ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research

Available Online at www.jbpr.in

CODEN: - JBPRAU (Source: - American Chemical Society)

Volume 5, Issue 3: May-June: 2016, 10-17

Research Article

COMPARISON OF THE THERAPEUTIC ACTIONS OF TAURINE, METHYLSULFONYLMETHANE AND SILYMARIN AGAINST ACETAMINOPHEN-INDUCED NEURO- AND HEPATO-TOXICITY IN ADULT MALE ALBINO RATS.

Omyma K. Radwan and Ahmed M. Shehata

Physiology department, National Organization for Drug Control and Research, Giza, Egypt

Received 10May 2016; Accepted 26 May 2016

ABSTRACT

To evaluate the neuro- and hepato-curative effects of taurine (TAU) and methylsulfonylmethane (MSM) in acetaminophen- induced neuro- and hepato- toxicity in adult male albino rats. Silymarin (SLN) was used as reference drug. **Methods**: A total of 40 albino rats were assigned into five groups of 8 rats in each group. Rats was administered a single daily dose of acetaminophen (APAP, 500 mg/kg body weight, p.o) for 14 days. Acetaminophen treated rats were divided into four groups, the positive control group, taurine treated group, MSM treated group and silymarin treated group. The Acetaminophen- intoxicated animals were treated with the tested drugs for two weeks. A group of untreated animals served as negative control. **Results**: There was significant ($P<0.05$) increase in levels of liver marker enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the actaminophen- treated rats, compared to the normal control. Moreover, acetaminophen induced oxidative stress in both liver and brain tissues in terms of increased MDA, GSSG and NO contents and decreased GSH; and caused oxidative DNA damage in terms of elevation of 8-hydroxy-2-deoxyguanine (8-OHdG). In brain, acetaminophen (APAP) moderately disturbed the normal levels of DA, NE and serotonin. Both silymarin, taurine and MSM normalized the serum activities of liver marker enzymes (ALT and AST) and restored the normal redox status and ameliorated acetaminophen induced DNA- oxidative damage in liver. In addition, both silymarin, taurine and MSM restored the normal redox status and the normal levels of brain monoamines (DA, NE and 5-HT) and ameliorated acetaminophen induced DNA- oxidative damage in brain. The efficiency of neuro- and hepatocurative effect was SLN> TAU > MSM.

Conclusion: the study shows that taurine, silymarin and MSM possess significant neuro- and hepato-curative attribute due to their antioxidant properties.

Keywords: Acetaminophen- oxidative stress-liver- brain- silymarin- taurine- MSM.

INTRODUCTION:

Paracetamol (APAP) is one of the most commonly used drugs for the treatment of pain and fever in adults and children, available without prescription, and one of the most prescribed drugs in hospitals [1,2]. In addition, paracetamol also may constitute an environmental pollutant as it is readily detected in the sewage effluent water and rivers in most areas of the world [3,4].

Despite its established safety profile at therapeutic doses, in overdose, acetaminophen is the leading cause of acute liver failure, hepatic necrosis that can be fatal [5-7]. Although recent studies indicated the implication of cellular redox changes and mitochondrial dysfunction in acetaminophen induced hepatotoxicity, however, the precise mechanisms of hepatocyte death are not fully understood.

In this context it is very important to pursue searching for effective curative remedy for acetaminophen-induced toxicity. Taurine (TAU) is an sulfonic acid containing amino group, that has a role in the regulation of oxidative stress and promoting mitochondrial normal functions [8], and possesses a number of cytoprotective properties through its actions as an antioxidant, osmoregulator, and intracellular calcium flux regulator [9,10]. Moreover,taurine plays multiple roles in the CNS including acting as a neuromodulator, a trophic factor in development

*Corresponding author: Omyma K Radwan| E-mail: Omymaradwan@yahoo.com/Mobile No.: 202 01006973291

and as a neuroprotectant against excitotoxicity [10].

