

## Protective Effect of Captopril against 5- Fluorouracil-Induced Hepato and Nephrotoxicity in Male Albino Rats

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**Abstract:** 5-fluorouracil (5-FU) is a potent anticancer agent; its clinical use is limited for its marked hepatotoxicity and nephrotoxicity. The present study investigated the possible protective effect of captopril, an angiotensin-converting enzyme (ACE) inhibitor, on 5-FU-induced hepatotoxicity and nephrotoxicity in male albino rats. 5-FU 20mg/kg b.wt injected i.p. caused a significant increase in serum urea, creatinine, uric acid, cortisol, and glucose levels, respectively. Also, a marked elevation in potassium (K), bilirubin,  $\alpha$  AFP, alanine aminotransferase (ALT), aspartat aminotransferase (AST) and malondialdehyde (MDA). 5-FU caused a significant decrease in Sodium (Na), magnesium (Mg), glutathione (GSH), protein and albumin. Captopril 20mg/kg b.wt. administered 1h before 5-FU ameliorated the biochemical toxicity induced by 5-FU, in the kidney and liver. This was evidenced by a significant reduction in serum urea, creatinine, uric acid, cortisol, glucose levels, K, bilirubin,  $\alpha$  AFP, ALT and AST, respectively, and a significant restoration in Na, Mg, GSH, protein and albumin. These results indicate that captopril, an Angiotensin-converting enzyme ACE activity, has a protective effect against 5-FU-induced damage to kidney and liver. This reflects the beneficial role of captopril in treatment of renovascular hypertension and congestive liver failure.

[Nora El-Hoseany M. Ali **Protective Effect Of Captopril Against 5- Fluorouracil-Induced Hepato And Nephrotoxicity In Male Albino Rats**] Journal of American Science 2012; 8(2):680-685]. (ISSN: 1545-1003). <http://www.americanscience.org>. 95

**Keywords:** 5-fluorouracil (5-FU), captopril, hepatotoxicity, nephrotoxicity, creatinine,,  $\alpha$  AFP & MDA.

### 1. Introduction

5-fluorouracil (5-FU), a fluorinated analogue of pyrimidine, traditionally is classified as an anti-metabolic agent and interferes with the synthesis of DNA and RNA both in normal cells and tumor cells. 5-FU and cisplatin have been extensively used as chemotherapy for various cancers, including that of the liver (**Lin et al., 2006**) and some solid tumors; including breast, colorectal, and head and neck carcinomas. Although they generate acceptable outcome they exhibit severe toxicity and undesirable side effects (**Cabellos et al., 2007**). Both 5-FU and cisplatin are considered nephrotoxic compounds (**Isaka, & Rakugi, 2009** and **Wu et al., 2009**). Furthermore, 5-FU shows hepatotoxic effect with increased activities of tissue aminotransferases, LDH and ALP, indicating hepatic damage (**Ray et al., 2007**).

5-FU is eliminated by liver metabolism, and only a small percentage undergoes renal excretion. Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in 5-FU catabolism. DPD is found in the liver, therefore it is hypothesized that no reduction in 5-FU dose needs to be made for patients with hepatic or renal dysfunction (**Tateishi et al., 1999**).

Captopril, an ACE containing sulfhydryl (-SH) group is widely used for cardiac disorders (**Jones et al., 1992**). Angiotensin-converting enzyme (ACE)

activity plays a major role in arterial hypertension and nephrotoxicity and hence ACE inhibitors such as captopril are effectively used as antihypertensive agents (**Habor, 1992**). **Al-Shabanah et al., 1998**, suggested that captopril ameliorates doxorubicin-induced cardio- and haematotoxicity in normal rats. The involvement of ACE inhibitors in amelioration of nephrotoxicity induced by doxorubicin is controversial and depends on the type and mode of action. Some studies reported that ACE inhibitors induced functional renal insufficiency, while others declared that captopril partially inhibits the development of the functional and morphological damage induced by doxorubicin. In view of the existing controversies on the role of ACE inhibitors as captopril on nephrotoxicity; it is worthwhile to gain further insight into the role of captopril as a free radical scavenger on the nephrotoxicity induced by doxorubicin (**Iacona, et al., 1991** and **Al-Shabanah et al., 1998**).

Therefore, this study was carried out to investigate the toxic effect of 5-FU on renal and hepatic male albino rats and efficacy of captopril to modulate these effects.

