

Hepatoprotective effects of simvastatin on paracetamol -induced hepatic damage in rats

Mahdi M. Thwaini¹ and Hanaa S. Kadhem²

¹College of Nursing, University of Thi Qar,

² College of Science, University of Basra

ABSTRACT: This study was designed to investigate the possibility of statins hepatoprotection in paracetamol toxicity. Paracetamol hepatotoxicity was associated with significant decrease in the serum total albumin(g/dl) $p < 0.05$ and total protein(g/dl) ($p < 0.05$). However, it significantly increased ($p < 0.0001$), ALT IU/L, AST IU/L, and ALP IU/L as shown in group 3 (positive control) in comparison with the negative control (group 1) (table 1). Using of simvastatin 10mg/kg in healthy animals (group 2) caused slight insignificant changes in serum total albumin(g/dl), total protein(g/dl), ALT IU/L, AST IU/L, and ALP IU/L. However, simultaneous administration of simvastatin 10mg/kg with paracetamol, significantly attenuate the adverse changes in the serum total albumin(g/dl), total protein (g/dl), ALT IU/L, AST IU/L, and ALP IU/L. However, it didn't bring them to the normal limits.

KEYWORDS: Paracetamol, hepatotoxicity, hepatoprotection, simvastatin

I. INTRODUCTION:

Paracetamol overdose is a major cause of acute liver failure. The glutathione (GSH) precursor *N*-acetylcysteine (NAC) is used to treat patients with paracetamol overdose for up to 48 hours. Although it is well established that early treatment with NAC can improve the scavenging of the reactive metabolite *N*-acetyl-*p*-benzoquinone imine, protective mechanisms at later times remain unclear [1].

However, many other drugs were used as hepatoprotective in paracetamol poisoning such as angiotensin converting enzyme inhibitors or angiotensin receptor II antagonists, Hydrogen-rich water, resveratrol, melatonin, quercetin and other flavonoids and many other drugs [2-7]. On the other hand modern research also showed that a wide range of plants can neutralize or detoxify toxins and protect hepatic system from the toxic effects of drugs and chemicals. These plants included: *Agrimonia eupatoria*, *Alhagi maurorum*, *Allium sativum*, *Alpinia galangal*, *Anchusa strigosa*, *Arctium lappa*, *Artemisia campestris*, *Asparagus officinalis*, *Astragalus hamosus*, *Bauhinia variegata*, *Benincasa hispida*, *Brassica nigra*, *Brassica rapa*, *Bryonia dioica*, *Bryophyllum calycinum*, *Caesalpinia crista*, *Calendula officinalis*, *Calotropis procera*, *Canna indica*, *Capparis spinosa*, *Capsella bursa-pastoris*, *Capsicum frutescens*, *Capsicum frutescens*, *Carthmus tinctorius*, *Carum carvi*, *Cassia occidentalis*, *Casuarina equisetifolia*, *Celosia cristata* and *Chenopodium album* [8-51]. The 3-hydroxy 3-methylglutaryl coenzyme A reductase inhibitors (*ie*, statins) are widely used for the treatment of patients with hyperlipidemia and ischemic heart diseases. But, there is growing interest in the use of statins, HMG-CoA reductase inhibitors, as neuroprotective and for treating specific neurodegenerative diseases (e.g., cerebrovascular disease, Parkinson's disease, Alzheimer's disease, multiple sclerosis) and possibly traumatic brain injury [52]. Furthermore, they were also used to protect lungs from emphysema and chronic obstructive pulmonary diseases occur as a result of smoking [53]. Statins also conferred 70-90% hepatic protection against ischemia-reperfusion injury in obese animals with steatosis or non-alcoholic steatohepatitis [54]. Therefore this study was designed to investigate the possibility of statins hepatoprotection in paracetamol toxicity.

Materials and Methods:

Paracetamol (SDI Co, Iraq) and Simvastatin (Actavis, Barnstaple, EX32, UK) were dissolved in normal saline before use.

