The Effect of *Capparis Spinosa* L. Plant on the Cytochrome and Glutathione to Reduce the Hepatotoxicity induced by Paracetamol in Mice

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Abstract

The acetaminophen is one of analgesics; non-steroidal anti-inflammatory drugs can cause the hepatotoxicity. Many of the hepatoprotective of plant use in medicine to treatment of hepatic disorders. The aqueous *Capparis spinosa* extract (CSE) (500 mg/kg) was used to reduce the hepatotoxicity induced by paracetamol (PARA) (300mg/kg). The current study, 70 male albino mice (25-30 g) were divided into five group; group I: It were received 0.9% sodium chloride (control), group II: It were given PARA intraperitoneally (IP) (300 mg/kg), single dose, group III: It were received PARA as a single dose (300 mg/kg) intraperitoneally (IP) directly followed by oral administration of the CSE (500 mg/kg) single dose per day for 21 days, group IV: It were received CSE (500 mg/kg) single dose per days for 21 days the injected by PARA intraperitoneally (IP) (300mg/kg), and Group V: It were administered orally of CSE only (500 mg/kg) per days for 21 days. The animals each groups above sacrificed at 1 h, 6h, 12h, 24h, 72h, 10 days and 21 days. Blood samples were collected to determine the serum of CYP450 2E1 and GSH. The PARA (300mg/kg) increased the CYP450 2E1 and reduced the GSH serum levels significantly when compared with the control group (P<0.05). The CSE showed non- significantly effect on these markers. The CSE showed the higher reducing effect on CYP450 2E1 and reduced the GSH serum levels significantly when compared with the control group (P<0.05). The CSE showed non- significantly effect on these markers. The CSE showed the higher reducing effect on CYP450 2E1 and reduced the GSH serum levels significantly when compared with the control group (P<0.05). This research conclude that the CSE (500 mg/kg) reduces the hepatotoxicity of paracetamol (300mg/kg IP) significantly.

Keywords: *Capparis Spinosa* L., Cytochrome, Glutathione, Reducing Hepatotoxicity of Paracetamol.
Introduction

The liver is the main organ for detoxification and disposal of external and internal compounds, and on the other hand, it is consider one of the causes of death and death in the world1. Which occurs due to damage or defect due to the accumulation of medicines or foreign objects2. Factors external to poisoning, in general, include clinical related factors such as drugs used for superfluous glomerular dosage such as acetaminophen etc3. The paracetamol (acetaminophen) is one of the most common medicines prescribed by physicians and its overdose is toxic to the liver4. Its affects by two mechanisms, direct and indirect hepatotoxicity 5, 6, and it is converted to NAPQI by CYP450 2E1, which is considered one of the causes of liver injury because it has a tendency to union with cysteine found in liver proteins7. In the recent period, there are many attempts to use medicinal plants as natural sources to reduce the toxic effects of drugs and have become a research material such as reducing the effect of doxorubicin on cardiac tissue and enzymes by using artemisinin compound 8 and reduce the toxic effects of Dichlorvos on liver enzymes using turmeric extract9. The caper plant Capparis Spinosa (Capparidaceae) used in traditional medicine to treat many diseases such as rheumatism, gastrointestinal problems headache, kidney and liver disease as well as toothache10, diabetes11, and Different parts of caper have antioxidant effects, potentially useful against some degenerative diseases12. Therefore, this research aimed to study the effect of capparis spinosa l. plant on the cytochrome and glutathione enzyme to treat and protect the liver from the toxic effects of paracetamol in mice.

Materials and Methods

Plant material and aqueous extract preparation

The fruits of the plant collected from the Afak of Al-Qadisiyah Governorate, Iraq in August-September 2019. It diagnosed in the biotechnology laboratory of the Biology Department, Faculty of Science/ University of Kufa, Iraq. The fruits collected, washed well, and then cut to get rid of the seeds, dried at 35 °C, and then grounded well using a laboratory blender. The aqueous extract occurred according to 13 with simple modified, the modified was taking a 500 g was extracted with ddH2O (10gm/100ml), then the extract was filtered using filter paper, dried in an oven at 50 °C. the dose admiration was 500gm/kg of mice.

Preparation of Paracetamol

The paracetamol (Sigma Aldrich Cat. NO. A3035) was prepared attended in 0.9% sodium chloride (NaCl 0.9%) at a concentration of 15 mg / mL at 30˚C in a water bath14.

