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Research Article

Protective effects of *Prunus laurocerasus* extracts against paracetamol-induced hepatotoxicity

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Abstract

Paracetamol, which is a good analgesic and antipyretic when used in therapeutic doses, becomes a very damaging drug in overdose. In particular, it causes hepatotoxicity and may cause liver failure in the future. *Prunus laurocerasus* extracts used in the study are a natural fruit with proven antioxidant capacity in previous studies and have protective properties in many diseases. The aim of the present study is to determine the hepatoprotective property of this extract in the damage induced by paracetamol. For this, the experiment was designed in 5 different groups and each group was determined to have 6 experimental animals. No treatment was given to the control group (Group 1). Group 2 was determined as positive control as N-acetyl-cysteine (NAC-150 mg/kg+paracetamol) in accordance with the literature. The treatment groups were determined as *Prunus laurocerasus* water and ethanol-water (400 mg/kg+paracetamol). The last group was paracetamol, which was given to animals at a dose of 2 mg/kg. Paracetamol administered in the liver and stomach significantly increased lipid peroxidation (LPO) and nitric oxide (NO) levels. It also caused a decrease in glutathione (GSH) levels, superoxide dismutase (SOD) and catalase (CAT) enzyme activities. With the applied extracts, these rates increased significantly to the level of healthy tissue. The obtained data reveal that these extracts have hepatoprotective properties.

Key Words: paracetamol; *prunus laourocerasus*; nitric oxide; lipid peroxidation; hepatotoxicity

Introduction

Paracetamol, a widely used analgesic and antipyretic drug, has a safe profile when used in therapeutic doses. The therapeutic dose has been determined as 4 g/day daily, but when this amount is doubled, dose-related hepatotoxicity develops and severe liver damage occurs. At the same time, it is not possible for other organs to be unaffected [1,2]. Paracetamol contributes to 80% of the liver failure cases associated with drugs [3]. Although there are many examples around the world, the most common cause of acute liver failure is the use of paracetamol. It has also been stated that overdose use in the United Kingdom has a high mortality rate [4-7]. The metabolite responsible for the damage caused by paracetamol is N-acetyl-p benzoquinone imine (NAPQI). Small amounts of paracetamol are converted to the highly reactive metabolite N-acetyl-p-benzoquinonimine (NAPQI) by cytochrome P450 enzymes in mitochondria. NAPQI is detoxified by conjugation with hepatic glutathione (GSH) to form conjugates of mercapturic acid and cysteine, which are non-toxic and excreted in the urine. However, at high doses, this metabolite saturates cellular glutathione and causes less NAPQI neuralization. The overproduction of NAPQI causes overproduction of reactive oxygen species, membrane damage, mitochondrial disorders, and many inflammatory reactions. Thus, in hepatocytes, NAPQI can form protein adducts with intracellular proteins. This structure can lead to DNA fragmentation, oxidative stress, liver failure and even hepatocyte death [8]. The current treatment for paracetamol toxicity is N-acetyl-L-cysteine (NAC). Being a very good glutathione precursor and store, NAC acts by increasing the GSH available for conjugation with NAPQI. Especially in experimental studies, paracetamol toxicity in rats has been tried to be expressed by determining it with oxidative stress parameters.

Recently, interest in natural dietary antioxidant compounds has increased in order to prevent damage in many health problems caused by oxidative stress [9,10]. For this reason, it has been stated in some studies that high fruit consumption can reduce the risk of certain diseases such as hepatotoxicity, cancer, cardiovascular and coronary heart diseases, diabetes and atherosclerosis. *Laurocerasus officinalis* Roem selected in the present study. (Cherry laurel) is grown as a native fruit on the Black Sea coast of Turkey and is called “Taflan” or “Karayemiş” in the region [11]. It is known that the fruit of L. officinalis is a rich source of natural antioxidant substances such as phenolics (chlorogenic acid, phenolic acids, anthocyanins, vanillic acid) and ascorbic acid [12,13]. Some studies have shown that L. officinalis fruit has a radical scavenging effect against superoxide and 2,2-diphenyl-1 picrylhydrazil (DPPH) radicals. In line with this information, the present study has been planned and it will be determined that liver damage caused by excessive use of paracetamol can be prevented with L. officinalis.

Materıals and Method

Chemicals

All chemicals for laboratory experimentation were purchased from Sigma Chemical (Germany). Ketamine (80 mg/kg) and xylazine (10 mg/kg) used in sacrification processes were obtained from a legal seller.

