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On the Protective Effects of IMOD and Silymarin Combination in a Rat Model of Acute Hepatic Failure Through Anti Oxidative Stress Mechanisms

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ABSTRACT

Hepatoprotective effect and mechanisms of a novel selenium/electromagnetically treated multiherbal mixture named Setarud (IMOD™) in combination with silymarin (SM) a known hepatoprotective compound was investigated in acetaminophen-induced acute hepatic failure rat model. Animals were divided into five groups and pre-induced with phenobarbital (0.1 mg kg^{-1}, i.p.) before administration of a single dose of acetaminophen (1 g kg^{-1}, i.p.) except group 1 which was considered as normal. Group 2 was remained without treatment and considered as control while groups 3 to 5 were treated with SM (50 mg kg^{-1}, p.o.), IMOD (30 mg kg^{-1}, i.p.) and IMOD+SM, respectively 24 h post administration of acetaminophen. Blood was collected at 0, 24 and 72 h post acetaminophen treatment. Elevated serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) confirmed hepatic failure induced by acetaminophen. After 48 h of treatment, the rats were anesthetized and the liver was removed and the right lobule was homogenized and then measured for catalase (CAT), malondialdehyde (MDA) and glutathione (GSH) value. Part of liver was left in paraffin for histopathology examination. CAT and GSH were significantly decreased in the acetaminophen-treated group while ALT, AST, ALP and MDA increased when compared to normals. Histopathological examination of acetaminophen-treated animals showed necrosis, inflammation, hyperplasia of kupffer and infiltration of mononuclear cells, dilation of sinusoids and disruption of hepatocytes, while treatment with IMOD+SM normalized protected hepatic architecture in accordance to biochemical results. Treatment of animals with IMOD and SM alone or in combinations considerably protected the hepatic failure by diminishing ALT, AST, ALP and MDA. Both IMOD and SM and their combination improved acetaminophen-induced histopathological hepatic damage. Conclusion is that combination of IMOD and SM considerably protect from acute hepatic failure via enzymatic and non-enzymatic mechanisms.

Key words: Acute hepatic failure, IMOD, silymarin, oxidative stress, cell damage

INTRODUCTION

Use of natural medicines was increased in the world because of low adverse effects, price and good efficacy in most human illnesses. Recent reviews of new experimental and clinical studies have

confirmed traditional thoughts about usefulness of most herbal medicines (Hasani-Ranjbar *et al.*, 2009). The novel natural drug Setarud (IMOD™) during it's around 6-year history of registration has shown promising effects in various inflammatory diseases especially through anti oxidative stress mechanisms as reviewed by Mohammadirad *et al.* (2011). IMOD is a combination of three ethanolic extracts of Rosa canina fruit, Tanacetum vulgar leaves and Utrica dioica leaves that were combined with selenium and urea and then exposed to a pulsed electromagnetic field (Novitsky *et al.*, 2007).

Silymarin (SM) is also a known natural compound with potent anti oxidative effects in various illnesses especially liver failure (Hasani-Ranjbar *et al.*, 2009).

Acetaminophen-induced hepatic failure is one of the best models for evaluating the hepatoprotective effect of natural and chemical compounds. Acetaminophen is metabolized by CYP450 to toxic compound N-acetyl-p-benzoquinoneimine (NAPQI) which causes oxidative damage and elevation of cellular lipid peroxidation and inadequacy of glutathione (GSH) to overcome free radical damage. Hepatic failure that is nowadays one of the most common debilitating illnesses of human (Khashab *et al.*, 2007) that may result in acute illness. Unfortunately, there is no exact cure for hepatic failure and thus it may directly lead to death.

Damage to the structural integrity of the liver is concluded from elevated of aspartat aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) in the blood as well as hepatic malondialdehyde (MDA) and glutathione oxide (GSSG). Defense mechanisms were performed through elevation of catalase (CAT) activity and restoration of GSH in the affected tissue. Considering above points we were interested to examine efficacy of IMOD and SM combination in a rat model of acetaminophen-induced acute hepatic failure.

MATERIALS AND METHODS

Chemicals: All chemicals were purchased from Merck Chemical Company (Darmstadt, Germany) unless otherwise stated. Enzymatic kits for AST, ALT, ALP and CAT were purchased from Ellitech diagnostic kits (Sees, France). Prepared IMOD and SM solutions were obtained from Parsrus Research Co. (Tehran, Iran). Acetaminophen and phenobarbital powders were obtained from local Pharmaceutical companies.

