The protective effects of naringin against 5-fluorouracil-induced hepatotoxicity and nephrotoxicity in rats

Volkan Gelen 1*, Emin Şengül 2, Serkan Yıldırım 3, Gözde Atila 1

1 Department of Physiology, Veterinary Faculty, Kafkas University, Kars, Turkey
2 Department of Physiology, Veterinary Faculty, Ataturk University, Erzurum, Turkey
3 Department of Pathology, Veterinary Faculty, Ataturk University, Erzurum, Turkey

ABSTRACT

Objective(s): 5-fluorouracil-induced (5-FU), an anticarcinogenic agent, is reported to have side-effects that include hepatotoxicity and nephrotoxicity. The study objective was to investigate the protective effects of naringin on 5-FU-induced hepatotoxicity and nephrotoxicity.

Materials and Methods: Thirty rodents were assigned to three groups. The control group received 1 ml of intragastric distilled water for 14 days. The 5-FU group received 1 ml of distilled water for 14 days as a placebo. On day 9, this same group received a 20 mg/kg dose of 5-FU administered intraperitoneally (IP) for a further five days. The naringin + 5-FU group received a 100 mg/kg dose of naringin (IP) for 14 days. On day 9, 20 mg/kg of 5-FU was administered (IP) to this group for a further five days. On day 15, the rats were decapitated, and blood and renal and hepatic tissues were taken.

Results: It was determined that serum creatinine, BUN, AST, ALT, ALP, and LDH levels, as well as cytokine levels in the liver and kidney tissues were significantly elevated in the 5-FU group, compared to the control group. The comparative values were similar in the control and naringin + 5-FU groups. When the liver tissue was examined histopathologically, in the control group, it was found to be normal in structure. However, necrosis was observed in the hepatocytes of the pericentric region in the 5-FU group. 8-OHdG cell density was significantly elevated in the 5-FU group, compared to the control and naringin + 5-FU groups.

Conclusion: Naringin was observed to have a protective effect on 5-FU-induced liver and kidney damage.

Introduction

5-fluorouracil (5-FU), a fluorinated pyrimidine, is classified as an antimetabolic agent and influences the synthesis of DNA and RNA in normal and tumor cells. The majority of 5-FU is abolished through liver metabolism and only a small portion is removed from the body via kidney excretion. 5-FU is widely used in chemotherapy for various cancers (1). As a fluoropyrimidine antimetabolite agent, it plays an important role in the treatment of colon, breast, gastrointestinal, head, neck, and pancreatic cancer (1). However, serious toxicity and unwanted side-effects occur following its use (2) and it is considered to be a nephrotoxic compound (3). In addition, it has hepatotoxic effects, with increased aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity in tissue (4, 5).

A number of studies have been conducted on the use of natural therapies to avoid the side-effects of anticancer agents (6-10). Naringin is a flavonoid that is usually found in grapefruit, orange and cooked tomato paste (11). It has antioxidant, immunomodulatory and anti-inflammatory properties. Flavonoids may have a protective effect against disease through various mechanisms, i.e., by activating and protecting antioxidant enzymes in the cells (12) or reacting directly or indirectly to reactive oxygen species (ROS) via the transfer of hydrogen atoms (13). Naringin was reported in various studies to be protective against hepatotoxicity and nephrotoxicity (14, 15).

The objective of the current study was to evaluate the hepatoprotective and nephroprotective potential of naringin against 5-FU-induced liver and renal toxicity.

Materials and Methods

Thirty male adult Sprague-Dawley rats, weighing 220–250 g, were included in the study. The animals were housed under adequate moisture and light conditions, at a suitable room temperature, and were provided with sufficient water and food until the day of the experiment. The study was performed in accordance with the national guidelines on the use and care of laboratory animals and was approved by the Animal Experiments’ Ethics Committee of Kafkas University.

The rats were divided into three groups (Table 1), consisting of a control and two experimental groups. The control group received 1 ml intragastric distilled water for 14 days. The 5-FU group received 1 ml of distilled water for 14 days as a placebo. On day 9, this same group received a 20 mg/kg dose of 5-FU that was administered (IP) for a further five days. The naringin + 5-FU group received a 100 mg/kg dose of naringin (dissolved in water oil) for 14 days. On day 9, 20 mg/kg 5-FU was administered (IP) to this group for a further five days.