Animal experimental design:

Experiments were performed in adult male albino Mice weighing 25 to 30 g. The animals were housed under standard environmental conditions (25 ± 2 °C, with 55 ± 5% humidity and a 12 h light/dark cycle) and maintained with free access to water and billet. The acclimation occurred in the laboratory condition for a week before. Five groups, fourteen animals for each group, the animals treated as the following:

Group I: 0.9% Sodium chloride, intraperitoneal injection (IP) as control animals. The animals sacrificed during 1 h, 6 h, 12h, 24 h, 72h, 10 days, and 21 days periods

Group II: 300 mg/kg, a single dose of paracetamol (IP). The animals sacrificed during 1 h, 6 h, 12h, 24 h, 72h, 10 days, and 21 days periods.

Group III: 300 mg/kg, a single dose of paracetamol (IP), and 500 mg/kg of aqueous extract Capparis spinosa oral administrated (OD), a single dose per 1 h, 6 h, 12h, 24 h, 72h, 10 days, and 21 days, and the animals sacrificed during these periods. This paracetamol dose was chosen as a standard dose to induce toxicity in C57BL/6 mice7.

Group IV: 500 mg/kg of aqueous extract Capparis spinosa (OD), a single dose per 1 h, 6 h, 12h, 24 h, 72h, 10 days, and 21 days. Followed the twenty-first day by a 300 mg/kg, a single dose of paracetamol (IP). Then, the animals sacrificed during 1 h, 6 h, 12h, 24 h, 72h, 10 days, and 21 days periods.
Group V: 500 mg/kg of aqueous extract *Capparis spinosa* (OD), a single dose per 1 h, 6 h, 12h, 24 h, 72h, 10 days, and 21 days, the animals sacrificed during these periods.

**Blood Collection**

The animal sacrificed to collect the blood sample from the mice. The serum was obtained by It is centrifugation (3000 rpm for 20 min), and stored at -20°C before analysis of CYP 450 2E1 and glutathione GSH (purchase from Bioassay Technology Laboratory, Shanghai Korain) by using the automated clinical chemistry analyzer.

**Bio-statistical Analysis**

The results were expressed as (mean ± standard Error and analyzed using Genstat program version 8. For all parameters, comparisons among groups carried out using two-way analysis of variance (ANOVA). P < 0.05 was considered significant.

**Results**

The CYP450 2E1 levels (mg/dl) in liver mice showed increased significantly in paracetamol group compared with the control group while *Capparis Spinosa* extract was not significantly different when compared with the control (P<0.05), Table (1).

The interaction effect CSE and PARA agents on this hepatic enzyme levels, the increasing percent of this enzyme by PARA alone compared with normal level in a control were reduced about 21.08% and 19.53% in PARA+CSE and CSE+PARA groups respectively, these reducing may be by CSE activity, Figure (1). The PARA showed a high level of CYP450 2E1 after 12 hours compared with after treated.

Table (1) showed the serum cytochrome P450 2E1 (CYP450 2E1) levels of albino mice (mg/dl), hepatotoxicity inducing by paracetamol, and reducing hepatotoxicity effect by *Capparis Spinosa* fruit extract.

| Period | Treatment | 1 h     | 6 h     | 12 h    | 24 h    | 72 h    | 10 d    | 21 d    | Mean b |
|--------|-----------|---------|---------|---------|---------|---------|---------|---------|--------|
|        | Control   | 1.13 ±0.09 | 1.72 ± 0.06 | 1.63 ± 0.05 | 1.23 ± 0.1 | 1.83 ± 0.02 | 1.73 ± 0.04 | 2.03 ± 0.19 | 1.61 ±0.06 |
|        | PARA      | 5.56 ±0.09 | 5.94 ± 0.02 | 6.89 ± 0.04 | 5.49 ± 0.09 | 5.50 ± 0.11 | 4.97 ± 0.01 | 3.06 ± 0.01 | 5.34 ±0.01 |
|        | PARA+ CSE | 4.89 ±0.15 | 4.82 ± 0.02 | 5.22 ± 0.02 | 4.03 ± 0.09 | 5.52 ± 0.21 | 4.82 ± 0.01 | 2.76 ± 0.01 | 4.58 ±0.01 |
|        | CSE +PARA | 5.30 ±0.25 | 5.67 ± 0.34 | 4.35 ± 0.21 | 5.02 ± 0.21 | 4.59 ± 0.12 | 4.02 ± 0.01 | 2.99 ± 0.01 | 4.56 ±0.01 |
|        | CSE only  | 1.08 ±0.32 | 1.39 ± 0.21 | 1.44 ± 0.03 | 1.21 ± 0.32 | 1.32 ± 0.12 | 1.24 ± 0.01 | 1.26 ± 0.01 | 1.31 ±0.01 |
|        | Mean a    | 3.592   | 3.908   | 3.906   | 3.448   | 3.752   | 3.356   | 2.42    |        |

LSD P < 0.05 a=0.475                      ab=1.343                      b=0.562

Key: PARA: Paracetamol (300 mg/kg, intraperitoneal injection IP), CSE: *Capparis Spinosa* fruit extract (500mg/kg, oral administrated OD).
Figure (1): Showed the effectiveness of cytochrome P450 2E1 (CYP450 2E1) Levels percent by agents compared with control.