Plant material and extraction of plant material

Prunus laurocerasus fruits were used as study material in the research and were obtained from a vendor selling dried fruit. The dried fruits obtained were freed from their seeds. After trituration with liquid nitrogen, it was placed in a Soxhlet instrument bottle. It was extracted with water and ethanol-water solvents separately in a shaking water bath for seven days. Then, the extracts, which were filtered and dried, were put into the test when they were turned into powder.

Animals, Treatments and Ethical Approval

30 Wistar albino experimental animals weighing 250-300 gr were obtained from Saki Experimental Animals in Ankara. The ethical approval was obtained from Giresun University Animal Experiments Local Ethics Committee for the applications (2019/14). The animals provided were kept in the same conditions and tested after seven days. First of all, the animals were divided into five different cages according to their weight, with six animals in each cage. The control group received no treatment. *Prunus laurocerasus* water and ethanol water extract were administered orally to two groups at a dose of 400 mg/kg. NAC, whose positive activity is known, was also added to another group was given orally at a dose of 150 mg/kg determined in the literature. The paracetamol group, which is the negative control group, was given orally at 2 g/kg dose (1% CMC in 1X PBS 2 ml per rat). The paracetamol was administered to all groups one hour after all treatments were completed. After 24 hours following the experiment, the all animals were sacrificed at a high dose and biochemical parameters were examined in the tissues.

Biochemical investigation

The NOx Level

NOx levels in the samples were determined by the spectrophotometric method of Miranda et al. (2001) [14].

Catalase (CAT) activity

Decomposition of H_2O_2 in presence of catalase was at 240 nm [15]. Catalase activity was defined as the amount of enzyme required to decompose 1 nmol of H_2O_2 per minute, at 25°C and pH 7.8. Results were expressed as mmol/min/mg tissue.

Superoxide dismutase activity

SOD activity was measured according to the principle of superoxide radical formation of xanthine [16]. SOD activity was then measured at 560 nm by the degree of inhibition of this reaction.

Total glutathione (GSH) determination

The amount of glutathione is determined according to the method of Sedlak et al. With homogenates compatible with the literature, glutathione in tissues is expressed as nmol/g [17].

Lipid peroxidation (LPO) determination

The level of lipid peroxidation was determined with the homogenates prepared in accordance with Ohkawa's method. Data obtained were expressed as nmol/g tissue [18].

Statistical analyses

The results were made using the appropriate SPSS program. Statistical differences were determined by the ANOVA test. Multiple comparisons were expressed by Duncan. Significance was determined according to $p<0.05$.

The effects of *Prunus laurocerasus* fruits extracts on liver and stomach tissue antioxidant levels on paracetamol-induced hepatotoxicity in rats

SOD, CAT enzyme activities and NOx, LPO and GSH levels were determined in all rat tissues. The antioxidant enzyme levels and the protective properties of the extracts were expressed. The data obtained are shown in Tables 1-2 and figures. First of all, the toxicity shown in Table 1 belongs to the liver tissues, and it was determined that the administered paracetamol caused a significant increase in NOx and LPO levels (Table 1-2, Fig. 1). In positive control NAC treated tissues, this increase was reduced to a level close to healthy. This decrease can be said for both types of extracts applied in the same way. Again, CAT and SOD enzymes and GSH levels were found to be quite low in the paracetamol group administered. The compared to healthy tissues, this level was significantly increased in tissues where NAC and extracts were applied ($p<0.05$). Antioxidant enzymes shown in Table 2 express the protection in gastric tissues. The NOx and LPO levels were found to be quite high compared to the healthy tissues with administered paracetamol (Fig. 1).

NAC and extracts showed their protective properties by reducing this increase significantly. SOD and CAT enzyme activities and GSH levels were found to be quite low with paracetamol applied (Table 1-2, Fig. 2). Again, both types of extracts NAC and increased this decrease significantly ($p<0.05$).

Treatment	N	NO Level (nmol/mg tissue)	Amount of LPO (nmol/g tissue)	Amount of GSH (nmol/mg tissue)	SOD Activity (mmol/min/mg tissue)	CAT Activity (mmol/min/mg tissue)
Healthy (control)	6	22.6±1.8a	91.1±0.5a	5.75±1.5d	5.0±0.3e	24.4±0.9d
Paracetamol (2g/kg)	6	69.5±0.9d	162.0±0.8d	2.90±0.2a	0.9±0.04a	10.6±0.6a
N-acetyl-cysteine (NAC 150 mg/kg)	6	29.0±1.1b	95.3±1.0b	4.74±0.1c	4.3±0.2d	19.8±0.6c
Prunus laurocerasus (ethanol-water exract 400mg/kg)	6	37.7±1.3c	99.9±1.0c	3.26±0.2a,b	3.8±0.2c	17.3±0.3b
Prunus laurocerasus (water exract 400mg/kg)	6	40.8±0.7c	100.6±1.2c	3.69±0.1b	2.9±0.1b	15.8±0.8b

Means in the same column by the same letter are not significantly different to the One-way ANOVA ($p<0.05$).Mean damage index ± SE of six animals in each group.