On day 15, the rats were anesthetized, intracardiac
blood samples were taken and the animals were sacrificed. Blood, kidney, and liver tissue samples were collected for biochemical analysis to determine the cytokine parameters (interleukin [IL]-1α, tumor necrosis factor-alpha [TNF-α], and IL-6). A histopathological examination was conducted.

**Blood sample collection**
Five days after taking the 5-FU treatment (i.e., on day 15), blood samples were separately collected from the liver and kidneys of each rat. Thereafter, the rodents were euthanized via cervical dislocation. The blood samples were centrifuged at 1,500 g for 12 min within 1 hr of collection to obtain serum samples, which were immediately analyzed.

**Oxidative parameters**
The hepatic and renal tissues were homogenized using TissueLyzer II® (Qiagen, Germantown, USA). The homogenates were centrifuged at 10,000 g for 20 min at 4 °C and supernatants were obtained. Superoxide dismutase (SOD) activity was assessed and thiobarbituric acid reactive substance and glutathione (GSH) levels were determined (16).

**Biochemical cytokine analysis**
Rat-specific cytokine levels were established for IL-6, TNF-α, and IL-1α using an immunoassay kit (Elabscience Biotechnology Co., Ltd, USA), according to manufacturer’s instructions. The results were expressed as mean ± standard deviation (SD) (pg/ml or ng/ml).

**Histopathological and immunohistochemical study**
The liver and kidney tissue samples were collected, placed in a 10% formalin solution for 48 hr and then washed under running tap water for histopathological evaluation. The tissue was routinely processed and was then buried in blocks of paraffin. Tissue sections cut to 4 µm thickness were taken from each block, placed on the slides, stained and examined under a Leica DM Laboratory Microscope (Leica Microsystems, Buffalo Grove, USA) to perform an accurate assessment of the hematoyxin-eosin and fibrous tissue staining and adhesion. 8-hydroxy-2'-deoxyguanosine (8-OhdG) staining was conducted with respect to hepatic and renal immunohistochemistry. 8-OhdG-positive cell intensity was scored as none = -; weak = +; moderate = ++ and strong = +++. 

**Statistical analysis**
All data were statistically evaluated by one-way ANOVA using SPSS 20.00, followed by Duncan post hoc test. The data were expressed as mean ± SD. P<0.05 was considered statistically significant.

**Results**

**Effect of naringin on liver and renal weight in g/kg body weight**
The weight of the liver and kidneys (g/kg of body

---

**Table 1. All groups of study and animal protocols**

| Groups            | Treatment                      | number of animals in groups |
|-------------------|--------------------------------|-----------------------------|
| Control           | Control (distilled water)      | 10                          |
| 5-FU              | 5-FU (20 mg/kg IP)             | 10                          |
| Naringin+ 5-FU    | 100 mg/kg naringin + 5-FU (20 mg/kg IP) | 10 |

---

**Table 2. Weight of organ in g/kg body weight in the experimental groups (P<0.05, n=10) the results are expressed as mean±SD**

| Groups            | weight of liver in g/kg body weight | weight of renal in g/kg body weight |
|-------------------|------------------------------------|------------------------------------|
| Control           | 0.44 ± 0.01                         | 0.16 ± 0.009                       |
| 5-FU              | 0.52 ± 0.02                         | 0.11 ± 0.008                       |
| Naringin+ 5-FU    | 0.39 ± 0.03                         | 0.07 ± 0.002                       |

---

**Figure 1. Illustration of levels of oxidative parameters (SOD, GSH, and TBARS) for all groups in the liver tissues. A; SOD activity, B; GSH activity, and C; TBARS levels, the letters indicate the statistical differences among groups (P<0.05, n=10), the results were expressed as mean±SD**

**Figure 2. Illustration of levels of oxidative parameters (SOD, GSH, and TBARS) for all groups in the renal tissues. A; SOD activity, B; GSH activity, and C; TBARS levels, the letters indicate the statistical differences among groups (P<0.05, n=10), the results were expressed as mean±SD**
Thiobarbituric acid reactive substance (TBARS) levels were higher in the 5-FU group than in the other groups ($P < 0.050$). However, treatment with a naringin dose of 100 mg greatly inhibited the increase in TBARS levels ($P < 0.050$). The data collected on TBARS levels for all groups are presented in Figures 1 C and 2 C.