Animals: Rats (185-200 g) were obtained from Animal House of Faculty of Pharmacy, Tehran University of Medical Science and quarantined for 1 week prior to use. The animals were kept under controlled environmental conditions of temperature (25°C), relative humidity of 50-55% and 12-h light/dark cycle. Animals had free access to stock laboratory diet and water.

Induction of acute hepatic failure: Acetaminophen was administered in a single dose of 1 g kg^{-1} via intraperitoneal (i.p.) injection. Four days prior to the injection of acetaminophen, animals received phenobarbital in a dose of 0.1 mg kg^{-1} (i.p.) to induce cytochrome P-450 enzymes and therefore potentiating subsequent hepatic failure by acetaminophen.

Experimental procedure: Animals were randomly divided into five groups, seven in each and pre-induced with phenobarbital (0.1 mg kg^{-1}, i.p.) before administration of a single dose of

acetaminophen (1 g kg^{-1}, i.p.) except group 1 which was considered as normal. Group 2 was remained without treatment and assigned as control. The groups 3 to 5 were treated with SM (50 mg kg^{-1}, p.o.), IMOD (30 mg kg^{-1}, i.p.) and combination of IMOD+SM, respectively 24 h post administration of acetaminophen. Effective doses of IMOD and SM were selected from authors' previous studies (Baghaei *et al*., 2010; Miroliaee *et al*., 2011). Blood samples at 0, 24 and 72 h post administration of acetaminophen were obtained from the rats under anesthesia with combination of ketamine (100 mg kg^{-1}) and xylosine (10 mg kg^{-1}, i.p.). The serum was separated and stored for biochemical assays. After killing animals by maximum ether inhalation, right lobule of the liver was dissected free, removed and fixed in 10% formalin for histopathology assay. Other part of the liver was homogenized and stored at -20°C for further assays.

Determination of biochemical parameters: ALT, ALP, AST and CAT were measured by kits according to the manufacturer brochure. The lipid peroxidation in hepatic homogenate was determined by the use of the method described by Esterbauer and Cheeseman (1990). The amount of MDA formed was measured at the wavelength 532 nm by reaction with thiobarbituric acid (TBA) as the red colored complex.

Liver GSH and GSSG were determined on the basis of production of a yellow color (colorimetric technique) using 5, 5-dithiobis nitrobenzoic acid (DTNB) as reagent. The method has been described previously (Soltan-Sharifi *et al*., 2007). Protein concentration was determined by Bradford reagent.

Histopathological examination: The fixed liver in 10% formalin was processed including embedded in paraffin, rehydrated and cut into 3 μm-thick sections using a rotary microtome. The sections were stained with hematoxylin-eosin (H and E) dye and mounted with Canada balsam and then evaluation by light microscope.

Statistical analysis: The data were expressed as Mean±SD and then compared using one way Analysis of Variance (ANOVA). Comparisons among groups were made according to Tukey's multiple comparisons test. The significance level was tested at $p<0.05$.

RESULTS

Results of serum AST, ALT and ALP activities following acetaminophen treatment are demonstrated in Table 1 and 3. A significant increase in the activities of AST, ALT and ALP were shown in acetaminophen group when compared to controls. The acetaminophen-induced elevation of AST, ALT and ALP was significantly restored following treatment with IMOD, SM or their combination. The best result was obtained from IMOD+SM. The combination of IMOD+SM decreased ALT more than SM ($p = 0.037$) or IMOD ($p = 0.017$). The same better effect of IMOD+SM was seen for AST in comparison to SM ($p = 0.036$) or IMOD ($p = 0.47$) and the same for ALP when compared to SM ($p = 0.34$) and IMOD ($p = 0.42$).