Animals Male Sprague-Dawley rats, weighing 250 ± 10 gr. were obtained from Basrah University laboratory animal house, Iraq. The animals were kept in standard conditions (23 ± 2 °C, 12 h light / dark cycle). Standard diet and water were given *ad libitum*. Rats were randomly divided into four groups (10 each). The first group received single daily oral dose of normal saline (vehicle) to serve as negative control. The second group was given simvastatin (10 mg/kg), as single oral dose. Hepatotoxicity was induced in animals of the third and fourth groups by a single oral dose of paracetamol 800 mg/kg. The third group of animals were treated

simultaneously with simvastatin (10 mg/kg), as a single daily oral dose. While the fourth group was given normal saline to serve as positive control. After 72 hours, the rats were anesthetized by diethyl ether; 5 ml of blood were taken by cardiac puncture. The abdomen was opened, and the livers were removed and cleaned. Liver tissue samples were fixed in 10% formalin solution and processed by routine histological technique for histopathology analysis.

The collected blood samples were allowed to clot. Sera were removed by centrifugation at 3000 rpm for 10 min. Then serum samples were processed to determine the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein and albumin, using a spectrophotometric autoanalyzer (Olympus AU-2700).

Results:

Paracetamol hepatotoxicity was associated with significant decrease in the serum total albumin (g/dl) $p < 0.05$ and total protein (g/dl) $(p < 0.05)$. However, it significantly increased $(p < 0.0001)$, ALT IU/L, AST IU/L, and ALP IU/L as shown in group 3 (positive control) in comparison with the negative control (group 1) (table 1). Using of simvastatin in healthy animals (group 2) caused slight insignificant changes in serum total albumin (g/dl), total protein (g/dl), ALT IU/L, AST IU/L, and ALP IU/L. However, simultaneous administration of simvastatin with paracetamol, significantly attenuate the adverse changes in the serum total albumin (g/dl), total protein (g/dl), ALT IU/L, AST IU/L, and ALP IU/L. However, it didn't bring them to the normal limits. Histopathological studies in hepatotoxicity induction untreated group showed that paracetamol caused pathological changes in liver consisted of congestions, hydropic degeneration and necrosis. In Simvastatin treated rats the liver sections were almost appeared in normal appearance with mild congestion, hydropic degeneration and necrosis.

Table 1: Effect of simvastatin on total albumin(g/dl), total protein(g/dl), ALT

Parameters	Total albumin (g/dl)	Total protein (g/dl)	ALT IU/L	AST IU/L	ALP IU/L
Group 1 negative control (treated by saline without induction)	5.21±0.91	6.44±1.22	33.41±3.34	38.81±2.96	112.81±6.83
Group 2 (without induction and treated with simvastatin)	5.01±0.81 ^{NS}	5.98±0.92 ^{NS}	34.21±3.29 ^{NS}	37.82±2.49 ^{NS}	114.85±5.93 ^{NS}
Group 3 positive control (induction and treated with saline)	3.75±0.72 ^{p<0.05}	4.62±0.88 ^{p<0.05}	76.82±5.63 ^{p<0.0001}	81.82±6.23 ^{p<0.0001}	173.44±7.49 ^{p<0.0001}
Group 4 (induction and treated with simvastatin)	4.12±0.75 ^{NS}	6.82±1.14 ^{NS}	56.92±4.44 ^{p<0.01}	64.91±4.78 ^{p<0.01}	162.52±6.75 ^{p<0.01}

IU/L, AST IU/L, and ALP IU/L in paracetamol induced hepatotoxicity in rats

P value in comparison with positive control (group 1), NS : not significant,

II. DISCUSSION:

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction. Paracetamol is commonly and widely used analgesic and antipyretic drug. It was safe when used in a therapeutic dose. It is detoxified mainly via formation of sulfate- and glucuronide-conjugates. When the enzymes saturated, paracetamol is increasingly metabolized into a reactive metabolite, Nacetyl-p-benzoquinone imine (NAPQI) by cytochrome P450 (CYP). The NAPQI is subsequently detoxified by glutathione (GSH) and the conjugated metabolite is excreted. When GSH is depleted, NAPQI is accumulated in the hepatocyte and interacts with thiol-containing proteins leading to hepatic necrosis . Paracetamol-induced liver injury is commonly used as models for investigation the efficacy of hepatoprotective drugs [55].