**Effect of naringin on liver marker enzymes (ALT, AST, and ALP)**

The liver enzyme activities in the 5-FU-treated animals were shown to be significantly increased when compared to the control group ($P < 0.050$). An inverse result was found for the 100-mg naringin-treated group when compared to the 5-FU group ($P < 0.050$). The liver enzyme activity in all groups is depicted in Figures 3 A, B, and C.

**Effect of naringin on renal markers (LDH, BUN, and Creatinine)**

The LDH, blood urea nitrogen (BUN), and creatinine levels of the 5-FU-treated animals were greatly elevated, compared to the control group ($P < 0.050$). By contrast, the BUN and creatinine levels in the 100-mg naringin-treated group were significantly decreased in the 5-FU group ($P < 0.050$). The data on the LDH, BUN, and creatinine levels for all the groups are shown in Figures 3 D, E, and F.

**Biochemical cytokine (IL-6, IL-1α, and TNF-α) levels in the liver and renal tissues**

Following cytokine analysis, the IL-6 levels were shown to be greatly higher in the 5-FU group than in the other groups ($P < 0.050$) (Figure 4 A). A statistically significant difference between the control and naringin+5-FU groups in this regard was also observed ($P < 0.050$). A marked difference was also applicable to the IL-1α and TNF-α levels in the 5-FU group, when compared to the other groups ($P < 0.050$). A statistically significant difference was not found between the control and the naringin+5-FU groups in this regard ($P < 0.050$) (Figures 4 B and C).

Similarly, IL-6, IL-1α, and TNF-α levels were significantly higher in the 5-FU group than in the other groups ($P < 0.050$). A statistically significant difference between the control and naringin+5-FU groups was reported ($P < 0.050$) (Figures 5 A, B, and C).

**Histopathological examination**

Following liver and kidney tissue analysis, normal structure and histology were determined in the control group. Hydropic degeneration and coagulative necrosis were observed in the hepatocytes in the psoriasis region, and mononuclear cell infiltration in the portal region, in the 5-FU group, when the liver tissue was examined.
Following a kidney tissue assessment, tubular dilation, glomerular atrophy, dilatation of the Bowman’s capsule, and degeneration and/or necrosis of the renal tubular epithelial cells were identified in the 5-FU group. However, following liver tissue analysis, necrotic cells were not identified when hydropic degeneration of the hepatocytes was observed in the pericentric region in the naringin+5-FU group. Mild hydropic degeneration was detected in the tubular epithelium cells when the kidney tissue was examined in the naringin+5-FU group (Figures 6(A, B, C), 8(A, B, C) and Table 3, 4).

**Immunohistochemical findings**

8-OHdG immunopositive reactions, with the use of 8-OHdG antibodies, were assessed in the liver and kidneys in all groups. 8-OHdG cell density was significantly elevated in the 5-FU group when compared with the control and naringin+5-FU groups (Figures 7-9).

**Discussion**

A widely used chemotherapeutic agent, 5-FU, has proven efficacy in human malignancy. However, its clinical utility is inhibited by hepatotoxic and nephrotoxic side-effects (17,18). ROS are produced when electrons from different systems penetrate oxygen in a living organism. The cellular antioxidant enzymatic and nonenzymatic defense plays a crucial role in alleviating tissue damage caused by free radicals (19-21). ROS has a direct effect on various biological components. It causes cellular damage and necrosis in the liver, kidney and other tissues (22-25).
The antioxidant defense system is the primary protective mechanism used to prevent cell damage to ROS. ROS damage increases portal and systemic endotoxin levels and translocation to the liver, resulting in ingestion by neutrophils and the release of ROS at higher levels (26-28). The elimination of ROS in normal healthy cells is accomplished by a radical scavenging healthy cells is accomplished by a radical scavenging system, comprising catalase (CAT), superoxide dismutase (SOD), and reduced GSH (29). Oxidative stress can occur as a consequence of increased ROS production or a reduced antioxidant defense (30, 31).

An increased amount of ROS has been reported in liver hepatic and renal toxicity induced by 5-FU (32). It has been shown that antioxidants protect against 5-FU-induced hepatic and renal damage in rats. A reduction in the activity of significant antioxidant enzymes in the kidneys and liver, including SOD, CAT, and GSH, was also demonstrated following treatment with 5-FU (33), as was an increase in serum malondialdehyde levels due to 5-FU-induced hepatotoxicity and nephrotoxicity.