Figure 1-4, respectively show the total GSH, GSSG, MDA and CAT changes in the hepatic homogenate. Acetaminophen induced a significant increase in GSH ($p<0.001$) in comparison to normal group (Fig. 1). GSSG and MDA were significantly increased ($p<0.001$) by acetaminophen in comparison to normal group (Fig. 2, 3). However, treatment with IMOD, SM and their combination restored GSH and significantly reduced GSSG and MDA. Furthermore, IMOD and SM in combination were much better in restoring the GSH when compared to IMOD ($p = 0.018$)

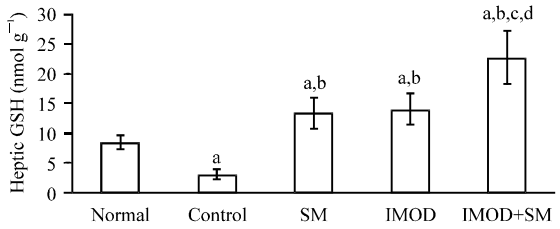

Fig. 1: Changes in hepatic glutathione (GSH). Data are Mean±SD of seven animals. [a]p<0.001, significantly different when compared with normal [b]p<0.001, significantly different from control group. [c]p<0.05 significantly different when IMOD+SM was compared with SM. [d]p<0.05 significantly different when IMOD+SM was compared with IMOD

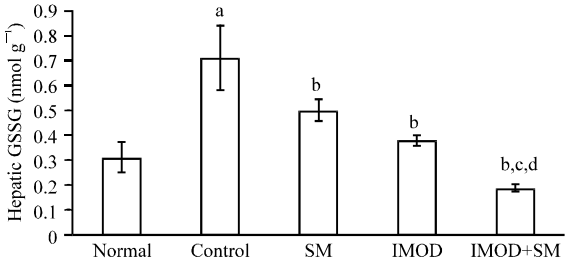

Fig. 2: Changes in hepatic glutathione oxide (GSSG). Data are Mean±SD of seven animals. [a]p<0.001, significantly different when compared with normal [b]p<0.001, significantly different from control group. [c]p<0.05 significantly different when IMOD+SM was compared with SM. [d]p<0.05 significantly different when IMOD+SM was compared with IMOD

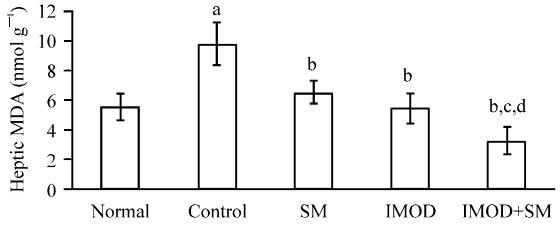

Fig. 3: Changes in hepatic malondialdehyde (MDA). Data are Mean±SD of seven animals. [a]p<0.001, significantly different when compared with normal [b]p<0.001, significantly different from control group. [c]p<0.05 significantly different when IMOD+SM was compared with SM. [d]p<0.05 significantly different when IMOD+SM was compared with IMOD

Table 1: Time course changes in serum alanine aminotransferase

Group	0 h	24 h	72 h
Normal	70.01±4.71	74.42±4.59	70.29±5.48
Control	72.50±6.24	287.26±15.13[a]	227.54±13.77[a]
SM	71.73±6.13	282.17±12.44[a]	97.32±9.84[b]
IMOD	69.16±5.68	283.37±11.81[a]	92.66±8.08[b]
IMOD+SM	70.37±5.28	284.17±13.29[a]	72.28±7.37[bcd]

Data are Mean±SD of seven animals. [a]p<0.001, significantly different when compared with normal. [b]p<0.001, significantly different from control group. [c]p<0.05 significantly different when IMOD+SM was compared with SM. [d]p<0.05 significantly different when IMOD+SM was compared with IMOD

Table 2: Time course changes in serum aspartate aminotransferase

Group	0 h	24 h	72 h
Normal	65.39±4.84	68.32±4.36	66.78±5.29
Control	69.44±5.13	178.11±12.37[a]	161.81±10.55[a]
SM	67.52±5.28	169.34±10.48[a]	86.52±.6.36[b]
IMOD	66.17±5.36	171.40±11.37[a]	84.06±7.17[b]
IMOD+SM	67.28±4.79	173.67±11.17[a]	62.48±6.29[bcd]

Data are Mean±SD of seven animals. [a]p<0.001, significantly different when compared with normal. [b]p<0.001, significantly different from control group. [c]p<0.05 significantly different when IMOD+SM was compared with SM. [d]p<0.05 significantly different when IMOD+SM was compared with IMOD