Table 1: *Effects of Prunus laurocerasus extracts and healthy groups on the amount of GSH, LPO, NO, activities of SOD and CAT in paracetamol-induced rat liver tissue.*

Treatment	N	NO Level (nmol/mg tissue)	Amount of LPO (nmol/g tissue)	Amount of GSH (nmol/mg tissue)	SOD Activity (mmol/min/mg tissue)	CAT Activity (mmol/min/mg tissue)
Healthy (control)	6	17.44±0.6a	34.2±1.4a	3.5±0.1c	4.13±0.3c	19.21±0.7d
Paracetamol (2g/kg)	6	62.95±5.9	72.7±3.02d	0.8±0.1a	1.50±0.2a	10.00±0.7a
N-acetyl-cysteine (NAC 150 mg/kg)	6	26.10±1.8a,b	42.0±0.6b	3.1±0.1c	3.79±0.1c	16.72±0.5c
***Prunus laurocerasus* (ethanol-water exract 400mg/kg)**	6	31.96±2.5b	48.4±0.7c	1.9±0.1b	3.03±0.1b	12.70±1.0b
***Prunus laurocerasus* (water exract 400mg/kg)**	6	26.33±1.5a,b	52.3±0.9c	1.5±0.3b	3.10±0.1b	13.00±0.6b

Means in the same column by the same letter are not significantly different to the One-way ANOVA ($p<0.05$).Mean damage index ± SE of six animals in each group.

Table 1: *Effects of Prunus laurocerasus extracts and healthy groups on the amount of GSH, LPO, NO, activities of SOD and CAT in paracetamol-induced rat stomach tissue.*

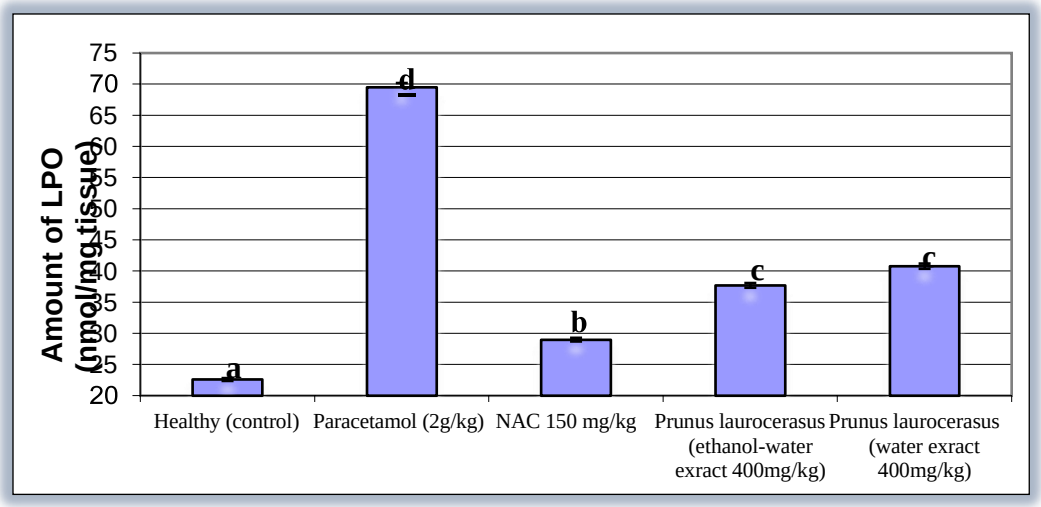

Figure (1): *Effects Prunus laourcerasus extracts on the amount of lipid peroxidation (LPO) in rat's paracetamol-induced in rat liver tissues. Means in the same column by the same letter are not significantly different to the One-way ANOVA*