The elevated serum liver enzymes such as ALT, AST and ALP in intoxicated rats can be attributed to the damage in the histostructural integrity of the liver cells (hepatocytes) [56].

It has been documented that covalent binding of N-acetyl-p-benzoquinone imine, an oxidation product of paracetamol, to sulphhydryl groups of protein resulted in cell necrosis and lipid peroxidation with concomitant decrease in glutathione levels in the liver [57-58]. In the assessment of liver damage by paracetamol, the determination of enzyme marker levels such as ALT and AST is often used. In necrosis or membrane damage, the enzymes are released into circulation and it can be therefore measured in serum as markers of hepatic damage. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [59]. However, serum ALP and bilirubin level were also related to the function of hepatic cell.

Numerous studies suggest inhibitory effects of statins on proinflammatory cytokine production, such as IFN- γ , tumor necrosis factor- α , interleukin (IL)-1 β , and IL-6 in several cells, including microglia, astrocytes, and mononuclear cells. Accordingly, statins possessed many protective effects including neuro and pulmonary protection. They were reduce neutrophil influx which might have a strong effect on attenuating the downstream inflammatory events, such as macrophage influx, lymphocyte activation and inhibition of cytokine release [60-62]. The inhibition of IL-6, IL-8 and GM-CSF expression by statins has been shown in human cell cultures . Statins also affected IL-6 levels in the systemic circulation exert anti-oxidative effects and inhibit apoptosis [56-58]. Statins could conceivably affect these pathways through their inhibition of intracellular prenylation and inhibition of the GTP-binding proteins that underlie these inflammatory pathways [61-63]. Furthermore, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) protect the brain against ischemic injury by upregulating endothelial nitric oxide synthase (eNOS). Ischemic lesion volumes and neurologic deficits were significantly reduced in mice by both simvastatin and atorvastatin. Statins increased eNOS and tPA mRNA levels but did not change mRNA levels of PAI-1[64]. Therefore, the hepatoprotective effects of simvastatin could be attributed to its interference with many pro- and inflammatory mediators which preceded hepatotoxicity.

III. CONCLUSION:

According to the results of this study, simvastatin possessed hepatoprotective characteristics against paracetamol induced hepatotoxicity.

REFERENCES:

- [1]. Satio C; Zwingmann C and Jaeschke H. Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and N-acetylcysteine. *Hepatology* 2010;51(1):246-254.
- [2]. Smilkstein MJ; Knapp GL; Kenneth Kulig KW; and Rumack BH. Efficacy of Oral N-Acetylcysteine in the Treatment of Acetaminophen Overdose. *N Engl J Med* 1988; 319:1557-1562.
- [3]. Suzuki A; Yuen N; Walsh J; Papay J; Hunt CM and Diehl AM. Co-medications That Modulate Liver Injury and Repair Influence Clinical Outcome of Acetaminophen-Associated Liver Injury. *Clin Gastroenterol Hepatol.* 2009;7(8):882-888.
- [4]. Zhang JY, Song SD, Pang Q, Zhang RY, Wan Y, Yuan DW, Wu QF, Liu C. Hydrogen-rich water protects against acetaminophen-induced hepatotoxicity in mice. *World J Gastroenterol.* 2015; 21(14): 4195-4209.
- [5]. Wang Y, Jiang Y, Fan X, Tan H, Zeng H, Wang Y, Chen P, Huang M, Bi H. Hepatoprotective effect of resveratrol against acetaminophen-induced liver injury is associated with inhibition of CYP-mediated bioactivation and regulation of SIRT1-p53 signaling pathways. *Toxicol Lett.* 2015; pii: S0378-4274.
- [6]. Matsura T; Nishida T; Togawa A; Horie S; Ohata S; Nakada J, Ishibe Y and Ohta Y. Mechanisms of protection by melatonin against acetaminophen-induced liver injury in mice. *Journal of Pineal Research* 2006; 41(3):211-219.
- [7]. Guzy J; Chovanová Z.; Mareková M; Chavková Z; Tomečková V; Mojžišová G and Kušník J. Effect of quercetin on paracetamol-induced rat liver mitochondria dysfunction. *Biologia, Bratislava* 2004; 59(3): 399-403.
- [8]. Al-Snafi AE. The miraculous nature of the prophet medicine: Analytical study. Al Dīaa Publication house, Iraq 2009.
- [9]. Al-Snafi AE. Chemical constituents and pharmacological activities of *Ammi majus* and *Ammi visnaga*. A review. *International Journal of Pharmacy and Industrial Research* 2013; 3 (3): 257-265.
- [10]. Al-Snafi AE. Pharmacological effects of *Allium* species grown in Iraq. An overview. *International Journal of Pharmaceutical and health care Research.* 2013;1(4):132-147.
- [11]. Al-Snafi AE. Chemical constituents and pharmacological activities of milfoil (*Achillea santolina*) . A Review. *Int J Pharm Tech Res.*2013,5(3): 1373-1377.
- [12]. Al-Snafi AE. The pharmaceutical importance of *Althaea officinalis* and *Althaea rosea* : A Review. *Int J Pharm Tech Res* 2013; 5(3): 1387-1385.
- [13]. Al-Snafi AE. The pharmacology of *Bacopa monniera*. A review. *International Journal of Pharma Sciences and Research* 2013; 4(12): 154-159.
- [14]. Al-Snafi AE. The Pharmacological Importance of *Bauhinia variegata*. A review. *Journal of Pharma Sciences and Research* 2013; 4(12): 160-164.
- [15]. Al-Snafi AE. The Pharmacological importance of *Benincasa hispida*. A review. *Int Journal of Pharma Sciences and Research* 2013; 4(12): 165-170.
- [16]. Al-Snafi AE. The Chemical constituents and pharmacological effects of *Bryophyllum calycinum*. A review. *Journal of Pharma Sciences and Research* 2013; 4(12): 171-176.