The serum of GSH level (mg/dl) in this study presented in Table (2), the PARA showed decreasing significantly of glutathione (GSH) level about 53.80% compared with the control group, while the CSE was showed increasing about 0.61% with not significantly compared with control (P<0.05), Figure (2). The interaction effect CSE and PARA agents on GSH levels, the reducing effect percent of GSH by PARA alone compared with normal level in control group were reduced about 62.52% and 24.73% in PARA+CSE and SCE+PARA groups respectively, Figure (2), Table (2).

Table (2): Showed the serum glutathione (GSH mg/dl) levels of albino mice, hepatotoxicity inducing by paracetamol, and reducing hepatotoxicity effect by Capparis Spinosa fruit extract.

| Period | Treatment  | 1 h     | 6 h     | 12 h    | 24 h    | 72 h    | 10 d    | 21 d    | Mean b |
|--------|------------|---------|---------|---------|---------|---------|---------|---------|--------|
|        | Control    | 120.10 ±| 118.13 ±| 119.70 ±| 119.02 ±| 120.19 ±| 118.90 ±| 121.10 ±| 119.59  |
|        | PARA       | 70.61 ± | 68.21 ± | 58.28 ± | 50.93 ± | 47.28 ± | 50.51 ± | 41.19 ± | 55.28  |
|        | PARA+CSE   | 95.73 ± | 92.92 ± | 94.13 ± | 97.98 ± | 90.27 ± | 97.78 ± | 100.59 ±| 83.48  |
|        | CSE+PARA   | 80.47 ± | 73.07 ± | 79.67 ± | 68.23 ± | 64.21 ± | 63.51 ± | 69.74 ± | 75.12  |
|        | CSE only   | 125.39 ±| 127.21 ±| 124.08 ±| 124.67 ±| 114.42 ±| 110.78 ±| 116.73 ±| 120.47 |
|        | Mean a     | 170.86  | 152.10  | 161.45  | 167.78  | 167.46  | 217.54  | 141.27  |

LSD P < 0.05      a=8.68  ab=19.33  b=10.26

Key: PARA; Paracetamol (300 mg/kg, intraperitoneal injection IP), CSE; Capparis Spinosa fruit extract (500mg/kg, oral administrated OD).

Figure (2): Showed the effectiveness of GSH Levels percent by agents compared with control.
Hepatotoxicity includes several types of injuries, depending on the type, nature, and dose of the cause, it can develop into more severe effects, such as necrosis, programed cell death and cholestasis, cirrhosis, liver tumors and hepatitis. The most common tests for detecting liver protection and activities are the size and weight of the liver and some biochemical estimates such as measurement of transaminase activity, SGPT, SCOT, alkaline phosphatase, serum bilirubin, total serum proteins, albumin, globulin and prothrombin time, functional parameters. Cytochrome is a group of enzymes that regulate pharmacokinetics within the body and response of 57 putatively functional human CYPs only about a dozen enzymes, belonging to the CYP 1, 2, and 3 families, it is responsible for the biological transformation of foreign substances, most of which are drugs. The CYP2E1 plays a role in the metabolism of internal source substances such as fatty acids and acetone and external source substances such as drugs, pollutants and ethanol. Its mechanism of action is to transfer electrons from the nicotinamide phosphate adenine dinonolotide as well as molecular oxygen to the substrates, the end product of this reaction is more polar groups within the substrates and the production of intermediate substances that are toxic, such as epoxide or aldehyde. It is responsible for the production of NAPQI, thus the metabolite binds either to glutathione within the detoxification mechanism or binds to the mitochondrial protein and thus leads to liver injury. An increased dose of acetaminophen increases the formation of NAPQI, which depletes the amount of glutathione, and instead binds to liver proteins. Its binding to hepatic proteins leads to increased oxidative stress, thereby weakening the mitochondrial function and loss adenosine triphosphate (ATP), increased oxidative stress and reduced ATP appear to lead to hepatic necrosis. In this study, the serum CYP450 2E1 level was increased at 1 h, 6 h, 12 h, 24 h, 72 hours (P <0.05) in comparison to control mice at zero time. Paracetamol is metabolized in the liver with three major pathways sulfonation, glucuronidation and oxidized. The oxidative process inside the hepatic microsomes is carried out by cytochrome p450. The metabolic activation of paracetamol was initiated by CYP-mediated conversion to NAPQI, which is the most proximal event in the toxicity mechanism. NAPQI causes oxidative stress and bind covalently to liver protein, which is normally detoxified by GSH both non-enzymatic and enzymatically in a reaction catalyzed by GSTs. The concurrent use of drugs and herbal products is becoming increasingly prevalent over the last decade. Several herbal products have been known to modulate cytochrome P450 (CYP) enzymes and P-glycoprotein (P-GP) which are recognized as representative drug-metabolizing enzymes and drug transporter, respectively. The main focus is the ability of herbal extracts and their phytochemicals to modulate the expression, and function of CYP and P-GP in several in vitro and in vivo animal and human systems. Glutathione is a tripeptide (cysteine, glycine, and glutamic acid) found in relatively high concentrations in many bodily tissues. It plays a pivotal role in reducing oxidative stress, maintaining redox balance, enhancing metabolic detoxification, and regulating the immune system. A major role of GSH is the antioxidant when acting as detoxification of hydrogen peroxide into the water by the non-enzymatic reaction. GSH protects cells against exogenous and endogenous toxins such as free radicals. C. Spinosa has been reported to possess profound anti-inflammatory and antioxidant activities. It protects against several models of oxidative stress. This research conclude that the CSE (500 mg/kg) which reduces the hepatotoxicity of paracetamol significantly.