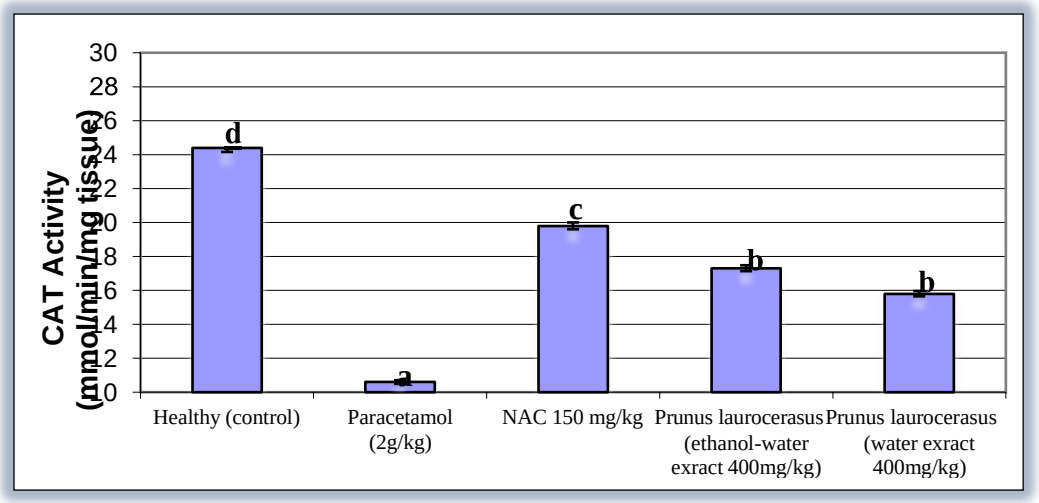

Figure (2): *Effects Prunus laourcerasus extracts on the enzyme activities catalase (CAT) in rat's paracetamol-induced in rat liver tissues. Means in the same column by the same letter are not significantly different to the One-way ANOVA.*

Discussion

In the present study, the effect of *P. Larurocerasus* fruit extracts (water and ethanol-water 400 mg/kg) on liver and stomach tissues was determined in the experimental toxicity model created with paracetamol. This effect was expressed biochemically by the antioxidant enzyme mechanism. Paracetamol is one of the widely used analgesics and antipyretics worldwide. It is a very reliable drug in therapeutic doses used to relieve mild to moderate pain. However, it is known to cause liver necrosis when used in excessive doses. In many studies, it has been determined that excessive use causes an increase in reactive oxygen species and a decrease in antioxidant enzyme levels due to this increase. Along with these, it causes strong damage to some tissues, especially the liver [19]. As a result of its oral use, the NAPQI compound is produced by the cytochrome p450 enzyme in the tissues. This compound is detoxified by glutathione, but in excessive use, glutathione deficiency causes damage to structures such as lipid, protein, and DNA [20,21]. (Sasidharan et al., Prescott, 2000).

The damage to tissues is explained by the oxidative stress marker. As shown in many studies, the use of paracetamol in high doses triggers oxidative stress. Therefore, it has been reported that there is an increase in lipid peroxidation due to increased oxidative stress [22,23]. This increase prevents the formation of antioxidant defense systems in tissues. The best indicator showing the onset of lipid peroxidation is the MDA level, and its level rises with the increase in stress. In the present study, it was determined that lipid peroxidation in liver and stomach tissues obtained was quite high in the paracetamol group. The high levels of MDA in the tissues prevented the antioxidant defense system from working with free radical production. However, it is seen that the extracts (400 mg/kg) applied to the treatment groups control this increase very well and reduce lipid peroxidation. We can say that the increased MDA level is activated and controlled by the antioxidant and phenolic compounds in the extracts.

The antioxidant defense systems in the body have different effects in all tissues. ROS usually attack macromolecules by producing superoxide anion ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2), hydrogen peroxide by-products. In the case of oxidative stress, although the body uses its defense system to scavenge radicals, it cannot completely eliminate them. In such cases, antioxidant enzymes try to prevent the effects of free radicals. The most important of these enzymes is the SOD enzyme, which tries to eliminate its harmful effects by converting the superoxide anion in the environment to hydrogen peroxide. The low SOD activity in the paracetamol administered group means that the hydrogen peroxide produced against the increased superoxide anion is not sufficient. The superoxide density in the environment means that the amount of SOD is low and the damage is high. In the treatment groups, the excess of antioxidant molecules was sufficient to remove the damage, and the enzyme defense system was activated by increasing the amount of SOD [24,25]. The extracts have been successful in preventing damage to the liver and stomach by the superoxide anion. Another enzyme that shows antioxidant defense by converting the hydrogen peroxide produced by SOD in the environment to molecular water and oxygen is catalase. They show defense by working in coordination with the CAT enzyme SOD. Therefore, it is normal to have low CAT activity like SOD in the administered paracetamol group. *Prunus laurocerasus* extracts protected the tissues from stress by converting the hydrogen peroxide produced in the environment to more water and oxygen. CAT activity was found to be quite high in the treatment groups and the NAC group. The data obtained are compatible with many studies [26,27].