Besides, methylsulfonylmethane (MSM) is a natural organosulfur compound that exhibits antioxidant where it is found in a wide range of human foods including fruits, vegetables, grains, and beverages [11]. Consistently, the anti-inflammatory effect of MSM on lipopolysaccharide-induced inflammatory responses in murine macrophages [12] and on experimental colitis in rats [13]. Nevertheless, MSM exhibited anti-oxidant effect on pitting edema [14], and exercise induced oxidative stress [15]. It was reported that MSM may be used as a precursor for the synthesis of methionine and cysteine, sulfur containing amino acids, and act as a source of sulfur [16].

Silymarin (SIL), a plant secondary metabolite, is a natural compound that is present in species derived from *Silybum marianum*, the scientific name for Milk thistle. The plant contains at least seven flavolignans and the flavonoid taxifolin. Silymarin is a complex mixture of four flavonolignan isomers, namely, silybin (60–70%), silychristin (20%), silydianin (10%), and isosilybin (5%) [17]. The most important flavolignan present include silybin. Silymarin has been reported to have antioxidative, anti-inflammatory, immunomodulatory, antilipid, liver-regenerating properties and neuroprotective potentials [17-19]. Silymarin has been reported to reduce the elevated levels of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) found in liver injuries and chronic diseases and liver fibrosis [20]. So, it has been used worldwide for many years as a complementary alternative medicine because of its beneficial effects associated with the treatment of hepatic diseases [19].

Materials and Methods

Chemicals:

Acetaminophen, Methylsulfonylmethane, taurine and silymarin were purchased from Sigma-Aldrich Biotechnology (St Louis, MO, USA). Assays kits for the detection of liver functions were purchased from Bio-diagnostic (Giza, Egypt). All other chemicals were of analytical grade for HPLC assay.

Experimental animals:

Male adult Sprague Dawely rats (150-200 g) were kindly provided from the breeding center at NODCAR and kept for a week for acclimatization before beginning of experiment under normal conditions with a 12 h/12 h light-dark cycle at room temperature (22ºC–25ºC) with ad libitum water and food until starting the experiment. Rats were grouped and housed in a conventional clean facility according to the protocol of the Institutional Animal Ethics Committee of NRC. All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals.

Experimental Design

A total of 40 albino rats were randomly divided into 5 groups (8/group):

Groups 1 (C-): Rats were fed on basal diet and orally received saline solution as vehicle and served as negative control group.

Groups 2 (C+): Rats were orally administered a single daily dose of acetaminophen dissolved in propanol (APAP, 500 mg/kg BW) for 14 days [21], then subdivided into three groups (G3, G4 and G5) and the rest left as positive control group (G2),

Groups 3 (Tau): Rats treated orally with taurine dissolved in water (500mg/ kg/day) for 14 days [21].

Groups 4 (MSM): Rats treated with MSM dissolved in saline administered intraperitoneal (i.p.) (400mg/ kg/day) for 14 days [16].

Groups 5 (SIL): Rats treated with silymarin orally, dissolved in water (300mg/ kg/day) for 14 days [17].

The whole blood was centrifuged at 3000 r.p.m for 10 min to separate the serum. Markers of liver function including AST and ALT were measured with colorimetric methods using commercially available kits. Liver tissues were homogenized in four volumes of ice-cold 150 mM Tris–HCl (pH 7.4) using Hiedolph homogenizer (SilentCrush M, Hiedolph, Germany). Brain tissues were homogenized at a concentration of 10% w/v in 70 % methanol. The homogenates were centrifuged at 3000 r.p.m for 15 min at 4Cº to obtain a supernatant for various biochemical analyses. Lipid peroxidation in the liver and brain homogenate was determined by measurement of MDA by HPLC –UV according to method of [22]. Liver and brain content of GSH and GSSG were determined as markers of antioxidant capacity using method

of[23].Nitric oxide (as nitrite and nitrate) was determined according to method of [24].Brain monoamines were determined by HPLC methods according to [25]. 8-hydroxy-2'-deoxyguanosine (8-OHdG) was determined by HPLC method according to [26].

Statistical analysis

Datawere done as means ± S.E. using SPSS ANOVA test version 11.5, P≤0.05 was considered significant difference.

RESULTS

Data depicted in table 1 shows that AST and ALT activities were significantly increased after the administration of APAP as compared with the normal group. Treatment with either silymarin, MSM or Taurine induced ameliorative effects and the difference was statistically significant ($p < 0.05$). The order of ameliorative potentials of both AST and ALT was silymarin > taurine > MSM.

