76%, respectively as compared to FED-C group (Table 2).

Effects of Pio, CA and their combination on lipid profile.

FED significantly elevated total cholesterol, LDL-C, TG levels and TG/HDL-C ratio as compared to NC group. Moreover, FED decreased HDL-C markedly when compared to NC group. Treatment of FED-fed rats with Pio or CA alone or in combination significantly decreased TG levels by 16.47%, 26.04% and 29.26%, respectively and TG/HDL-C ratios by 49.66%, 52.35% and 58.50%, respectively as compared to FED-C group. Treatment of FED-fed rats with CA alone or in combination with Pio significantly reduced total cholesterol levels by 20.25% and 30.48%, respectively as well as LDL-C levels by 33.51% and 48.97%, respectively as compared to FED-C group (Table 3).

Effects Pio, CA and their combination on liver and kidney function tests.

FED significantly increased urea, creatinine and uric acid levels as well as ALT and AST activities when compared to NC-group. Treatment of FED-fed rats with Pio or CA alone or in combination markedly reduced urea levels by 36.77%, 26.01% and 32.92%, respectively as compared to FED-C group. Likewise, treatment of FED-fed rats with Pio alone or combined with CA significantly decreased creatinine levels by 26.44% and 14.42%, respectively as well as ALT activities by 39.01% and 26.47%, respectively and AST activities by 22.17% and 17.32, respectively as compared to FED-C group (Table 4). Moreover, administration of CA alone or in combination with Pio significantly decreased serum uric acid levels by 39.81% and 44.75%, respectively when compared to FED-C group (Table 4).

Effects of Pio, CA and their combination on leptin and TNF-α.

As shown in Figure 1, feeding rats with FED for 15 weeks showed significant increases in serum leptin and TNF-α levels when compared to NC-group. Treatment of FED-fed rats with CA alone or in combination with Pio, significantly reduced serum leptin levels by 26.91% and 36.11%, respectively as compared to FED-C group (Figure 1A). Treatment of FED-fed rats with Pio or CA alone or in combination markedly reduced TNF-α levels by 53.09%, 51.50% and 62.30%, respectively when compared to FED-C group (Figure 1B).

Table (1): Effects of Pio, CA and their combination on body weight gain serum glucose and insulin levels as well as liver and kidney indices in normal and FED-fed rats.

Groups / Parameters	NC	NC + Pio	NC + CA	FED-C	FED + Pio	FED + CA	FED + Pio + CA
Body weight gain (%)	29.28±1.57	32.52±3.46	25.71±1.34	57.03*±4.92	60.68*±4.06	43.21$^{@}$±2.33	40.57$^{\dagger@}$±3.53
Liver index (%)	2.94±0.13	2.97±0.20	2.98±0.25	4.22*±0.39	3.37±0.22	4.12±0.27	3.98±0.32
Kidney index (%)	0.53±0.08	0.40±0.05	0.48±0.04	0.77±0.09	0.52±0.08	0.44†±0.07	0.52±0.05

Results were expressed as mean ± S.E.M. (n = 8).
Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test.
∗ p <0.05 versus NC group.
† p <0.05 versus FED-C group.
@ p < 0.05 versus pioglitazone treated group.
NC, Normal control; FED-C, Fructose-enriched diet control; Pio, Pioglitazone; CA, Caffeic acid.

Effects of Pio, CA and their combination on oxidative stress biomarkers.

FED significantly decreased TAC level and GPx activity as compared to NC- group (Figure2A & B). Moreover, FED elevated MDA and NO contents significantly when compared to NC- group (Figure 2C & D). Treatment of FED-fed rats with Pio or CA alone or in combination markedly increased the activities of GPx significantly by 58.22%, 47.95% and 67.96%, respectively and decreased MDA contents by 39.26%, 68.38% and 64.16%, respectively parallel to reduced NO contents by 34.74%, 49.54% and 52.80%, respectively as compared to FED-C group (Figure2 B-D). Treatment of FED-fed rats with CA alone or combined with Pio significantly elevated TAC contents by 61.70% and 55.61%, respectively as compared to FED-C group (Figure 2A).