### 2. Material and Methods

#### Materials

Captopril was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. 5-FU was

donated from Choongawa Pharma Corporation, Seoul, Korea. The therapeutic dose of 5-FU (20 mg/kg) was given intraperitoneally(i.p) according to **El-Sayyad et al. (2009)**. Captopril was administered by oral gavage at a dose of 20 mg/kg/day, based on the findings of the previous study (**Shin et al., 2009**).

### Animals

Forty adult male Sprague–Dawley rats, each weighing  $140 \pm 10$  g, were obtained from the Breeding Unit of the Egyptian Organization for Biological and Vaccine Production, A.R.E. The rats were housed in stainless steel cages under conventional and controlled laboratory conditions of relative humidity ( $55 \pm 5\%$ ) and temperature ( $25 \pm 5$  °C). Standard laboratory rodent chow and tap water were provided *ad libitum*. The animals were acclimatized for a period of one week prior to the commencement of the experiment.

### Experimental design:

The animals were divided into four groups each of ten rats as follows:

**Group I (Control):** The animals were given normal saline (2ml/kg b.w./day), parallel to the drug treated groups, throughout the course of the study of 14 days.

**Group II (5-FU group):** The animals first received normal saline (2ml/kg b.w./ day) orally for 9 days, and subsequently received 5-FU (20mg in 2ml normal saline per kg b.w.) once daily by intraperitoneal injection in association with normal saline for a 5 days.

**Group III (Captopril group):** The animals were given captopril alone (20 mg in 2ml normal saline per kg b.w.) once daily by oral intubation for 9 days and subsequently received normal saline by intraperitoneal injection in association with captopril for 5 days.

**Group IV (5-FU + captopril group):** The animals first received captopril alone at a dose of (20 mg/kg b.w./day) orally for 9 days, and subsequently received 5-FU(20 mg/kg b.w./day) in association with captopril for 5 days.

At the end of the experimental period all rats were sacrificed and blood was collected, by carotid bleeding, in centrifuge tubes. Serum was separated and used freshly for the assessment of kidney and liver function tests. Kidneys and livers were quickly harvested and immediately stored at  $-20^{\circ}\text{C}$  till further biochemical estimations.

### Biochemical analysis:-

Serum ALT and AST activities were measured colorimetrically according to the method of **Reitman and Frankel (1957)**.  $\alpha$ -AFP was estimated by using the method of **Wepsic (1981)**. Total bilirubin was measured photometry by using the method of **Rolinski et al. (2001)**. Serum urea was estimated by using the method of **Pathson and Nauth (1977)** and creatinine was determined according to the method of **Rock et al. (1987)**. Uric acid and cortisol were determined by method of the **Barham and Trinder (1972)** and **Coolidge (1939)**. Serum glucose level in blood was determined using the method of **Trinder (1969)**. Mg was measured by using the method of **Zettner and Seligson (1964)**. Sodium and potassium were measured by using the method of **Kokko and Tannen, 1986**. Determination of total proteins and albumin were estimated depending on the assays of **Doumas et al. (1981)** and **Doumas et al. (1971)**, GSH was assayed by the method of **Hissin and Hilf (1976)**. MDA in the tissue liver and kidney was determined using the method of **Mihara and Uchiyama (1978)**.

### Statistical analysis

Statistical analyses were done using InStat version 2.0 (GraphPad, ISI Software, Philadelphia, PA, USA, 1993) computer program. The results were expressed as mean  $\pm$  SE. Multiple comparisons were done using one-way ANOVA followed by Tukey-Kramer as a post-ANOVA test.

## 3. Results

### The effect of 5-FU

5-FU administration significantly increased serum ALT, AST, bilirubin,  $\alpha$  AFP, glucose concentrations and hepatic MDA in the male albino rats. Also, a marked elevation in serum urea, creatinine, uric acid, corticoid, K and renal MDA ( $P<0.01$ ) whereas, these concentrations tended to increase highly significant compared to the values in the control group, thus, 5-FU-induced increase in the renal and liver function injury.

On the other hand, 5-FU caused a highly significant decrease in serum protein, albumin, Na, Mg, and GSH of renal tissue ( $P<0.01$ ) compared to the values in the control group.

### Treatment with captopril.

Treatment with captopril succeeded to inhibit these high concentrations of serum ( $P<0.01$ ). They decreased after captopril administration significantly compared to the values in the control group. Also, these changes were statistically significant as compared to the values obtained after injection with 5-FU alone group (Tables 1&2).

After treated with captopril serum protein, albumin, Na, Mg and GSH of renal tissue enhanced significantly ( $P < 0.01$ ) (Tables 1&2).