GSH, which acts as a radical scavenger found in many mammalian tissues, is an intermediate scavenger of paracetamol. Hydrogen perocists support the antioxidant defense system by removing superoxide anions and free radicals. The fact that the GSH level of paracetamol administered in the current study is quite low compared to healthy tissues is due to the release of too much acetaminophen metabolite NAPQI in this group. Since it plays a role in the detoxification of NAPQI, the GSH level was found to be quite high in the treatment groups [8].

The another oxidant induced by paracetamol is NO. In the presence of superoxide, it causes the formation of peroxynitrite, which is a strong oxidant. Thus, an excessive amount of NO synthesis occurs through iNOS in tissues given paracetamol. The peroxynitrite, which can easily react with lipids in the cell membrane, causes damage. Normally, peroxynitrides can be detoxified with GSH, but they cannot prevent this in overdose. The existing GSH cannot keep up to prevent the damage of peroxynitrites. Thus, it is expected that NO level is high in the groups given paracetamol. However, this level was significantly reduced in both types of the applied extracts. Both ethanol-water and water extract reduced the damagein peroxynitrite formation by supporting antioxidant activity.

The data obtained with some tudies are compatible[28-30].

In line with all this information, it has been determined that reactive oxygen species have a great contribution in the pathogenesis of liver and stomach damage induced by paracetamol. The natural products that can be used can help to remove this damage. In the present study, the protectiveness was clearly determined at appropriate doses of *Prunus laurocerasus* ethanol-water and water extracts. We can say that this fruit can be easily consumed for liver and stomach protection.

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The author's contribution

A.K: The care of the animals, drug administration, pharmacological support,
O.A.B: The biochemical analysis, statistics of data and interpretation of results
G.G.P: The biochemical analysis, drug administration

Conflict to interest

All authors declare that there are no conflicts of interest.

References

1. Dart, R.C., Erdman, A.R., Olson, K., Christianson, G., Manoguerra, A.S., Chyka, P.A., et al. (2006). Acetaminophen poisoning: an evidence-based consensus guideline for out-ofhospital management. Clin. Toxicol. 44 (5):1-18.
2. Jaeschke, H. (2015). Acetaminophen: dose-dependent drug hepatotoxicity and acute liver failure in patients. Dig. Dis. 33(4):464-471.
3. Larson, A.M., Polson, J., Fontana, R.J., Davern, T.J., Lalani, E., Hynan, L.S., et al. (2005). Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology. 42(6):1364-1372.
4. Ostapowicz, G., et al. (2012). Results of a prospective study of acute liver failure at 17 tertiary care centers in the united states. Ann. Intern. Med. 137:947-954.
5. Bateman, D.N., Carroll, R., Pettie, J., Yamamoto, T., Elamin, M.E., Peart, L., et al. (2014). Effect of the UK's revised paracetamol poisoning management guidelines on admissions,adverse reactions and costs of treatment. Br J Clin Pharmacol. 78(3):610-618.
6. Wong, A., Graudins, A., Kerr, F., Greene, S.L. (2014). Paracetamol toxicity: What would be the implications of a change in Australian treatment guidelines? EMA – Emerg. Med. Australas. 26(2):183-187.