IV. DISCUSSION

Results of the present study revealed that feeding rats with FED for 15 weeks induced body weight gain, hyperglycemia, dyslipidemia, hyperinsulinemia, hyperuricemia, oxidative stress, pancreatic dysfunction and insulin resistance. The present results are in harmony with those of other investigators **(Alzamendi et al., 2009 and 2012)** which make this animal model a useful tool

Table (2): Effects of Pio, CA and their combination on parameters related to insulin level and insulin resistance in normal and FED-fed rats.

Groups / Parameters	NC	NC + Pio	NC + CA	FED-C	FED + Pio	FED + CA	FED + Pio + CA
Insulin resistance	2.14±0.16	2.05±0.10	2.23±0.09	15.25*±1.05	4.63*†±0.12	5.08*†±0.37	7.05$^{*\dagger@}$± 0.30
Insulin sensitivity	0.34±0.003	0.34±0.002	0.33±0.002	0.26*±0.002	0.31*†±0.001	0.30*†±0.002	0.29$^{*\dagger@}$±0.002
β-cell function	215.31±36.11	243.43±31.70	250.70±37.96	88.88*±10.47	175.80†±15.61	165.10†±13.68	129.60*±6.67
FBG (mg/dl)	82.80±3.72	75.80±4.12	83.00±3.67	195.00*±6.08	101.80*†±2.38	105.80*†±1.86	126.20$^{*\dagger@}$± 1.39
Fasting serum insulin (µIU/ml)	10.42±0.40	11.02±0.58	10.94±0.59	31.94*±2.72	18.50*†±0.75	19.45*†±1.39	22.64*†±0.98

Results were expressed as mean ± S.E.M. (n = 8).
Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test.
∗ p <0.05 versus NC group.
† p <0.05 versus FED-C group.
@ p < 0.05 versus pioglitazone treated group.
NC, Normal control; FED-C, Fructose-enriched diet control; Pio, Pioglitazone; CA, Caffeic acid; FBG, Fasting blood glucose.

to either study the underlying mechanism of such changes or to test the effectiveness of different treatment strategies.

Micronutrients such as natural antioxidants have received recently a great deal of attention with respect to their efficacy in treating the insulin-resistance syndrome complications. Caffeic acid is a phenolic acid compound which is naturally found in many plant foods such as carrots, tomatoes, strawberries and blueberries **(Sellappan et al., 2002)**. It has been documented that these phenolic acids possess anti-oxidative activities such as scavenging free radicals and chelating metal ions (**Makena and Chung, 2007; Prakash et al., 2007)**.

The present results revealed that CA significantly decreased kidney index as compared to FED-C group which might be attributed to the reduction in serum uric acid level, which in turn leads to attenuation of uric acid-induced alterations in the renal tissue.

Findings of the present study showed that treatment of insulin-resistant rats with CA for 5 weeks significantly decreased FBG, insulin and leptin levels when compared to FED-C rats, these results are in harmony with **Okutan et al. (2005) and Park and Min (2006)** who reported that CA has an antidiabetic effect in streptozotocin-induced diabetic rats. The hypoglycemic effect of CA may be due to enhanced transport of blood glucose to adipose tissue. Moreover, CA seemed to suppress the hepatic glucose output by enhancing hepatic glucose utilization and inhibiting glucose over-production **(Jung et al., 2006)**. A significant decrease in insulin by CA is due to improving peripheral insulin action and exerting beneficial effects on pancreatic β-cells **(Guerre-Millo et al., 2000; Kim et al., 2003; Bergeron et al., 2006)**. This in turn improves leptin level as it is positively correlated with the insulin level **(Cho et al., 2010)**. Likewise, CA significantly decreased insulin resistance as well as enhanced insulin sensitivity and β-cells function as compared to FED-C group. The initial adaptation to the insulin resistance is one of islet β-cell hyperplasia resulting in marked hyperinsulinemia (**Orland and Permutt, 1987**) and as mentioned above and reported by **Jung et al. (2006)**, CA decreased insulin and insulin resistance significantly which in

turn preserved β-cell architecture. The authors also reported that islet surface area in pancreas is relatively greater in CA-supplemented rats.