Table 2 shows that acetaminophen treated rats exhibited elevated levels of liver MDA, NO and GSSG and decreased levels of GSH in comparison to control group and the difference was statistically significant ($p < 0.0$)5. Silymarin, taurine and MSM minimized the effect of acetaminophen on the redox status in liver. Their effects are comparable to each other and to control group.

In addition, acetaminophen induced oxidative stress in rat brain and increased levels of MDA, NO and GSSG and decreased levels of GSH in comparison to control group (Table 3). Silymarin, taurine and MSM minimized the effect of acetaminophen on the redox status and the difference was statistically significant ($p < 0.05$). The antioxidant effect of taurine was more remarkable than silymarin and MSM (Table 3).

Acetaminophen treatment induced neurochemical changes in rat brain in terms of increased levels of dopamine and norepinephrine and decreased serotonin level compared to control group (Table 4) and the difference was statistically significant ($p < 0.05$). Silymarin, taurine and MSM minimized the neurochemical effect of acetaminophen on brain monoamines levels (Table 4).

Table 1: Effect of Silymarin (SIL), Taurine (TAU) and Methylsulphonylmethane (MSM) on liver Function in acetaminophen (APAP) induced heptotoxicity in Rats.

Groups / Parameter	Control	APAP	SIL	TAU	MSM
AST (U/L)	60.6 ± 2.41	119.7 ± 2.32*	72.4 ± 3.93$^{*\$}$	82.2 ± 2.40$^{*\$a}$	89.6 ± 1.85$^{*\$a}$
ALT (U/L)	40.2 ± 0.95	72.2 ± 3.41*	50.1 ± 2.92$^{*\$}$	53.6 ± 3.01$^{*\$}$	56.3 ± 0.92$^{*\$}$

Data are expressed as Mean ± S.E. for 6-rats/group
* significant difference from control group at P < 0.05.
$ significant difference from acetaminophen group at P < 0.05.
a significant difference from silymarin group at P < 0.05.

Table 2: Effect of Silymarin (SIL), Taurine (TAU) and Methylsulphonylmethane (MSM) on liver Oxidative Stress Parameters in acetaminophen induced heptotoxicity in Rats

Groups / Parameter	Control	APAP	SIL	TAU	MSM
MDA (nmol / g)	112.2 ± 5.542	215.9 ± 5.773*	117.5 ± 9.273$^{\$}$	101.9 ± 7.807$^{\$a}$	127.9 ± 11.83$^{\$ab}$
GSH (µmol / g)	49.7 ± 0.629	28.9 ± 2.257*	49.8 ± 1.352$^{\$}$	49.5 ± 1.518$^{\$}$	50.0 ± 2.42$^{\$}$
GSSG (µmol / g)	12.5 ± 0.548	18.5 ± 1.516*	14.5 ± 1.042$^{*\$}$	11.3 ± 0.534$^{\$a}$	11.3 ± 0.84$^{\$a}$
NO (µmol / g)	16.7 ± 0.927	19.8 ± 1.623*	17.3 ± 0.929	17.6 ± 0.691	12.5 ± 0.71$^{*\$ab}$

Data are expressed as Mean ± S.E. for 6-rats/group
* significant difference from control group at P < 0.05.
$ significant difference from acetaminophen group at P < 0.05.
a significant difference from silymarin group at P < 0.05.
b significant difference from taurine group at P < 0.05.

Table 3: Effect of Silymarin (SIL), Taurine (TAU) and Methylsulphonylmethane (MSM) on Brain Oxidative Stress Parameters in acetaminophen- treated Rats.