Table 3: Time course changes in serum alkaline phosphatase

Group	0 h	24 h	72 h
Normal	51.23±5.71	49.32 4.36	54.27±4.83
Control	54.57±5.31	141.72±10.43[a]	127.22±9.87[a]
SM	52.14±4.87	137.15±9.72[a]	75.51±9.18[b]
IMOD	55.19±5.93	139.03±10.38[a]	73.01±8.48[b]
IMOD+SM	50.43±4.22	143.23±11.06[a]	46.25±5.31[bcd]

Data are Mean±SD of seven animals. [a]p<0.001, significantly different when compared with normal. [b]p<0.001, significantly different from control group. [c]p<0.05 significantly different when IMOD+SM was compared with SM. [d]p<0.05 significantly different when IMOD+SM was compared with IMOD

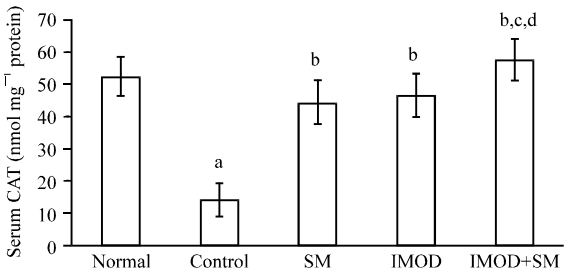

Fig. 4: Changes in serum catalase (CAT). Data are Mean±SD of seven animals. [a]p<0.001, significantly different when compared with normal [b]p<0.001, significantly different from control group. [c]p<0.05 significantly different when IMOD+SM was compared with SM. [d]p<0.05 significantly different when IMOD+SM was compared with IMOD

or SM (p = 0.011). The combination of IMOD and SM reduced GSSG and MDA more than that of IMOD (p<0.001, p = 0.039) or SM (p = 0.47, p = 0.021).

As shown in Fig. 4, CAT was reduced by acetaminophen treatment in comparison to normal group (p<0.001) while treatment with IMOD and SM alone or in combination restored CAT activity. Optimum result was observed with IMOD+SM better than that of IMOD or SM groups (p = 0.43, p = 0.013, respectively).

Histopathology results: Figure 5 demonstrates necrosis, inflammation, disrupter of hepatocytes, hyperplasia of kupffer cells and infiltration of mononuclear cells and increased diameter of hepatocytes in liver of rats treated with acetaminophen. Treatment with IMOD, SM and/or their combination markedly maintained the normal architecture of liver sections. Of course, no significant changes were distinguishable between IMOD, SM or their combination.

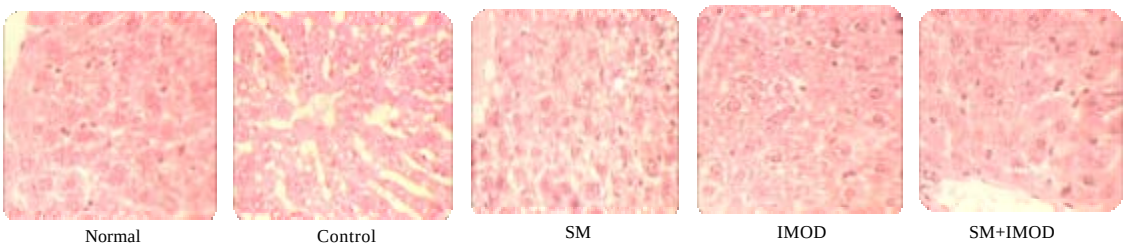

Fig. 5: Hepatic histopathology images, normal architecture i.e., normal limited plate in a classic lobule and normal central vein with radiating hepatocyte are evident in normal group, in the control group, necrosis, inflammation, degeneration, hyperplasia of kupffer cell with infiltration of mononuclear cell and disruption of radiated hepatocyte, limited plate of lobule and dilation of sinusoids are evident, in groups of SM, IMOD and their combination, a mild structure change of liver with presence of mild inflammation and decreased mononuclear cell and kupffer cell hyperplasia are evident. Magnification for all images was 400x

DISCUSSION

In the present study, we showed for the first time the hepatoprotective effect of IMOD alone or in combination with SM. Also we specified the biochemical mechanisms by which this combination works in acute hepatic failure. Furthermore, histopathological findings confirmed positive biochemical changes in favor of protection of acute hepatic failure.