The present study revealed that CA decreased total cholesterol, TG and TG/HDL-ratio, significantly as well as it markedly increased serum LDL-C level when compared to FED-C group. These results are in harmony with **Chao et al. (2009) and Cho et al. (2010)**. The mechanism of CA effect on lipid profile could be attributed to the significant inhibition of fatty acid synthase, 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase and cholesterol acyltransferase (acyl-CoA) activities, while it increased fatty acid β-oxidation activity and peroxisome proliferator-activated receptors-α (PPAR α), one of nuclear transcription factors that act as lipid sensors and regulate lipid metabolism **(Willson and Wahli, 1997)** expression in the liver, which suggests that CA inhibited fatty acid and cholesterol synthesis and

Table (3): Effects of Pio, CA and their combination on lipid profile in normal and FED-fed rats.

Groups / Parameters	NC	NC + Pio	NC + CA	FED	FED + Pio	FED + CA	FED + Pio + CA
Total Cholesterol (mg/dl)	124.90±4.06	135.70±4.85	120.10±4.10	245.40*±14.74	249.00*±11.53	195.69$^{*\dagger@}$±11.56	170.63$^{*\dagger@}$±10.91
LDL-C (mg/dl)	43.59±6.32	60.42±7.04	52.58±5.39	173.10*±10.66	163.51*±3.15	115.10$^{*\dagger@}$±10.43	88.33$^{*\dagger@}$±5.61
TG (mg/dl)	76.60±4.91	64.40±5.98	49.60±4.17	195.51*±8.02	163.33*†±7..31	144.61*†±6.50	138.30*†±6.15
HDL-C (mg/dl)	66.00±2.93	62.40±4.74	57.60±5.24	33.40*±5.13	53.60±5.77	51.60±5.80	54.60±4.80
TG / HDL-C ratio	1.17±0.08	1.07±0.16	0.90±0.15	6.32*±0.77	3.18*†±0.35	3.01*†±0.49	2.62†±0.26

Results were expressed as mean ± S.E.M. (n = 8).
Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test.
∗ p <0.05 versus NC group.
† p <0.05 versus FED-C group.
@ p < 0.05 versus pioglitazone treated group.
NC, Normal control; FED-C, Fructose-enriched diet control; Pio, Pioglitazone; CA, Caffeic acid; LDL-C, Low density lipoprotein-cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein-cholesterol.