#### 4. Discussion

5-Fluorouracil (5-FU), a widely used chemotherapeutic agent, has proven efficacy in a variety of human malignancies. However, the clinical

usefulness of 5-FU has been precluded because of its hepatotoxic and nephrotoxic side effects (Skretkiewicz *et al.*, 1996; El-Sayyad *et al.*, 2009). In the present study, injection of 5-FU in rats resulted in deterioration of hepatic function as indicated by elevation in ALT, AST,  $\alpha$  AFP, bilirubin and glucose and by a significant decrease in total proteins and albumin. Furthermore,

**Table (1): The effect of captopril on 5-FU- induced changes on serum ALT, AST,  $\alpha$  AFP, Bil, total protein, albumin, glucose levels and hepatic MDA.**

Groups	Parameters							
	ALT IU/ml	AST IU/ml	$\alpha$ -AFP IU/ml	Bil. mg/dl	Total Protein g/dl	Albumin g/dl	Glucose mg/dl	MDA nmol/g
Control	22.56±0.143	29.66± 0.08	8.67 ± 0.09	0.83± 0.01	7.24±0.09	3.30±0.07	67.40 ± 0.13	83.86 ± 0.36
5-FU	37.83± 0.318 a*	62.14 ± 0.35 a*	23.68 ± 0.11 a*	1.68 ± 0.06 a*	4.43±0.08 a*	1.84±0.05 a*	87.376 ± 0.17 a*	146.13 ± 0.35 a*
Captopril	21.74 ± 0.089 b*	30.15 ± 0.31 b*	7.59 ± 0.07 a b*	0.74 ± 0.01 b*	7.05±0.05 b*	3.30±0.14 b*	68.22 ± 0.42 b*	82.24 ± 0.19 ab*
5-FU + captopril	30.78± 0.041 abc*	41.61 ± 0.14 a b*	11.68 ± 0.15 abc*	0.95 ± 0.01 bc*	5.73±0.05 abc*	2.61±0.11 abc*	83.12 ± 0.13 abc*	103.10 ± 0.21 abc*

Data are expressed as mean ± S.E. (n = 10 in each group).

a: Significant change at  $p < 0.05$  with respect to control group.

b: Significant change at  $p < 0.05$  with respect to 5-FU group. c: Significant change at  $p < 0.05$  with respect to captopril group.

\*Highly significant change at  $p < 0.01$ .

**Table (2): The effect of captopril on 5-FU- induced changes on serum urea, creatinine, uric acid, cortisol, Na, K, Mg levels and renal GSH&MDA.**

Groups	Parameters								
	Urea mg/dl	Creatinine mg/dl	Uric acid mg/dl	Cortisol mg/dl	Na mmol/L	K mmol/L	Mg mg/dl	GSH mmol/100g	MDA nmol/g
Control	21.35±0.04	0.96± 0. 01	21.50 ± 0.80	5.79 ± 0.05	136.32±0.08	4.13±0.05	7.66 ± 0.08	2.39±0.06	191.44 ± 0.17
5-FU	39.71± 0.08 a*	02.63 ± 0.08 a*	50.10 ± 1.30 a*	22.47 ± 0.10 a*	129.19±0.96 a*	8.12±0.08 a*	5.58± 0.15 a*	1.22±0.09 a*	231.22± 0.12 a*
Captopril	19.28 ± 0.08 ab*	0.93± 0.01 a*b*	28.00 ± 1.10 a b*	6.52± 0.16 b*	139.97±0.14 ab*	4.33±0.06 b*	73.00 ± 0.08 b*	2.33±0.09 b*	190.19± 0.11 ab*
5-FU + captopril	25.60± 0.14 abc*	1.98± 0.07 abc*	35.00 ± 1.03 abc*	15.41 ± 0.11 abc*	138.50±0.18 ab*	6.21±0.12 abc*	80.20 ± 0.10 ab*	2.43±0.05 b*	201.00 ± 0.16 abc*

Data are expressed as mean ± S.E. (n = 10 in each group).

a: Significant change at  $p < 0.05$  with respect to control group.

c: Significant change at  $p < 0.05$  with respect to captopril group.

b: Significant change at  $p < 0.05$  with respect to 5-FU group.