7. Hawton, K., Bergen, H., Simkin, S., Dodd, S., Pocock, P., Bernal, W., et al (2013). Long term effect of reduced pack sizes of paracetamol on poisoning deaths and liver transplant activity in England and Wales: interrupted time series analyses. BMJ. 346(7895):1-9.
8. Jaeschke, H., McGill, M.R., Ramachandran, A. (2012). Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. Drug Metab Rev. 44 (1):88-106.
9. Ben Amara I, Soudani N, Troudi A, Bouaziz H, Boudawara T. (2011). Zeghal N: Antioxidant effect of vitamin E and selenium on hepatotoxicity induced by dimethoate in female adult rats. Ecotoxicol Environ Saf. (74): 811-819.
10. Al-Awthan YS, Al-Douis MA, El-Sokkary GH. (2012). Aqlan EM: Dimethoateinduced oxidative stress and morphological changes in the liver of guinea pig and the protective effect of vitamin C and E. Asian J Biol Sci. 5(1):9-19.
11. Beyhan O: (2010). A study on selection of promising native cherry laurel (Prunus laurocerasus L.) genotypes from Sakarya, Turkey. J Anim Plant Sci. 20(4):231-233.
12. Alasalvar C, Al-Farsi M, Shahidi F: Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez. J Food Sci. 70(1):47-51.
13. Halilova H. (2010). Ercisli S: Several physico-chemical characteristic of cherry laurel (Laurocerasus officinalis Roem.) fruits. Biotechnol Biotechnol Eq. 24:1970-1973.
14. Miranda, K.M., Espey, M.G., Wink, D.A, A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 2001. (5), 62-71.
15. Aebi H. (1984). Catalase Methods. Enzymol. 105:121-126.
16. Sun Y., Larry W.O., & Ying L. (1988). A simple method for clinical assay of superoxide dismutase. Clin Chem. 34:497-500.
17. Sedlak J., & Lindsay R.H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryls groups in tissue with Ellman's reagent. Anal Biochem, 25:192-205.
18. Ohkawa H., Ohishi H., & Yagi K. (1979). Assay for lipid peroxide in animal tissues by thiobarbutiric acid reaction. Anal Biochem. 95:351–358.
19. Abdel Zaher AO, Abdel Rahman MM, Hafez MM, Omran FM. (2007). Role of nitric oxide and reduced glutathione in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against acetaminophen-induced hepatic and renal damage. Toxicology. 234:124-134.
20. Sasidharan S, Aravindran S, Latha LY, Vijenthi R, Saravanan D, Amutha S. (2010). In vitro antioxidant activity and hepatoprotective effects of Lentinula edodes against paracetamol-induced hepatotoxicity. Molecules. 15:4478-4489.
21. Prescott LF. (2000). Paracetamol, alcohol and the liver. British Journal of Clinical Pharmacology. 49:291-301.
22. Younes M, Cornelius S, Siegers CP. (1986).Ferrous ion supported in vivo lipid peroxidation induced by paracetamol – its relation to hepatotoxicity. Res Commun Chem Pathol Pharmacol. 51:89-99.
23. Muriel P, Garciapina T, Perez-Alvarez V, Mourelle M. (1992). Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. J Appl Toxicol. 12:439-442.
24. Lee DM, Hoffman WH, Carl GF, et al. (2002). Lipid peroxidation and antioxidant vitamins prior to, during, after correction of diabetic ketoacidosis. J Diabetes Complications. 16:294-300.
25. Curtis SJ, Moritz M, Snodgrass PJ. (1972). Serum enzymes derived from liver cell fractions. I. The response to carbon tetrachloride intoxication in rats. Gastroenterology. 62:84-92.
26. Eldutar E, Kandemir FM, Kucukler S, Caglayan C. (2017). Restorative effects of Chrysin pretreatment on oxidant–antioxidant status, inflammatory cytokine production, and apoptotic and autophagic markers in acute paracetamol-induced hepatotoxicity in rats: An experimental and biochemical study. Journal of Biochemical and Molecular Toxicology. 31:21-60.
27. El-Maddawy ZK, El-Sayed YS. Comparative analysis of the protective effects of curcumin and N-acetyl cysteine against paracetamol-induced hepatic, renal, and testicular toxicity in Wistar rats. Environmental Science and Pollution Research, 2018, 25: 3468-3479.Jaeschke, H., McGill, M.R. (2012). Ramachandran, A, Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. Drug Metab. Rev. 44 (1):88-106.
28. Laskin DL, Rodriguez del Valle M, Heck DE, Hwang S-M, Ohnishi ST, Durham SK, Goller NL, et al. (1995). Hepatic nitric oxide production following acute endotoxemia in rats is mediated by increased nitric oxide synthase gene expression. Hepatology. 22:223-34.
29. Gardner CR, Heck DE, Feder LS, Mc Closkey TW, Laskin JD, Laskin DL. (1992). Differential regulation of reactive oxygen and nitrogen intermediate production by hepatic macrophages and endothelial cells. In: Jesaitus A, Dratz E, eds. The molecular basis of oxidative damage by leukocytes. Boca Raton, FL: CRC Press. 267-272.
30. Hinson JA, Pike SL, Pumford NR, Mayeux PR. (1998).Nitrotyrosine-protein adducts in hepatic centrilobular areas following toxic doses of acetaminophen in mice. Chem Res Toxicol. 11:604-607.

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