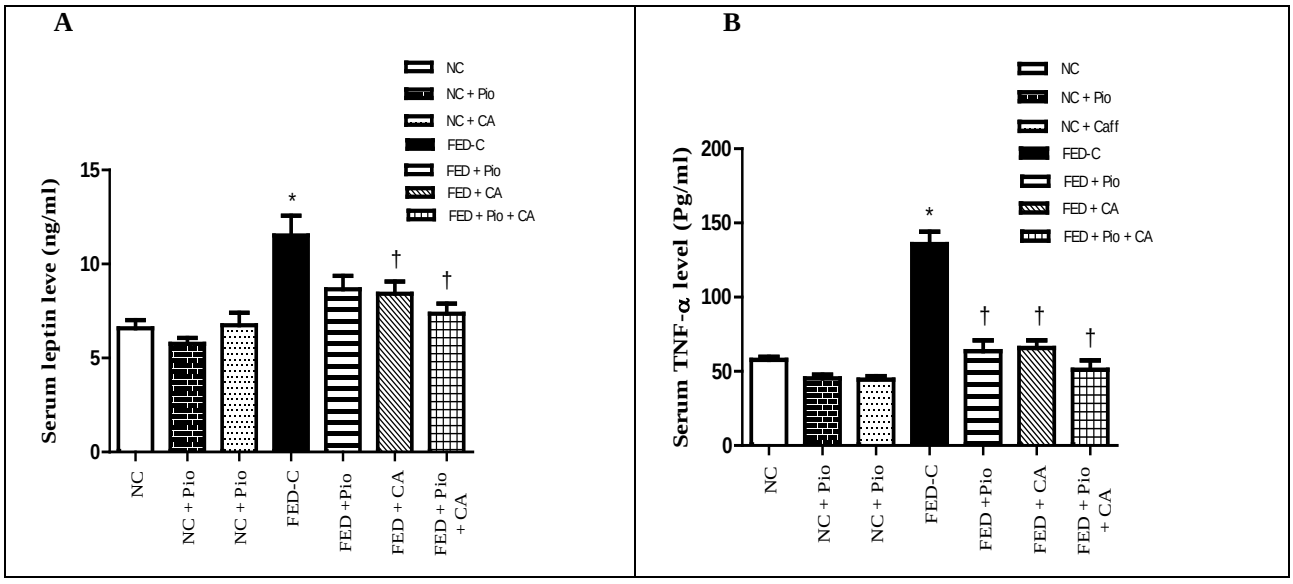

Figure (1): Effects of Pio, CA and their combination on serum levels of: A- Leptin, B- TNF-α in normal and FED-fed rats.
Results were expressed as mean ± S.E.M. (n = 8).
Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test.
* p <0.05 versus NC group.

† p <0.05 versus FED-C group.
@ p < 0.05 versus pioglitazone treated group.
NC, Normal control; FED-C, Fructose-enriched diet control; Pio, Pioglitazone; CA, Caffeic acid, TNF-α, Tumor necrosis factor- alpha

stimulated fatty acid oxidation in the liver **(Cho et al., 2010)**. Moreover, CA significantly, reduced serum TNF-α level as compared to FED-C group, this result is in accordance with **Guerriero et al. (2011)**, who reported that CA decreased the production of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-8 and the increased that of anti-inflammatory cytokines such as IL-10.

Likewise, CA reduced serum urea and uric acid significantly as compared to FED-C group. The present results are in line with that of **Rehman and Sultana (2011)**. In addition, **Pari and Karthikesan (2007)** reported that CA seems to maintain the structural integrity of liver and kidney membrane against alcohol challenge. Moreover, ferulic acid, a metabolite of CA significantly reduced the alcohol-induced toxicity in rats **(Rukkumani et al., 2004)**.

In the current study, CA significantly elevated plasma GPx activity as well as TAC in liver tissue. In addition it reduced MDA and NO contents in liver tissue. These results are in accordance with those of **Rehman and Sultana (2011) and Chao et al. (2009)**. CA has the ability to scavenge the free radicals **(Jung et al., 2006)** and attenuate the lipid peroxidation as indicated by increased levels of enzymatic antioxidants in the liver **Özyurt et al., (2006)** via the ability of its phenolic hydroxyl group to quench free radicals **(Devipriya et al., 2008**). In addition, CA may also act as a chain breaking antioxidant, thereby terminating the chain reaction of lipid peroxidation and minimizing its deleterious effects **(Nardini et al., 1998)**.

Treatment of insulin resistant rats with Pio was associated with significant decreases in serum glucose and insulin levels as well as improved insulin sensitivity when compared to FED-C group; these results are in harmony with **Schaalan (2012) and Singh et al. (2014).** Pioglitazone caused a reduction in serum glucose levels in various animal species by acting as an insulin sensitizer **(Murakami et al., 1998)**. Moreover, the positive effect of Pio on glucose was previously documented in several insulin resistance models **(Ding et al., 2005; Vijay et al., 2009)** and can be attributed to its agonistic activity on PPARγ which impart metabolic effects that ultimately mitigate insulin resistance in adipose tissues and liver **(Westerink and Visseren, 2011)**.