In the current study lipid peroxidation was determined by measuring TBARS levels in rodent liver and kidney tissues. TBARS levels were higher in the 5-FU group than in the other groups, thereby explaining accelerated peroxidation levels in the liver and kidneys. SOD activity and GSH concentrations were significantly decreased in the hepatic and renal tissue following treatment with 5-FU. In contrast, a marked increase in SOD activity and GSH levels were detected when naringin was given to this group. The findings suggest that 5-FU-induced hepatotoxicity and nephrotoxicity arises from ROS, which disrupts the antioxidant system. It is thought that naringin prevents damage due to its antioxidant properties. This result is consistent with that reported in a previous study (33).

Treatment with 5-FU resulted in a significant increase in serum ALT, AST, and ALP activities in the animals, in whom severe hepatotoxicity was established. These data are consistent with those reported elsewhere (34). Excessive oxidative production and the accumulation of oxidation products in the liver damaged the biological membranes and the endothelial lining of the liver in the current study. This was probably due to liver damage, causing elevated ALT, AST, and ALP concentrations in the blood. ALT, AST, and ALP are considered to be the most important biological markers of cellular damage and toxicity (35). A significant elevation in serum AST, ALT, and ALP activity has been used as an indicator of acute liver injury in other studies (36, 37).

ALT is a cytosolic enzyme that targets the liver rather than other tissues, and AST is found in the mitochondria and targets the liver, skeletal muscles, and kidneys. ALP activity increases due to obstruction and inflammation of the biliary tract. To a great extent, its increase in the blood occurs when ALT and AST infiltrate the hepatocytes. This is considered to be a marker of liver damage or dysfunction. The administration of naringin resulted in the considerable recovery of and a significant reduction in the activity of these enzymes, suggestive of hepatotoxicity in animals exposed to 5-FU.

LDH, BUN, and creatinine are commonly used as markers in the analysis of kidney injury (38). High BUN and serum creatinine levels indicate 5-FU-induced kidney damage. The results of the current study were consistent with the findings of previous studies (32). Lower LDH, BUN, and serum creatinine levels, when compared with the 5-FU treated group, were found in rats to whom naringin had been administered. A reduction in LDH, BUN, and creatinine levels is possibly caused by the nephroprotective efficacy of naringin. A significant increase in LDH was seen in the 5-FU group, compared to the other groups (32). However, the present findings revealed that naringin treatment significantly attenuated LDH, demonstrating that it has the potential to prevent renal damage.

The role of proinflammatory cytokines in the pathogenesis of hepatic and renal toxicity in relation to the cellular signaling pathways is still being researched. Cytokine secretion is the mediator of inflammation and contributes to the pathogenesis of tissue injury (39, 40). It has been reported that proinflammatory cytokines are associated with a significant increase in serum IL-1ß, TNF-α, and IL-6 levels following 5-FU treatment in rats (41).

The inhibitory effect of flavonoids in the release of chemical mediators, including IL-1α, TNF-α, and IL-6, was also reported (42). Previous studies reported the inhibitory effect of naringin on IL-1α, TNF-α, and IL-6 (43). In the current study, 5-FU treatment markedly increased IL-1α, TNF-α, and IL-6 levels. Conversely, naringin treatment caused a significant reduction in IL-1α, TNF-α, and IL-6 levels in 5-FU-induced experimental rats. This is possibly owing to its anti-inflammatory properties. In the light of these findings, it can be deduced that naringin prevents kidney and liver inflammation caused by 5-FU.

Histopathological examination of liver and renal tissues in the 5-FU-treated group suggested cell injuries and necrosis in the liver and renal tissues. In previous studies degeneration and coagulative necrosis were observed in the hepatocytes and mononuclear cell infiltration in the portal region (4, 44). Kidney tissue assessment, degeneration, and/or necrosis of the renal tubular epithelial cells were identified due to 5-FU administration (44). Thus, the results of the present

| Table 3. Histopathological evaluation of liver tissues | Control | 5-FU | Naringin + 5-FU |
|------------------------------------------------------|---------|-----|---------------|
| Degeneration in hepatocytes                          | -       | +++| ++            |
| Necrosis in hepatocytes                              | -       | +++| +            |
| Dilatation and hyperemia in sinusoids                 | -       | ++| +            |
| 8-OHdG                                               | +       | +++| ++           |