As mentioned in introduction, the metabolite of acetaminophen (NAPQI) reacts with GSH and reduces its store in body which results in an oxidative damage mediated by Reactive Oxygen Species (ROS). The MDA is one of the lipid peroxides that is produced after reaction of ROS with cellular lipids. In general, the effects of ROS can be controlled by a wide spectrum of enzymatic and non-enzymatic defense mechanism including the enzyme CAT or restoration of GSH (Abdollahi *et al*., 2004). Therefore, measuring MDA, GSH and CAT are useful to decide about rate of tissue toxicity. Hepatic failure shows itself as elevated blood level of ALT, AST and ALP happening by administration of acetaminophen in the present study. The present results also indicated the synergistic effects of IMOD and SM that is mediated through their anti oxidative effects.

IMOD is famous for its strong anti-oxidative effects as evidenced in various oxidant-related disease models (Mohammadirad *et al*., 2011). The components of IMOD have previously demonstrated good anti oxidative stress effects when used alone (Khorram-Khorshid *et al*., 2008; Mehri *et al*., 2011; Babaie *et al*., 2007). Selenium the another component of IMOD plays an important role in protective metallo-enzymes including Glutathione Peroxidase (GPx) and superoxide dismutase (SOD) that work with CAT in protection against oxidative stress and ROS (Pourkhahli *et al*., 2011). Therefore, there is no doubt that IMOD mediates its effects by reduction of oxidative stress biomarkers as previously confirmed in various disease models including diabetes (Mohseni-Salehi-Monfared *et al*., 2010), colitis (Baghaei *et al*., 2010), pancreatic Langerhans islet transplant (Larijani *et al*., 2011), acute sepsis (Mahmoodpoor *et al*., 2010), oral lichen planus, (Agha-Hosseini *et al*., 2011) and polycystic ovary syndrome (Rezvanfar *et al*., 2011).

On the other hand, antioxidant effects of SM have been confirmed for many years. SM, a flavolignan component of Silybium marianum seeds, has been studied extensively for treatment

of hepatic diseases (Das and Vasudevan, 2006). This beneficial property of SM is derived from its ability to scavenge free radicals and chelation of metal ions. Hopefully, SM has been reconsidered in the recent years and its efficacy in inflammatory oxidative-mediated diseases like colitis has been confirmed (Esmaily *et al*., 2009; Malihi *et al*., 2009; Miroliaee *et al*., 2011).

The present study showed that generation of free radicals is a major determinant involved in the acetaminophen-induced acute hepatic failure. The free radicals and further lipid peroxidation went through mitochondria where cause further toxicity. Thus additive antioxidant properties of SM in combination with IMOD as shown by their effects on GSH, GSSG, MDA and CAT in acetaminophen-treated animals suggest that administration of this combination results in easier reaching to mitochondria and improving hepatic cells antioxidant capacity.

Therefore all evidences support the benefit of IMOD+SM combination in acute hepatic failure. The next step is to trial this mixture in patients with acute, subacute or chronic hepatic failure.

REFERENCES

Abdollahi, M., A. Ranjbar, S. Shadnia, S. Nikfar and A. Rezaie, 2004. Pesticides and oxidative stress: A review. Med. Sci. Monit., 10: RA141-RA147.

Agha-Hosseini, F., I. Mirzaii-Dizgah, M. Abdollahi and N. Akbari-Gillani, 2011. Efficacy of IMOD in the treatment of oral lichen planus. Open J. Stomatol., 1: 13-17.

Babaie, M., N. Yasa, A. Mohammadirad, R. Khorasani and M. Abdollahi, 2007. *In vivo* antioxidant potentials of rosa damascene petal extract from Guilan, Iran, comparable to α-tocopherol. Int. J. Pharmacol., 3: 510-514.

Baghaei, A., H. Esmaily, A.H. Abdolghaffari, M. Baeeri, F. Gharibdoost and M. Abdollahi, 2010. Efficacy of setarud (IMOD), a novel drug with potent anti-toxic stress potential in rat inflammatory bowel disease and comparison with dexamethasone and infliximab. Indian J. Biochem. Biophys., 47: 219-226.

Das, S.K. and D.M. Vasudevan, 2006. Protective effects of silymarin, a milk thistle (*Silybium marianum*) derivative on ethanol-induced oxidative stress in liver. Indian J. Biochem. Biophys., 43: 306-311.

Esmaily, H., A. Hosseini-Tabatabaei, R. Rahimian, R. Khorasani and M. Baeeri *et al*., 2009. On the benefits of silymarin in murine colitis by improving balance of destructive cytokines and reduction of toxic stress in the bowel cells. Cent. Eur. J. Biol., 4: 204-213.