Likewise, Pio enhanced β-cell function significantly as compared to FED-C group; this result is in harmony with that of **González-Ortiz et al. (2007)**.

PPAR-γ agonist protects pancreatic islets under conditions of islet fat accumulation from lipotoxicity by regulating TG partitioning among tissues **(Matsui et al., 2004)**. This opinion supports the present results about TG, as Pio significantly reduced serum TG with no significant changes in serum HDL-C, LDL-C and total cholesterol levels. Similar results have been reported by **Bhosale et al. (2013)**.

The present study revealed that treatment of FED-fed rats with Pio nearly normalized serum TNF-α. Similarly, **Collino et al. (2010)** reported that daily administration of Pio to high cholesterol and fructose diet-rats restored TNF-α to the basal levels.

The present study showed that treatment of FED-fed rats with Pio significantly decreased serum AST and ALT activities as compared to FED-fed rats. Likewise, **Shokouh et al. (2013)** reported that Pio has beneficial effects on both ALT and AST activities in non-diabetic MS patients. This beneficial effect of Pio could be explained by its anti-inflammatory effect mediated by activation of PPARγ on macrophages **(Awara et al., 2005)** resulting in decreased production of the pro-inflammatory cytokines as TNF-α. Moreover, Pio significantly decreased serum urea and creatinine levels in insulin resistant-rats when compared to FED-control group, these results are in harmony with those of **Grover and Bafna (2013) and Peng et al. (2014)**.

The current results have confirmed the potential antioxidant effect of Pio in MS. It was found that treating insulin-resistant rats with Pio was accompanied by a marked reduction in NO and MDA contents in the liver and a significant elevation in plasma GPx activity. These results are in harmony with **Rabbani et al. (2010) and Vandana et al. (2014)**. Antioxidant activity of Pio is reported to be mediated by blocking the vicious cycle of ROS production, improving insulin sensitivity and halting the pro-inflammatory signaling transduction **(Hsiao et al., 2008)**.

The present results revealed that the combination of CA with Pio in FED-fed rats significantly reduced body weight and serum total cholesterol, LDL-C as well as uric acid levels as compared to Pio-treated group.

In the same pattern, the combination of CA and Pio had a marked antioxidant effect as it significantly elevated TAC and reduced MDA contents, significantly when compared to Pio-treated group.

The present results provide an evidence that both drugs enhance the effect of each other on MS symptoms as well as oxidative stress biomarkers.

The current results are supported by those of **Kumar et al. (2010)**, who reported that combination of Pio and CA produces better results as compared to each of them alone.

V. Conclusions

In conclusion, CA alone or combined with Pio, could improve certain metabolic risk factors in experimentally induced MS by reducing glucose, insulin resistance as well as oxidative stress and inflammatory biomarkers. Further studies are warranted to validate the conclusions.

Table (4): Effects of Pio, CA and their combination on liver and kidney function tests in normal and FED-fed rats.

Group / Parameters	NC	NC + Pio	NC + CA	FED-C	FED + Pio	FED + CA	FED + Pio + CA
Urea (mg/dl)	14.61±0.66	12.34±0.56	13.88±0.70	30.68*±1.17	19.40*†±1.07	22.70*†±1.14	20.58*†±1.80
Creatinine(mg/dl)	0.48±0.02	0.42±0.03	0.52±0.02	0.83*±0.02	0.61*†±0.025	0.74*@±0.03	0.71*†±0.01
Uric acid (mg/dl)	3.76±0.22	3.86 ±0.17	3.02±0.18	6.48*±0.44	5.40*±0.48	3.90†±0.35	3.58†@±0.34
ALT (U/L)	37.60±2.48	32.83±3.04	41.76±3.40	85.23*±7.11	51.98†±5.74	71.63*±5.08	62.67*†±4.32
AST (U/L)	61.30±3.22	59.55±2.78	65.39±3.25	157.02*±6.39	122.20*†±6.04	140.40*±7.71	129.81*†±5.18