\*Highly significant change at  $p < 0.01$ .

our results are supported by a significant increase in malondialdehyde concentration in the hepatic tissues after treatment with 5-FU. These results are in agreement with the previous reports on 5-FU-induced hepatotoxicity (El-Sayyad *et al.*, 2009; Mikalauskas *et al.*, 2011). Also, King & Perry (2001) and Zorzi *et al.* (2007) reported that when 5-FU has been given intravenously in the treatment of breast and gastrointestinal cancers, it is metabolized in tissues to its active form, 5-fluoro-deoxyuridine-monophosphate, which inhibits thymidylate synthase. 5-FU is also catabolized primarily in the liver, as dihydrouracil, and the reduced compound is then cleaved to  $\alpha$ -fluoro- $\beta$ -alanine, ammonia, urea, and carbon dioxide which cause the hepatic and nephrotoxicity. The toxicity may be decreased if the

catabolism is blocked by inhibiting dihydrouracil dehydrogenase. Davis *et al.*, (1993) showed that protein depletion results in increased toxicity to 5-FU, which is associated with a significantly decreased rate of hepatic metabolism and clearance of 5-FU and a significant decrease in hepatic DPD activity.

On the other hand studies showed that treatment with captopril significantly inhibited increase in proteinuria and blood pressure, so restore the protein content and may be useful for progressive renal failure (Tomita *et al.*, 2005).

In this study 5-FU was found to cause significant kidney injury manifested biochemically manifested by a significant increase in serum urea, creatinine, uric acid, cortisol and potassium and by a

significant decrease in sodium and magnesium. Additionally, our results are confirmed by a significant increase in malondialdehyde and a significant decrease in glutathione concentrations in the renal tissues after treatment with 5-FU. These results are consistent with previous studies reported by other investigators (Skretkiewicz *et al.*, 1996; Gao *et al.*, 2006) that 5-FU-induced nephrotoxicity in normal rats.

In this study it was shown that injection of captopril before 5-FU, protected the liver from damage induced by 5-FU. This protection was clearly reflected by a decrease in serum ALT, AST,  $\alpha$  AFP, bilirubin and glucose and by a significant increase in total proteins and albumin. The results also reveal that serum urea, creatinine, uric acid, cortisol, sodium and magnesium returned approximately to the normal control levels when animals were injected with captopril 5 days before 5-FU. These indicate that captopril has a protective potential on 5-FU-induced hepatotoxicity and nephrotoxicity.

Reduced glutathione (GSH) has a multiple role as an antioxidant agent. It functions as a scavenger of reactive oxygen species (ROS), including hydroxyl radicals, singlet oxygen, nitric oxide and peroxynitrite (Halliwell and Gutteridge, 1989). GSH was reported to protect the cells from cytotoxic damage induced by many compounds and it is generally known as a potent factor in the control of lipid peroxidation (Mansour *et al.*, 1999). Data of this study indicate that GSH increased when animals were injected with captopril before 5-FU administration. Captopril is known to increase GSH content in erythrocytes and brain (deCavanagh *et al.*, 1995 & 2000). The data of the antioxidant effects of captopril against 5-FU-induced hepatotoxicity are in agreement with Kalia *et al.* (2007), who observed a significant valuable effect of captopril on hepatic oxidative stress induced by arsenite in rats. Moreover, the results are consistent with Mansour *et al.* (1999) who revealed the promising protective effect of captopril against doxorubicin-induced nephrotoxicity. The possibility that captopril protects the renal tissues against the 5-FU-induced toxicity may be through scavenging the superoxide anion radicals formed by 5-FU. These results are in agreement with Al-shabana *et al.*, 1999 who reported that captopril totally abolished leukotriene B<sub>4</sub>, highly superoxide anion generator, formation from calcium ionophore-stimulated neutrophil. Therefore, it is possible that captopril may protect the renal tissues against 5-FU-induced renal toxicity by indirectly inhibiting the generation of superoxide anion via inhibition of Leukotriene B<sub>4</sub> formation.

Captopril has been reported to react rapidly with hydroxyl radicals and hypochlorous acid at

micromolar concentrations. Moreover, captopril was found to enhance the enzymatic activity of superoxide dismutase and selenium-dependent glutathione-peroxidase. Thus, the possible hydroxyl radical and hypochlorous acid scavenging ability, together with the interference of captopril with the binding of 5-FU to DNA may explain at least in part, the observed protective effect of captopril against undesirable side-effects of 5-FU (Mansour *et al.*, 1999; El-Sayed *et al.*, 2008; Ibrahim *et al.*, 2009).

In conclusion, Captopril, an ACEI, has a protective effect against 5-fluorouracil-induced nephrotoxicity and hepatotoxicity. The protective effect of captopril relies, at least in part, on its free radicals scavenging and antioxidant effects which are sulfhydryl dependent. Further investigations are needed to explore the possible mechanism of the protective actions of captopril.

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2/1/2012