Esterbauer, H. and K.H. Cheeseman, 1990. Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. Methods Enzymol., 186: 407-421.

Hasani-Ranjbar, S., B. Larijani and M. Abdollahi, 2009. A systematic review of the potential herbal sources of future drugs effective in oxidant-related diseases. Inflamm. Allergy Drug Targets, 8: 2-10.

Khashab, M., A.J. Tector and P.Y. Kwo, 2007. Epidemiology of acute liver failure. Curr. Gastroenterol. Rep., 9: 66-73.

Khorram-Khorshid, H.R., J.A. Azonov, Y.A. Novitsky, B. Farzamfar and M.H. Shahhosseiny, 2008. Hepatoprotective effects of setarud against carbon tetrachloride-induced liver injury in rats. Indian J. Gastroenterol., 27: 110-112.

Larijani, B., M. Salimi, N. Pourkhalili, A. Mohammadirad, M. Baeeri, A. Nili-Ahmadabadi and M. Abdollahi, 2011. Positive response of isolated rat pancreatic islets to IMOD; hopes for better transplant outcome and graft function. Asian J. Anim. Vet. Adv., 6: 1019-1025.

Mahmoodpoor, A., M. Mojtahedzadeh, A. Najafi, A. Ahmadi and A. Dehnadi-Moghadam *et al.*, 2010. Examination of setarud (IMOD™) in the management of patients with severe sepsis. DARUJ. Pharm. Sci, 18: 23-28.

Malihi, F., A. Hosseini-Tabatabaei, H. Esmaily, R. Khorasani, M. Baeeri and M. Abdollahi, 2009. Improvement of inflammatory and toxic stress biomarkers by silymarin in a murine model of type one diabetes mellitus. Cent. Eur. J. Biol., 4: 369-380.

Mehri, A., S. Hasani-Ranjbar, B. Larijani and M. Abdollahi, 2011. A systematic review of efficacy and safety of *Urtica dioica* in the treatment of diabetes. Int. J. Pharmacol., 7: 161-170.

Miroliaee, A.E., H. Esmaily, A. Vaziri-Bami, M. Baeeri, A.R. Shahverdi and M. Abdollahi, 2011. Amelioration of experimental colitis by a novel nanoselenium-silymarin mixture. Toxicol. Mech. Methods, 21: 200-208.

Mohammadirad, A., H.R. Khorram-Khorshid, F. Gharibdoost and M. Abdollahi, 2011. Setarud (IMOD™) as a multiherbal drug with promising benefits in animal and human studies: A comprehensive review of biochemical and cellular evidences. Asian J. Anim. Vet. Adv., 6: 1185-1192.

Mohseni-Salehi-Monfared, S.S., E. Habibollahzadeh, H. Sadeghi, M. Baeeri and M. Abdollahi, 2010. Efficacy of Setarud (IMOD™), a novel electromagnetically-treated multi-herbal compound, in mouse immunogenic type-1 diabetes. Arch. Med. Sci., 6: 663-669.

Novitsky, Y., H. Madani, F. Gharibdoust, M. Farhadi, B. Farzamfar and M. Mohraz, 2007. European patent application. EP Patent No. EP 2 087 825 A3.

Pourkhalili, N., A. Hosseini, A. Nili-Ahmadabadi, S. Hassani and M. Pakzad *et al.*, 2011. Biochemical and cellular evidence of the benefit of a combination of cerium oxide nanoparticles and selenium to diabetic rats. World J. Diabetes, 2: 204-210.

Rezvanfar, M.A., A. Ahmadi, M. Baeeri, A. Kebriaeezadeh and M. Abdollahi, 2011. Pioglitazone ameliorates letrozole-induced hyperandrogenism and oxidative stress-mediated polycystic ovary in female rat. Toxicol. Lett., 205: S247-S247.

Soltan-Sharifi, M.S., M. Mojtahedzadeh, A. Najafi, M.R. Khajavi and M.R. Rouini *et al.*, 2007. Improvement by N-acetylcysteine of acute respiratory distress syndrome through increasing intracellular glutathione and extracellular thiol molecules and antioxidant power: Evidence for underlying toxicological mechanisms. Hum. Exp. Toxicol., 26: 697-703.