Groups / Parameters	Control	APAP	SIL	TAU	MSM
MDA (nmol / g)	53.3 ± 4.18	135.1 ± 12.488*	83.06 ± 6.439$^{*\$}$	75.5 ± 3.291$^{*\$}$	102.3 ± 8.09$^{*\$ab}$
GSH (µmol / g)	26.84 ± 1.205	8.78 ± 0.195*	19.76 ± 0.997$^{*\$}$	21.83 ± 0.529$^{*\$}$	17.31 ± 1.253$^{*\$}$
GSSG (µmol / g)	3.36 ± 0.218	7.86 ± 0.375*	4.77 ± 0.125$^{*\$}$	4.45 ± 0.327$^{*\$}$	4.46 ± 0.157$^{*\$}$
NO (µmol / g)	13.02 ± 1.016	19.38 ± 1.125*	15.68 ± 0.782$^{\$}$	14.71 ± 1.086$^{\$}$	14.86 ± 0.475$^{\$}$

Data are expressed as Mean ± S.E. for 6-rats/group
* significant difference from control group at P < 0.05.
$ significant difference from acetaminophen group at P < 0.05.
a significant difference from silymarin group at P < 0.05.
b significant difference from taurine group at P < 0.05.

Table 4: Effect of Silymarin (SIL), Taurine (TAU) and Methylsulphonylmethane (MSM) on Brain monoamines in acetaminophen-treated Rats.

Groups / Parameter	Control	APAP	SIL	TAU	MSM
NE (µg/g)	0.873 ± 0.017	1.251 ± 0.114*	1.005 ± 0.086*	0.841 ± 0.061$^{\$}$	0.842 ± 0.005$^{\$}$
DA µg/g)	1.241 ± 0.058	1.616 ± 0.093*	0.977 ± 0.063$^{\$}$	1.398 ± 0.127$^{\$a}$	1.57 ± 0.053^{*a}
5HT(µg/g)	0.555 ± 0.029	0.391 ± 0.041*	0.412 ± 0.036$^{*\$}$	0.506 ± 0.029$^{\$}$	0.419 ± 0.03$^{*\$}$

Data are expressed as Mean ± S.E. for 6-rats/group
* significant difference from control group at P < 0.05.
$ significant difference from acetaminophen group at P < 0.05.
a significant difference from silymarin group at P < 0.05.
b significant difference from taurine group at P < 0.05.

Table 5: Effect of Silymarin (SIL), Taurine (TAU) and Methylsulphonylmethane (MSM) on Liver and Brain DNA fragmentation in acetaminophen- treated Rats.

Groups / Parameter	Control	APAP	SIL	TAU	MSM
Liver 8HDG (pg/g)	193.1 ± 4.538	221.5 ± 12.601*	181.1 ± 4.986$^{\$}$	183.9 ± 11.661$^{\$}$	179.8 ± 4.3$^{\$}$
Brain 8HDG (pg/g)	71.4 ± 3.295	223.6 ± 13.635*	155.8 ± 15.0$^{*\$}$	191.1 ± 16.87$^{*\$a}$	110.5 ± 5.85$^{*\$ab}$

Data are expressed as Mean ± S.E. for 6-rats/group
* significant difference from control group at P < 0.05.

$ significant difference from acetaminophen group at P < 0.05.
a significant difference from silymarin group at P < 0.05.
b significant difference from taurine group at P < 0.05.

Table 5 shows that acetaminophen induced DNA degradation in terms of elevated level of 8-hydroxy-2- deoxyguanosine in both liver and brain of treated rats. Silymarin, taurine and MSM minimized the degenerative effect of acetaminophen on DNA (Table 5) and the difference was statistically significant ($p < 0.05$). MSM treatment was the most effective.

DISCUSSION

The liver, as the first organ to come into contact with absorbed nutrients, represents a major sensor of substrate, electrolyte and water input. In addition, the liver is the central organ of the body's detoxification processes of many xenobiotics (drugs and environmental chemicals). The liver is innervated by both afferent and efferent autonomic nerves, which are associated with the portal vein, hepatic artery, bile ducts and liver hilus [27]. Therefore, liver sends information via sensory, afferent nerves to the brain and thence, via efferent nerves to other peripheral organs, particularly the liver [28], to maintain whole body homeostasis. Hence, cerebral and autonomic nervous dysfunction accompanying various forms of liver disease have long been known [29]. Accordingly, the present study aimed to investigate the potential toxic effect of APAP on liver and brain and the possible curative effect of TAU, MSM and SIL.