- [17]. Al-Snafi AE. The Pharmacological activities of *Alpinia galangal* - A review. International Journal for Pharmaceutical Research Scholars 2014; 3(1-1): 607-614.
- [18]. Al-Snafi AE. Chemical constituents and pharmacological activities of *Arachis hypogaea* - A review. International Journal for Pharmaceutical Research Scholars 2014; 3(1-1): 615-623.
- [19]. Al-Snafi AE. The Pharmacological importance and chemical constituents of *Arctium Lappa*. A review. International Journal for Pharmaceutical Research Scholars 2014; 3(1-1): 663-670.
- [20]. Al-Snafi AE. The pharmacology of *Apium graveolens*. - A review. International Journal for Pharmaceutical Research Scholars 2014; 3(1-1): 671-677.
- [21]. Al-Snafi AE. The pharmacology of *Anchusa italica* and *Anchusa strigosa* – A review. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(4): 7-10.
- [22]. Al-Snafi AE. The pharmacological importance of *Anethum graveolens* – A review. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(4): 11-13.
- [23]. Al-Snafi AE, Wajdy JM and Tayseer Ali Talab. Galactagogue action of *Nigella sativa* seeds. IOSR Journal of Pharmacy 2014; 4(6): 58-61.
- [24]. Al-Snafi AE. The chemical constituents and pharmacological effects of *Adiantum capillus-veneris* - A review. Asian Journal of Pharmaceutical Science and Technology 2015; 5(2):106-111.
- [25]. Al-Snafi AE. The pharmacological and therapeutic importance of *Agrimonia eupatoria*- A review. Asian Journal of Pharmaceutical Science and Technology 2015; 5(2): 112-117.
- [26]. Al-Snafi AE. The chemical constituents and pharmacological effects of *Ammannia baccifera* - A review. International Journal of Pharmacy 2015; 5(1): 28-32.
- [27]. Al-Snafi AE. The chemical contents and pharmacological effects of *Anagallis arvensis* - A review. International Journal of Pharmacy 2015; 5(1): 37-41.
- [28]. Al-Snafi AE, Hanaon RM, Yaseen NY, Abdulalhussain WS. Study the anticancer activity of plant phenolic compounds. Iraqi Journal of Cancer & Medical Genetics 2011; 4(2): 66-71.
- [29]. Al-Snafi AE. The pharmacological importance of *Artemisia campestris*- A review. Asian Journal of Pharmaceutical Research 2015;5(2): 88-92.
- [30]. Al-Snafi AE. Chemical constituents and pharmacological effects of *Asclepias curassavica* – A review. Asian Journal of Pharmaceutical Research 2015;5(2): 83-87.
- [31]. Al-Snafi AE. The pharmacological importance of *Asparagus officinalis* - A review. Journal of Pharmaceutical Biology 2015; 5(2): 93-98.
- [32]. Al-Snafi AE. The medical importance of *Betula alba* - An overview. Journal of Pharmaceutical Biology 2015; 5(2): 99-103.
- [33]. Al-Snafi AE. Bioactive components and pharmacological effects of *Canna indica*- An Overview. International Journal of Pharmacology and toxicology 2015; 5(2):71-75.
- [34]. Al-Snafi AE. The chemical constituents and pharmacological effects of *Capsella bursa-pastoris* - A review. International Journal of Pharmacology and toxicology 2015; 5(2): 76-81.
- [35]. Al-Snafi AE. The pharmacological importance of *Ailanthus altissima*- A review. International Journal of Pharmacy Review and Research 2015; 5(2): 121-129.
- [36]. Al-Snafi AE. *Alhagi maurorum* as a potential medicinal herb: An overview. International Journal of Pharmacy Review and Research 2015; 5(2): 130-136.
- [37]. Al-Snafi AE. The pharmacological importance of *Aloe vera*- A review. International Journal of Phytopharmacy Research 2015; 6(1): 28-33.
- [38]. Al-Snafi AE. The constituents and biological effects of *Arundo donax* - A review. International Journal of Phytopharmacy Research 2015; 6(1): 34-40.
- [39]. Al-Snafi AE. The nutritional and therapeutic importance of *Avena sativa* - An overview. International Journal of Phytotherapy 2015; 5(1): 48-56.
- [40]. Al-Snafi AE. The Pharmacological Importance of *Bellis perennis* - A review. International Journal of Phytotherapy 2015; 5(2): 63-69.
- [41]. Al-Snafi AE. The chemical constituents and pharmacological effects of *Carum carvi* - A review. Indian Journal of Pharmaceutical Science and Research 2015; 5(2): 72-82.
- [42]. Al-Snafi AE. The pharmacological importance of *Casuarina equisetifolia* - An overview. International Journal of Pharmacological Screening Methods 2015; 5(1): 4-9.
- [43]. Al-Snafi AE. The chemical constituents and pharmacological effects of *Chenopodium album*- An overview. International J of Pharmacological Screening Methods 2015; 5(1): 10-17.
- [44]. Al-Snafi AE, Bahaadeen EF, Marbeen MI and Marbut MM. The effect of date palm pollens and zinc sulphate in the treatment of human male infertility. Tikrit Journal of Pharmaceutical Sciences 2006; 2(1): 31-34.
- [45]. Al-Snafi AE. Encyclopedia of the constituents and pharmacological effects of Iraqi medicinal plants. Thi qar University 2013.
- [46]. Al-Snafi AE. Pharmacology and medicinal properties of *Caesalpinia crista* - An overview. International Journal of Pharmacy 2015; 5(2): 77-89.
- [47]. Al-Snafi AE. The chemical constituents and pharmacological effects of *Calendula officinalis* - A review. Indian Journal of Pharmaceutical Science & Research 2015; 5(3):172-185.
- [48]. Al-Snafi AE. The constituents and pharmacological properties of *Calotropis procera* - An Overview. International Journal of Pharmacy Review & Research 2015; 5(3): 259-275.
- [49]. Al-Snafi AE. The pharmacological importance of Capsicum species (*Capsicum annuum* and *Capsicum frutescens*) grown in Iraq. Journal of Pharmaceutical Biology 2015; 5(3): 124-142.
- [50]. Al-Snafi AE. The chemical constituents and pharmacological importance of *Carthamus tinctorius* - An Overview. Journal of Pharmaceutical Biology 2015; 5(3): 143-166.
- [51]. Al-Snafi AE. The therapeutic importance of *Cassia occidentalis* - An overview. Indian Journal of Pharmaceutical Science & Research 2015; 5 (3): 158-171.
- [52]. Wood WG; Eckert G; Igbavboa U and Muller W. Statins and neuroprotection. Annals of the New York Academy of Sciences 2010; 1199:69-76.
- [53]. Young RP and Hopkins RJ. Update on the Potential Role of Statins in Chronic Obstructive Pulmonary Disease and its Comorbidities. Expert Rev Resp Med. 2013; 7(5): 533-544.