Conclusions: This research conclude that the CSE (500 mg/kg) which reduces the hepatotoxicity of paracetamol significantly.

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Conflicts of interest: Declared none.
Ethics Statement: The research was conducted within the conditions of research ethics and approved by the Central Committee for Research Ethics at the University of Kufa / College of Science, no:722, 13/1/2020

Reference

1. Wang, F. S., Fan, J. G., Zhang, Z., Gao, B., & Wang, H. Y. (2014). The global burden of liver disease: the major impact of China. *Hepatology, 60*(6), 2099-2108.
2. Bahar, E., Ara, J., Hossain, M., Nath, B., & Runi, N. (2013). Cytotoxic (In-Vitro) effect of methanol & petroleum ether extracts of the Aerva lanata. *Journal of Pharmacognosy and Phytochemistry, 2*(1).
3. Pandit, A., Sachdeva, T., & Bafna, P. (2012). Drug-induced hepatotoxicity: a review. *J Appl Pharm Sci, 2*(5), 233-43.
4. Aborehab, N. M., & Boshra, S. A. (2019). Hepatoprotective effect of ginger and grape seed, alone and in combination orally, in paracetamol induced acute liver toxicity in rats.
5. Bigoniya, P., Singh, C. S., & Shukla, A. (2009). A comprehensive review of different liver toxicants used in experimental pharmacology. *International Journal of Pharmaceutical Sciences and Drug Research, 1*(3), 124-135.
6. Maqbool, M., Rasool, S., Dar, M. A., Bashir, R., & Khan, M. (2019). Hepatotoxicity and Hepatoprotective agents: A Mini review. *PharmaTutor, 7*(9), 34-40.
7. McGill, M. R., & Jaeschke, H. (2013). Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. Pharmaceutical research, 30(9), 2174-2187.
8. Hashym, Q.M., Al-Zahra, J.M.A. and Kadhim, N.J., (2019), REDUCING THE HEART BIOCHEMICAL AND HISTOLOGICAL EFFECT OF DOXORUBICIN BY ARTEMISININ COMPOUND, Plant Archives Vol. 19, Supplement 1, pp. 268-271.
9. Hadi, M.A., Hameedi, E.H., Kadhum, N.J., Aziz, D.Z., Al-Saddi, A.H. and Zaidan, H.K., 2016. Ameliorative Effect of Curcuma longa L. Rhizomes against Biochemical Toxicity Induced by Dichlorvos in Female Albino Rats. *J. Chem. Pharm. Sci, 9*, pp.1098-1106.
10. Lansky, E. P., Paavilainen, H. M., & Lansky, S. (2013). *Caper: the genus Capparis*. CRC Press.
11. Tlili, N., Elfalleh, W., Saadaoui, E., Khaldi, A., Triki, S., & Nasri, N. (2011). The caper (Capparis L.): Ethnopharmacology, phytochemical and pharmacological properties. *Fitoterapia, 82*(2), 93-101.
12. Siracusa, L., Kulisic-Bilusic, T., Politeo, O., Krause, I., Dejanovic, B., & Ruberto, G. (2011). Phenolic composition and antioxidant activity of aqueous infusions from Capparis spinosa L. and Crithmum maritimum L. before and after submission to a two-step in vitro digestion model. *Journal of agricultural and food chemistry, 59*(23), 12453-12459.
13. Parekh, A.B. and Putney Jr, J.W., 2005. Store-operated calcium channels. *Physiological reviews, 85*(2), pp.757-810.
14. Mossanen, J. C., & Tacke, F. (2015). Acetaminophen-induced acute liver injury in mice. *Laboratory animals, 49*(1_suppl), 30-36.
15. Lee, W. M. (2003). Drug-induced hepatotoxicity. *New England Journal of Medicine, 349*(5), 474-485.
16. Das, A., Biswas, P., & Chakrabarty, P. (2011). HEPATOTOXICITY AND HEPATOPROTECTISM HERBS: HERBAL REMIDIES. *International Journal of Research in Ayurveda & Pharmacy, 2*(4).
17. Vargas-Mendoza, N., & Madrigal-Santillán, E. (2014). y González-Rubio MG-L, et al. Hepatoprotective effect of silymarin. World J Hepatol, 6(3), 144.