In the present study, the administration of acetaminophen to rats resulted in rises in circulating liver enzymes, serum ALT and AST compared to control and significantly increased hepatic DNA fragmentation ($P < 0.05$ vs. control group), indicating that acetaminophen is capable of inducing hepatocyte apoptosis. The observation that acetaminophen decreased the content of GSH and increased the levels of hepatic MDA, GSSG and NO might suggest that oxidative stress is the culprit of acetaminophen-induced hepatotoxicity. In accordance, recent in vitro and in vivo studies indicated that acetaminophen induced inflammation and oxidative stress [30-32]. It is likely that acetaminophen toxicity is related to the accumulation of toxic intermediate metabolites such as N-acetyl-p-benzoquinoneimine(NAPQI) [33]. Normally, acetaminophen is metabolized into NAPQI by cytochrome P450-dependent mixed-function oxidase in the liver and by the prostaglandin synthetase system in the kidney [34]. Then, these metabolites are detoxified by reduced glutathione (GSH) [35]. When glutathione levels are depleted, this intermediate metabolite can covalently bind to nucleophilic targets of macromolecules in cells that eventually cause cell death [35].

Moreover, acetaminophen treatment induced neurochemical changes in treated rats in terms of increased brain levels of dopamine and norepinephrine and decreased serotonin content. In addition, acetaminophen induced oxidative stress and DNA degeneration in brains of treated rats. This effect might be directly due to the generation of ROS and the subsequent cell apoptosis and neurochemical imbalance in the brain or indirectly through liver dysfunction. Consistently, most diseases afflicting the nervous system are rooted in improperly formed blood, brought about by an unwell liver. The observation that acetaminophen increased both dopamine and/or epinephrine level might indicate that acetaminophen enhanced the transmitters' synthesis and/or inhibited their degradation. In accordance, a recent study indicated that acetaminophen inhibited the enzymatic activities of monoamine oxidase and ChE, but not ATPase enzyme,
suggestingthatacetaminophenneurotoxicity is at least partly- due to neurotransmission disturbance [36], which supports the opinion of present study. Moreover, the oxidative stress effect of APAP on the brain might be to the depleting effect of the drug on reduced glutathione and its disturbing effect on liver function including glutathione synthesis and DNA degradation and apoptotic cell death [37].

In the present study SIL, TAU and MSM significantly attenuated acetaminophen induced liver injury and restored the normal levels of serum AST, ALT, restored the normal redox status and mend APAP-induced hepatic DNA fragmentation. In addition, SIL, TAU and MSM significantly attenuated APAP induced-

neurotoxicity and restored the normal redox status, minimized the disturbing effect of APAP on brain monoamines and mitigates APAP-induced brain DNA fragmentation in rats. It's worthy to note that both SIL and TAU were more efficient than MSM. The variation of their beneficial effect might be proportionated with their respective antioxidant potentials. Consistently, a previous study indicated that hepatoprotective effect of SIL is due to the antioxidant properties of flavonoids, and stimulating of hepatic synthesis of RNA proteins. Moreover, SIL also stimulates the RNA polymerase I, and further the ribosomal RNA and synthesis of protein [17, 38]. This might suggest that SIL posses multiple mechanism of action against hepatotoxic agents. The antioxidant property and the active cell regeneration through inhibiting the free radicals that are produced from the metabolism of toxic substances, protecting the brain and spinal nerves against free radical damage and increase synthesis of RNA and proteins are considered the most important actions [39- 41].

Meanwhile, taurine possesses a number of cytoprotective properties through its actions as an antioxidant, osmoregulator, and intracellular calcium flux regulator [40]. Moreover, taurine exhibited both prophylactic and therapeutic effects in acetaminophen-induced hepatic injury [40]. It has been suggested that taurine administration could affect the disposition of APAP by enhancing its metabolism through the GSH-dependent pathway and also by increasing the biliary excretion of this drug and its metabolites [42]. In addition, taurine, hypotaurine and N-acetylcysteine exhibited equipotent protective actions against the toxic actions of APAP in the liver when tested in equimolar doses and under the same conditions [21]. Moreover, it seems that the antioxidant property of MSM is underlying mechanisms of its hepatoprotective and curative effect against APAP induced liver and brain dysfunction [43]. In accordance, a previous study indicated that MSM increased the level of GSH, and antagonized acetaminophen induced hepatotoxicity, may be through its sulfur donating and antioxidant effects [16, 44].