- [54]. Ajamieh H; Farrell G; Wong HJ; Chen J and Teoh N. Atorvastatin protects obese mice against hepatic ischemia-reperfusion injury by Toll-like receptor-4 suppression and endothelial nitric oxide synthase activation. *J Gastroenterol Hepatol.* 2012; 27(8): 1353-1361
- [55]. Dixon MF; Nimmo J and Prescott LF. Experimental paracetamol-induced hepatic necrosis: A histopathological study. *J Pathol* 1971; 103: 225-229.
- [56]. Kaplowitz N. Drug-induced liver disorders: Implications for drug development and regulation. *Drug Safety* 2001; 24: 483-490.
- [57]. Jollow DJ; Thorgeirsson SS; Potter WZ; Hashimoto M and Mitchell JR. Acetaminophen induced hepatic necrosis VI. Metabolic disposition of toxic and non-toxic doses of Acetaminophen. *Pharmacology* 1974; 12: 251-271.
- [58]. Handa SS and Sharma A. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride. *Indian J Med Res* 1990; 92: 276-283.
- [59]. Drotman RB and Lawhorn GT. Serum enzymes as indicators of chemically induced liver damage. *Drug Chem Toxicol* 1978; 1: 163-171.
- [60]. Takahashi S; Nakamura H; Seki M; *et al.* Reversal of elastase-induced pulmonary emphysema and promotion of alveolar epithelial cell proliferation by simvastatin in mice. *Am J Physiol Lung Cell Mol Physiol* 2008; 294: L882-L890.
- [61]. Maher BM; Ni Dhonnchu T; Burke JP; *et al.* Statins alter neutrophil migration by modulating cellular Rho activity – a potential mechanism for statins-mediated pleiotropic effects? *J Leukoc Biol* 2009; 85: 186-193.
- [62]. Chello M; Patti G; Candura D; *et al.* Effects of atorvastatin on systemic inflammatory response after coronary bypass surgery. *Crit Care Med* 2006;34: 660-667.
- [63]. Guasti L; Marino F; Cosentino M; *et al.* Simvastatin treatment modifies polymorphonuclear leukocyte function in high-risk individuals: a longitudinal study. *J Hypertens* 2006; 24: 2423-2430.
- [64]. Asahi M; Huang Z; Thomas S; Yoshimura S; Sumii T; Mori T; Qiu J; Amin-Hanjani S; Huang PL; Liao JK; Lo EH and Moskowitz MA. Protective effects of statins involving both eNOS and tPA in focal cerebral ischemia. *J Cereb Blood Flow Metab* 2005; 25(6):722-729.