Results were expressed as mean ± S.E.M. (n = 8).
Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test.
∗ p <0.05 versus NC group.
† p <0.05 versus FED-C group.
@ p < 0.05 versus pioglitazone treated group.
NC, Normal control; FED-C, Fructose-enriched diet control; Pio, Pioglitazone; CA, Caffeic acid; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.

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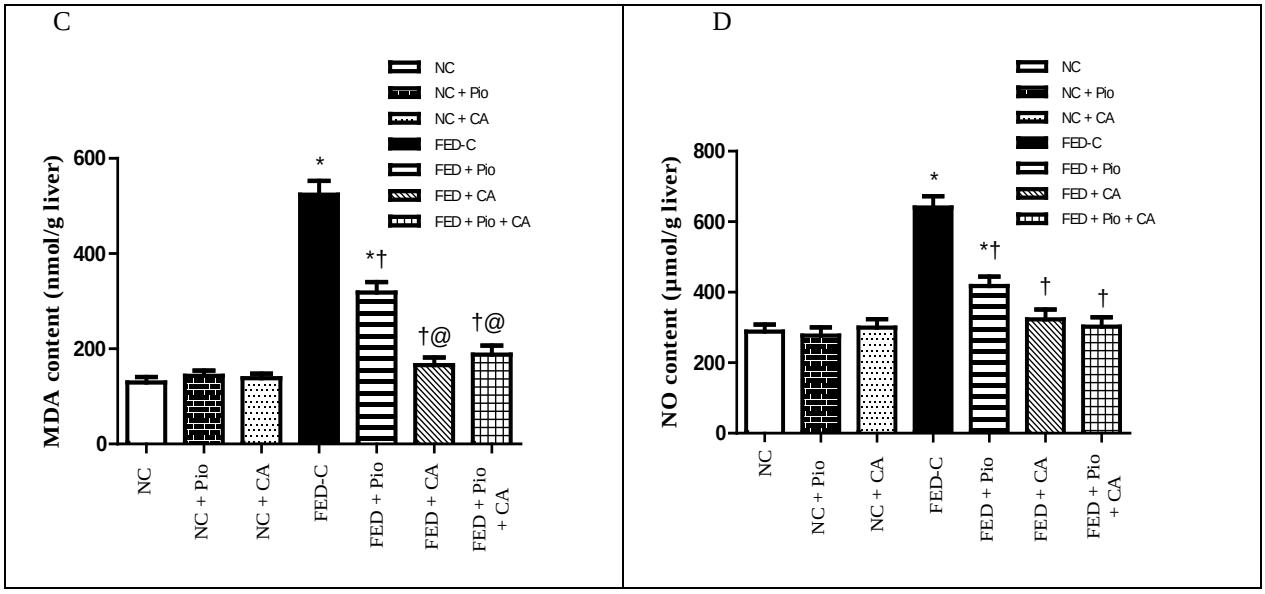

Figure (2): Effects of Pio, CA and their combination on: A- TAC content, B- GPx activity, C- MDA and D- NO contents in normal and FED-fed rats.

Results were expressed as mean ± S.E.M. (n = 8).
Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparison test.
∗ p <0.05 versus NC group.
† p <0.05 versus FED-C group.
@ p < 0.05 versus pioglitazone treated group.
NC, Normal control; FED-C, Fructose-enriched diet control; Pio, Pioglitazone; CA, Caffeic acid, TAC, Total antioxidant capacity, GPx, Glutathione peroxidase, MDA, Malondialdehyde, NO, Nitric oxide.