18. Zanger, U. M., & Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacology & therapeutics, 138(1), 103-141.

19. Hartman, J. H., Martin, H. C., Caro, A. A., Pearce, A. R., & Miller, G. P. (2015). Subcellular localization of rat CYP2E1 impacts metabolic efficiency toward common substrates. Toxicology, 338, 47-58.

20. Quintanilha, J. C. F., de Sousa, V. M., Visacri, M. B., Amaral, L. S., Santos, R. M. M., Zambrano, T., & Moriel, P. (2017). Involvement of cytochrome P450 in cisplatin treatment: implications for toxicity. Cancer chemotherapy and pharmacology, 80(2), 223-233.

21. Jiang, S., Vozmediano, V., Abdel-Rahman, S. M., Schmidt, S., & James, L. P. (2019). Acetaminophen protein adducts in hospitalized children receiving multiple doses of acetaminophen. The Journal of Clinical Pharmacology, 59(10), 1291-1299.

22. Barbier-Torres, L., Iruzubieta, P., Fernández-Ramos, D., Delgado, T. C., Taibo, D., Guiñérez-de-Juan, V., & Zubiete-Franco, I. (2017). The mitochondrial negative regulator MCJ is a therapeutic target for acetaminophen-induced liver injury. Nature communications, 8(1), 1-11.

23. Mazaleuskaya, L. L., Sangkuhl, K., Thorn, C. F., FitzGerald, G. A., Altman, R. B., & Klein, T. E. (2015). PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. Pharmacogenetics and genomics, 25(8), 416.

24. Jaeschke, H., Xie, Y., & McGill, M. R. (2014). Acetaminophen-induced liver injury: from animal models to humans. Journal of clinical and translational hepatology, 2(3), 153.

25. Zheng, L., et al. (2018). Protective effect of dioscin against thioacetamide-induced acute liver injury via FXR/AMPK signaling pathway in vivo. 97, 481-488.

26. Cho, H. J., & Yoon, I. S. (2015). Pharmacokinetic interactions of herbs with cytochrome p450 and p-glycoprotein. Evidence-Based Complementary and Alternative Medicine, 2015.

27. Zhou, S., et al. (2003). Interactions of herbs with cytochrome P450. 35(1), 35-98.

28. Pizzorno, J. (2014). Glutathione!. Integrative Medicine: A Clinician's Journal, 13(1), 8.

29. Sciuto, A. M. (2017). Antioxidant properties of glutathione and its role in tissue protection. In Oxidants, antioxidants and free radicals (pp. 171-191). Routledge.

30. Bonina, F., Puglia, C., Ventura, D., Aquino, R., Tortora, S., Sacchi, A., ... & de Caparisi, P. (2002). In vitro antioxidant and in vivo photoprotective effects of a lyophilized extract of Capparis spinosa L. buds. Journal of cosmetic science, 53(6), 321-336.

31. Germano, M. P., De Pasquale, R., D'angelo, V., Catania, S., Silvari, V., & Costa, C. (2002). Evaluation of extracts and isolated fraction from Capparis spinosa L. buds as an antioxidant source. Journal of agricultural and food chemistry, 50(5), 1168-1171.