Scored as: none = -; weak = +; moderate = ++; strong = +++

| Table 4. Histopathological evaluation of renal tissue | Control | 5-FU | Naringin + 5-FU |
|------------------------------------------------------|---------|-----|---------------|
| Degeneration in tubal epithelium                     | -       | +++| ++            |
| Necrosis in tubal epithelium                         | -       | +++| +            |
| Hyperemia                                            | -       | +++| +            |
| 8-OHdG                                               | +       | +++| ++           |

Scored as follows: none = -; weak = +; moderate = ++; strong = +++
study revealed that naringin treatment resulted in minimal liver and renal damage and no necrosis in the liver and kidneys of 5-FU-induced rats.

8-OHdG is considered to be the most important indicator of DNA damage (45, 46). Hydroxyl radicals destroy the hydrogenation of nucleic acid or react with double bonds, leading to 8-OHdG (47). El-Sayad et al. suggested that DNA damage intensified in rat testes following the administration of 5-FU (48). 8-OHdG was shown to be a good indicator of tissue damage following 5-FU-induced nephrotoxicity in experimental rats in the current study. It has been reported elsewhere that DNA damage was diminished following the administration of antioxidants (49). This supports the current study finding that naringin had an antioxidant effect, thereby reducing ROS-mediated 8-OHdG levels and preventing oxidative DNA damage. This suggests that naringin treatment has an important restorative effect on renal and hepatic injury induced by 5-FU.

Conclusion

Naringin treatment can mitigate renal and hepatic damage caused by 5-FU-induced renal and hepatic toxicity in rats. Naringin also enhances the restoration of biochemical oxidative enzymes as it has anti-inflammatory, antioxidant, and DNA-protective properties. It was found in the current study that naringin was protective against 5-FU-induced renal and hepatic toxicity. Further studies are warranted to investigate its future clinical applications.