On conclusion the present study shows that acetaminophen overdose induced neuro- and hepato-toxicity. The suggested mechanisms of cell injury are metabolic activation of APAP, glutathione depletion and formation of reactive oxygen/nitrogen species. In addition, TAU, SIL and MSM possess significant neuro- and hepato-curative attribute due to their antioxidant properties.

REFERENCES

1. Vargas-Mendoza, N., Madrigal-Santillán, E., Morales-González, A., Esquivel-Soto,J.,Viberg, H., Eriksson, P., Gordh, T. and Fredriksson, A., 2014, Paracetamol (Acetaminophen) administration during neonatal brain development affects cognitive function and alters its analgesic and anxiolytic response in adult male mice. toxicological sciences 138(1):139–147.
2. Friedrichsdorf, S.J., Postier, A., Eull, D., Weidner, C., Foster, L., Gilbert, M. and Campbell, F., 2015, Pain outcomes in a US children's hospital: A prospective cross-sectional survey. Hosp Pediatr. 5(1):18-26.
3. Roberts, P. H. and Thomas, K. V., 2006, The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci. Total Environ. 356, 143–153.
4. Wu, S., Zhang, L. and Chen, J., 2012, Paracetamol in the environment and its degradation by microorganisms. Appl. Microbiol. Biotechnol. 96, 875–884.
5. Bronstein, A.C., Spyker, D.A., Cantilena, L.R. Jr, Green, J, Rumack, B.H. and Heard, S.E., 2007, Annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS). Clin Toxicol 45: 815917.
6. Papazoglu, C., Ang, J.R., Mandel, M., Basak, P. and Jesmajian, S., 2015, Acetaminophen overdose associated with double serum concentration peaks. J Community Hosp Intern Med Perspect.; 5(6):29589
7. Khayyat, A., Tobwala, S., Hart, M. and Ercal, N., 2016, N-acetylcysteine amide, a promising antidote for acetaminophen toxicity. Toxicol Lett.;241:133-42.
8. Alkholifi, F.K. and Albers, D.S., 2015, Attenuation of rotenone toxicity in SY5Y cells by taurine and N-acetyl cysteine alone or in combination. Brain Res.;1622:409-13.
9. Cuttitta, C.M., Guariglia, S.R., Idrissi, A.E. and L'amoreaux, W.J., 2013, Taurine's effects on

the neuroendocrine functions of pancreatic β cells. Adv Exp Med Biol.;775:299-310.

10. Kumari, N., Prentice, H. and Wu, J.Y., 2013, Taurine and its neuroprotective role. Adv Exp Med Biol.;775:19-27.
11. Amirshahrokhi, K. and Bohlooli, S., 2013, Effect of methylsulfonylmethane on paraquat-induced acute lung and liver injury in mice. Inflammation.;36(5):1111-21.
12. Kim, Y.H., Kim, D.H., Lim, H., Baek, D.Y., Shin, H.K. and Kim, J.K., 2009, The anti-inflammatory effects of methylsulfonylmethane on lipopolysaccharide-induced inflammatory responses in murine macrophages. Biol Pharm Bull; 32:651-656.
13. Amirshahrokhi, K., Bohlooli, S. and Chinifroush, M.M., 2011, The effect of methylsulfonyl-methane on the experimental colitis in the rat. Toxicol Appl Pharmacol 253:197-202.
14. Tripathi, R., Gupta, S., Rai, S. and Mittal, P.C., 2011, Effect of topical application of methylsulfonylmethane (MSM), EDTA on pitting edema and oxidative stress in a double blind, placebo-controlled study. Cell Mol Biol (Noisy.-le-grand) 57:62-69.
15. Nakhostin-Roohi, B., Barmaki, S., Khoshkhahesh, F. and Bohlooli S., 2011, Effect of chronic supplementation with methylsulfonylmethane on oxidative stress following acute exercise in untrained healthy men. J Pharm Pharmacol. ; 63(10):1290-1294.
16. Bohlooli, Sh., Mohammadi, S., Amirshahrokhi, K., Mirzanejad-asl, H., Yosefi, M., Mohammadi-Nei, A. and Chinifroush, M.M., 2013, Effect of methylsulfonylmethane pretreatment on acetaminophen induced hepatotoxicity in rats. Iran J Basic Med Sci; 16: 896-900.
17. Rasool, M., Iqbal, J., Malik, A., Ramzan, H.S., Qureshi, M.S., Asif, M., Qazi, M.H., Kamal, M.A., Chaudhary, A.G., Al-Qahtani, M.H., Gan, S.H. and Karim, S., 2014, Hepatoprotective effects of silybum marianum (Silymarin) and glycyrrhiza glabra (Glycyrrhizin) in combination: A possible synergy. Evid Based Complement Alternat Med.;641597
18. Borah, A., Paul, R., Choudhury, S., Choudhury, A., Bhuyan, B., Das Talukdar, A., Dutta Choudhury, M. and Mohanakumar, K.P., 2013, Neuroprotective potential of silymarin against CNS disorders: insight into the pathways and molecular mechanisms of action. CNS Neurosci Ther.;19 (11):847-53.
19. Burczynski, F.J., Yan, J., Gong, Y., Nguyen, D., Wang, G., Burczynski, S.D., Smith, H.J. and Gong, Y., 2013, The Hepatoprotective Effect of Diltiazem and Silymarin. Nat Prod Chem Res., 1:3
20. Surai, P.F., 2015, Silymarin as a natural antioxidant: An overview of the current evidence and perspectives. Antioxidants (Basel).; 4(1):204-47.
21. Acharya, M. and Lau-Cam, C.A., 2010, Comparison of the protective actions of N-acetylcysteine, hypotaurine and taurine against acetaminophen-induced hepatotoxicity in the rat. J Biomed Sci. 17(Suppl 1):S35.
22. Karatepe, M., 2004, Simultaneous determination of ascorbic acid and free malondialdehyde in human serum by HPLC-UV. Lc Gc North America.; 22(4):362-365
23. Jayatilleke, E. and Shaw, S., 1993, A high-performance liquid chromatographic assay for reduced and oxidized glutathione in biological samples. Analyt. Biochem., 214(2): 452-457
24. Everett, S.A., Dennis, M.F., Tozer, G.M., Prise, V.E., Wardman, P. and Stratford, M.R., 1995, Nitric oxide in biological fluids: analysis of nitrite and nitrate by high-performance ion chromatography. J Chromatogr A, 706(1-2):437-42
25. Pagel, P., Blome, J. and Wolf, H.S., 2000, High-performance liquid chromatographic separation and measurement of various biogenic compounds possibly involved in the pathomechanisms of Parkinson's disease. J. Chromatogr.B., 746: 297-304.
26. Zhang, S.W., Xing, J., Cai, L.S. and Wu, C.Y., 2009, Molecularly imprinted monolith in-tube solid-phase microextraction coupled with HPLC/UV detection for determination of 8-hydroxy-2'-deoxyguanosine in urine. Anal Bioanal Chem.;395(2):479-87
27. Jensen, K., Afroze, S., Ueno, Y., Rahal, K., Frenzel, A., Sterling, M., Guerrier, M., Nizamutdinov, D., Dostal, D.E., Meng, F. and Glaser, S.S., 2013, Chronic nicotine exposure stimulates biliary growth and fibrosis in normal rats. Dig Liver Dis.; 45(9):754-61.
28. O'Hare, J.D. and Zsombok, A., 2016, Brain-liver connections: role of the preautonomic PVN

neurons. Am J Physiol Endocrinol Metab.;310(3):E183-9.

29. Butterworth, R.F., 2015, The concept of "the inflamed brain" in acute liver failure: mechanisms and new therapeutic opportunities. Metab Brain Dis. 30(6): 1-5.