Acknowledgment

The authors thank the staff of the Veterinary Physiology Department of Kafkas University, Kars, Turkey, due to laboratory support.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Longley DB, Harlín DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 2003;3:330-338.
2. Cabellos R, Garcia-Carbonero R, Garcia-Lacalle C, Gomez P, Tercero A, Sanchez D, et al. Histopathological effects of ascorbic acid and water extract of Spirulina plantesis (blue green algae) on 5-fluorouracil-induced lipid peroxidation. Acta Pol Pharm 2007;64: 35-44.
3. Isaka Y, Raługi H. Severe adverse effects of 5-fluorouracil in S-1 were lessened by haemodialysis due to elimination of the drug. NDT Plus Advance Access 2009;18:152-154.
4. Gelen V, Şengül E, Gedikli S, Atıla G, Uslu H, Makav M. The protective effect of rutin and quercetin on 5-FU-induced hepatic toxicity in rats. Asian Pac J Trop Biomed 2017;7:647-653.
5. Ray S, Roy K, Sengupta C. In vitro evaluation of protective effects of ascorbic acid and water extract of Spirulina platensis (blue green algae) on 5-fluorouracil-induced lipid peroxidation. Acta Pol Pharm 2007;64: 35-44.
6. Şengül E, Gelen V, Gedikli S, Özkanlar S, Gür S, Çelebi F, et al. The protective effect of quercetin on cyclophosphamide-induced lung toxicity in rats. Biomed Pharmacother 2017;92:303-307.
7. Choi JAS, Piao YJ, Kang KW. Effect of quercetin on the bioavailability of doxorubicin in rats: role of CYP3A4 and Pgp inhibition by quercetin. Arch Pharm Res 2011;34:607-620.
8. Swamy AH, Patel UM, Koti BC, Gadad PC, Patel NL, Thippeswamy AH. Cardioprotective effect of Saracindicavagainst cyclophosphamide induced cardiac toxicity in rats: a biochemical, electrocardiographic and histopathological study. Indian J Pharmacol 2013;45:48-56.
9. Raskovic A, Stilinovic N, Kolarovic J, Vasovic V, Vukmirovic S, Mikov M. The Protective Effects of Silymarin against Doxorubicin-Induced Cardiotoxicity and Hepatotoxicity in Rat. Molecules 2011;16: 8601-8614.
10. Matouk AI, Taye A, Heeba GH, El-Moselhy MA. Quercetin augments the protective effect of losartan against chronic doxorubicin cardiotoxicity in rats. Environ Toxicol Pharmacol 2013;36: 443-449.
11. Igual M, Garcia-Martinez E, Camacho MM, Martinez-Navaanrete N. Jam processing and storage effects on β-carotene and flavonoids content in grapefruit. J Funct Foods 2013;5:736-744.
12. Orsolić N, Gajski G, Garaj-Vrhovac V, Bikik D, Prskalo ZS, Sirovina D. DNA-protective effects of quercetin or naringin in alloxan-diabetic mice. Eur J Pharmacol 2011;110:110-118.
13. Leopoldini M, Russo N, Toscano M. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chem 2011;125:288-306.
14. Lee MH, Yoon S, Moon J. O. The flavonoid naringin inhibits dimethylnitrosamine-induced liver damage in rats. Biol Pharm Bull 2004;27:72-76.
15. Singh D, Chander V, Chopra K. Protective effect of naringin, a bioflavonoid on ferric nitrotriacetate-induced oxidative renal damage in rat kidney. Toxicology 2004; 201:1-8.
16. Shi Z, Cao J, Chen J, Li S, Zhang Z, Yang B, et al. Butenolide induced cytotoxicity by disturbing the prooxidant- antioxidant balance, and antioxidants partly quench in human chondrocytes. Toxico in Vitro 2009;23:99-104.
17. Skretkowicz J, Sekulmska M, Danilewicz M, Wagrowska-Danilewicz M, Polakowski P. Effect of some antioxidant drugs and combined chemotherapy on renal toxicity. Biol Signals Recept 1996;5:51-58.
18. El-Sayad HL, Ismail ME, Shalaby FM, Abou-El- Magd RE, Gaur RL, et al. Histopathological effects of cisplatin, doxorubicin and 5-fluorouracil (5-FU) on the liver of male albino rats. Int J Toxicol 2009;36: 443-493.
19. Gulcin I, Berashvili D, Gepdiremen A. Antiradical and antioxidant activity of total anthocyanins from Perilla pankinensis decne. J Ethnopharmacol 2005;101:287-293.
20. Polidoro G, Dillio C, La Rovere G, Fedrici GS. Superoxide dismutase, reduced glutathione and TBA-reactive products in erythrocytes of patients with multiple sclerosis. Int J Biochem 1984;16:505-509.
21. Polat Köse L, Gülcin I, Gören AC, Namiesnik J, Martinez-Ayalà AL, Gorinstein S. LC-MS/MS analysis, antioxidant and anticholinergic properties of galanga (Alpinia officinarum Hance) rhizomes. Ind Crops Prod 2015;74:712-721.
22. Gulcin I, Beydemir S. Phenolic compounds as antioxidants: Carbonic anhydrase isoenzymes inhibitors. Mini Rev Med Chem 2013;13:408-430.
23. Sehitioglu MH, Han H, Kalin P, Gulcin I, Ozkan A, Aboul-Enein HY, et al. Gum A potent inhibitor of reactive oxygen species. J Enzym Inhib Med Chem 2015;30:264–269.
24. Bae JH, Park YJ, Namiesnik JJ, Gülcin I, Kim TC, Kim HC, et al. Effects of artificial lighting on bioactivity of sweet red pepper (Capsicum annuum L.). Int J Food Sci Technol 2016;51:1378–1385.
25. Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity. A review. Am J Med Sci 2007;334:115–124.
26. Ren W, Wang X, Zhang A, Li C, Chen G, Ge X, et al. Selective bowel decontamination improves the survival of 90% hepatectomy in rats. J Surg Res 2015;195:454–464.
27. Alwahsh SM, Xu M, Seyhan SA, Ahmad S, Mihm S, Ramadori G, et al. Diet high in fructose leads to an overexpression of lipocalin-2 in rat fatty tissue. World J Gastroenterol 2014;20:1807–1821.

28. Godos J, Federico A, Dallio M, Scaglione F. Mediterranea diet and nonalcoholic fatty liver disease: Molecular mechanisms of protection. Int J Food Sci Nutr 2017;68:18–27.

29. Gulcin I. Antioxidant and antiinflammatory activities of L-Carnitine. Life Sci 2006;78:803–811.

30. Buyukokuroglu ME, Gulcin I, Oktay M, Kufrevioglu O. In vitro antioxidant properties of dantrolene sodium. Pharmacol Res 2001;44:491–494.

31. Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI. In vitro antioxidant properties of dantrolene sodium. Antioxidant and analgesic activities of turpentine of Pinus nigra Arn. Subsp. pallasiana (Lamb.) Holmboe. J Ethnopharmacol 2003;86:51–58.

32. Nora El-Hoseany MA. Protective Effect of Captopril against 5-Fluorouracil-Induced Hepato and Nephrotoxicity in Male Albino Rats. J American Sci 2012;8:680-685.

33. Afolabi OK, Adeleke1 GE, Ugbaja RN. Crocin Alleviates 5-Fluorouracil-induced Hepatotoxicity through the abrogation of Oxidative Stress in Male Wistar rats. Asian Pac J Health Sci 2016;3:58-68.

34. Dimitriu D, Lupusoru C, Cojocaru I, Gafitanu C, Palade L, Lupusoru R. Assessing biochemical and oxidative stress parameters after vaginal and oral administration of 5-fluorouracil in laboratory animals. Farmacia 2015; 63:230-233.

35. Stockham SL, Scott MA. Fundamentals of Veterinary Clinical Pathology. Ames, Iowa State University Press 2002;52:434–459.

36. Zeashan H, Amresh G, Singh S, Rao CV. Hepatoprotective and antioxidant activity of Amaranthus spinosus against CCl4 induced toxicity. J Ethnopharmacol 2009;125:364–366.

37. MacNamara E, Goldberg DM. Serum enzymes and enzyme profiles in the diagnosis of liver and biliary tract diseases. Surv Dig Dis 1985;3:165–186.

38. Nada O. A review of propolis antitumour action in vivo and in vitro. J Api Product Api Med Sci 2010;2:1–20.

39. Laverty HG, Antoine DJ, Benson G, Chaponda M, Williams D, Park BK. The potential of cytokines as safety biomarkers for drug-induced liver injury. Eur J Clin Pharmacol 2010;66:961–976.

40. Lacour S, Gautier JC, Pallardy M, Roberts R. Cytokines as potential biomarkers of liver toxicity. Cancer Biomarkers 2005;1:29–39.

41. Chang CT, Ho TY, Lin H, Liang JA, Huang HC. 5-Fluorouracil induced intestinal mucositis via nuclear factor-kB activation by transcriptomic analysis and in vivo bioluminescence imaging. PLoS ONE 2017;7: e31808.

42. Peluso I, Raguzzini A, Serafini M. Effect of flavonoids on circulating levels of TNF-α and IL-6 in human: a systematic review and meta-analysis. Mol Nutr Food 2015;57:784-801.

43. Pinho-Ribeiro FA, Zarpelon AC, Mizokami SS, Borghi SM, Bordignon J, Silva RL, et al. The citrus flavonone naringin reduces lipopolysaccharide-induced inflammatory pain and leukocyte recruitment by inhibiting NF-κB activation. J Nutr Biochem 2016;33:8-14.

44. Hak, Heba Nageh Gad EL, Tarek Ibrahim Saad M, Gamal Abdel-A. Histological study of the effect of chemotherapy with 5-Fluorouracil on normal liver and kidney of mice. International J Novel Res Life Sci 2015;2:8-13.

45. Cadet J, Douki T, Gasparutto D, Ravanat JL. Oxidative damage to DNA: formation, measurement and biochemical features. Mutat Res Fund Mol M 2003; 531:5–23.

46. Stepniak J, Karbownik-Lewinska M. 17 β-estradiol prevents experimentally-induced oxidative damage to membrane lipids and nuclear DNA in porcine ovary. Sys Biol Reprod Med 2016;62:17–21.

47. Cadet J. Oxidative degradation pathways of cellular DNA: product formation and mechanistic insights. Free Radical Biol Med 2016;75:S2.

48. El-Sayyad IH, Hassan A. Effects of adriamycin, cisplatin, and 5-fluorouracil on the testes of albino rats. Br J Med Health Sci 2013;1:45-62.

49. Ince S, Arslan Acaroz D, Neuwirth O, Hasan Hüseyin D, Bars D, Ismail K, et al. Protective effect of polydatin, a natural precursor of resveratrol, against cisplatin-induced toxicity in rats. Food Chem Toxicol 2014;72:147-153.