30. Maes, M., McGill, M.R., da Silva, T.C., Lebofsky, M., Maria Monteiro de Araújo, C., Tiburcio, T., Veloso Alves Pereira, I., Willebrords, J., Crespo Yanguas, S., Farhood, A., Zaidan Dagli, M.L., Jaeschke, H., Cogliati, B. and Vinken, M., 2016. Connexin32: a mediator of acetaminophen-induced liver injury? Toxicol Mech Methods.:1-9.

31. Mathur, A., Rizvi, F. and Kakkar, P., 2016, PHLPP2 down regulation influences nuclear Nrf2 stability via Akt-1/Gsk3β/Fyn kinase axis in acetaminophen induced oxidative renal toxicity: Protection accorded by morin. Food Chem Toxicol.; 89:19-31. Molecules.;21(2). pii: E161.

32. Palabiyik, S.S., Karakus, E., Halici, Z., Cadirci, E., Bayir, Y., Ayaz, G. and Cinar, I., 2016, The protective effects of carvacrol and thymol against paracetamol-induced toxicity on human hepatocellular carcinoma cell lines (HepG2). Hum Exp Toxicol. pii: 0960327115627688.

33. Leeming, M.G., Gamon, L.F., Wille, U., Donald, W.A. and O'Hair, R.A., 2015, What are the potential sites of protein rrylation by N-Acetyl-p-benzoquinone imine (NAPQI)? Chem Res Toxicol.;28(11):2224-33.

34. Lőrincz, T., Jemnitz, K., Kardon, T., Mandl, J. and Szarka, A., 2015, Ferroptosis is involved in acetaminophen induced cell death. Pathol Oncol Res.;21(4):1115-21.

35. Ilavenil, S., Al-Dhabi, N.A., Srigopalram, S., Ock Kim, Y., Agastian, P., Baru, R., Choi, K.C. and Valan Arasu, M., 2016, Acetaminophen induced hepatotoxicity in Wistar rats-A proteomic approach. Molecules, 21(2), 161.

36. Wu, J.P. and Li, M.H.. 2015, Inhibitory effects of pain relief drugs on neurological enzymes: implications on their potential neurotoxicity to aquatic animals. Environ Toxicol Pharmacol.; 39(2):898-905.

37. Lu, S.C., 2013, Glutathione synthesis. Biochim Biophys Acta.; 1830(5): 3143–3153.

38. Ramadan, S.I., Shalaby, M.A., Afifi, N. and El-Banna, H.A., 2011, Hepatoprotective and antioxidant effects of silybum marianum Plant in Rats. IJAVMS, 5 (6): 541-547.

39. Pandey Govind and Sahni, Y.P., 2011, A review on hepatoprotective activity of silymarin. IJRAP, 2(1) 75-79.

40. Waters, E., Wang, J.H., Redmond, H.P., Wu, Q,D., Kay, E. and Bouchier-Hayes, D., 2001, Role of taurine in preventing acetaminophen-induced hepatic injury in the rat. Am J Physiol Gastrointest Liver Physiol.;280(6):G1274-9.

41. Vijay kranti.m*1, Mahesh.v1, Srinivas.p1, Y.v.ganesh1 ,Ajay Godwin.p2 and dr. Mangala lahkar3 (2013): Evaluation of the protective effect of silymarin on doxorubicin induced chronic testicular toxicity in rats. Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 473 – 484.

42. Samy Ali Hussein*; Omnia M. Abdel-Hamid and Hatem Fathy Elgemezy. Metabolic Effects Of Taurine on Experimentally Induced-hyperlipidemia In RatsBenha Veterinary Medical Journal, VOL. 27, NO. 2:76-90, December 2014

43. Valantina Al Bitar, Dr Shaza Laham Methylsulfonylmethane and Green Tea Extract Reduced Oxidative Stress and Inflammation In An Ulcerative Colitis. Asian Journal of Pharmaceutical and Clinical Research, Vol 6, Suppl 2, 2013, 153-158

44. Aycan IÖ1, Tüfek A2, Tokgöz O2, Evliyaoğlu O3, Fırat U4, Kavak GÖ2, Turgut H5, Yüksel MU2 (2014): Thymoquinone treatment against acetaminophen-induced hepatotoxicity in rats. Int J Surg. 2014;12(3):213-8.