Asian Pac. J. Health Sci., **2016; 3 (2):58-68** **e-ISSN: 2349-0659, p-ISSN: 2350-0964**

Crocin Alleviates 5-Fluorouracil-induced Hepatotoxicity through the abrogation of Oxidative Stress in Male Wistar rats

O. K. Afolabi[*1], G. E. Adeleke[1], R. N. Ugbaja[2]

[1]Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
[2]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria

ABSTRACT

5-Fluorouracil (5-FU) is a pyrimidine analogue that is used as an anti-cancer drug but its therapeutic potential is limited by its hepatotoxicity. This study therefore, aimed at investigating the protective effect of crocin, a natural supplement, against 5-FU-induced liver injury. Male rats were administered with 5-FU (30 mg/kg b.wt i.p.) for 5 days. Two groups of animals were either orally treated 3 days prior to or 3 days after 5-FU administration with crocin (40 mg/kg bwt). Another group was concurrently treated with crocin along with 5-FU administration. Biochemical variables indicative of liver injury, oxidative stress and liver histopathological alterations were thereafter determined. 5-FU administration markedly increased oxidative stress and markers of hepatic injury. Pre-, concurrent and post-treatment with crocin significantly reduced MDA, AOPP and LOOH levels and increased total antioxidant capacity compared to the 5-FU alone group, with improvement in the activities of the antioxidant enzymes, SOD, CAT and PONase. Liver injury was markedly reversed when AST, ALT and ALP activities estimated in the three treatment groups were compared to 5-FU group, but the restoration was incomplete, compared to the control. These biochemical variables were supported by histopathological examinations. The results indicate that crocin supplementation can ameliorate 5-FU-induced oxidative stress and liver injury in rat.

Key words: 5-Fluorouracil, Crocin, Hepatotoxicity, oxidative stress

Introduction

5-Fluorouracil (5-FU) is one of the most widely used antineoplastic drugs, mainly because of its efficacy against various malignancies [1, 2]. It is a fluoropyrimidine antimetabolite agent that plays a significant role in the treatment of cancers such as, colon and breast cancers, gastrointestinal cancers, head and neck cancers, and pancreatic cancer [2]. In the cancerous cell, it interferes with nucleoside metabolism and is incorporated into ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), resulting in cytotoxicity and cell death [3, 4]. 5-FU is extensively metabolized in the liver and the production of toxic intermediate may trigger liver injury. Fluorouracil is reported to exhibit severe toxicity and adverse effects which have restricted its chemotherapeutic use, limiting its potential as an effective anti-cancer agent [5]. 5-FU severe side effects are based on its systemic toxicity, including hepatotoxicity [6]. The pathophysiological mechanisms underlying this toxicity is yet to be elucidated but increased oxidative stress, apoptosis and inflammation have all been associated with 5-FU toxicity [6].A number of natural agents have been reported to possess therapeutic properties. Notable among them is crocin, a hydrophilic carotenoid compound found in the perennial herb, *Crocus sativus* [7]. Several studies have shown that crocin displays important biological and pharmacological properties, exerting beneficial effects on many systems and organs [8,9]. These important properties include neuroprotection [10], anti-atherogenic [11], hypolipidemic [12], hypotensive [13], cardioprotective [15], antitumor [15] among others. Some of the proposed mechanisms by which crocin impacts its beneficial effects are linked to its antioxidant activity [8], cytotoxic effect [16], anti-inflammatory and immunomodulatory actions [17, 18]. Naghizadeh et al.

**Correspondence*
Dr. O. K. Afolabi
Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
E Mail: okafolabi@lautech.edu.ng

[20] reported that pretreatment with crocin lowers blood urea and creatinine in cisplatin-induced renal failure. Marked increase in total thiol and glutathione peroxidase concentrations, as well as, enhanced renal total antioxidant capacity have also been reported following oral administration of crocin [8]. Furthermore, crocin also protects against aflatoxin B1- and iron- induced hepatic damage [20, 21]. Although the exact hepatoproptective mechanisms of crocin have not been well elucidated, its protective actions have been proposed to be predicated on its antioxidant properties [7]. There is, however, a dearth of information about its ability to protect against 5-FU-induced liver injury. But given that the efficacy of crocin as a therapeutic agent is based, among others, on its potential as an antioxidant and the advent of oxidative stress have been associated with hepatic toxicities, there is therefore a possibility that crocin, given its free radical scavenging and anti-oxidant properties, could prevent tissue oxidation generated by 5-FU induced toxicity. The current study thus, aimed at evaluating the protective effect of crocin against 5-FU-induced hepatic toxicity by the quantitative analyses of liver function enzymes in the plasma and investigating the oxidative imbalance of some oxidative stress indices, in addition to monitoring the level of intracellular antioxidant capacity. Furthermore, histopathological studies were carried out to assess ultrastructural changes in the liver.

Materials and method

Chemicals

Crocin was purchased from Sigma –Aldrich, Germany. 5-Fluorouracil was manufactured by Fidson Healthcare Pharmaceuticals, Nigeria. All other chemicals used were of analytical grade from Merck, India and Sigma-Aldrich, Germany.

Animals and treatment

Male Wistar rats (150 – 180 g) were obtained from the animal house of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The animals were kept in plastic cages under controlled conditions, fed with standard chow diet and water *ad libitum*, and acclimatized for 2 weeks before the commencement of the experiment.

Experimental design

The rats were divided into 6 groups of 7 animals each. Control animals (Group I) received normal feed and water. 5-FU at therapeutic dose (20 mg/kg bwt) was administered intraperitoneally to Group II for 5 days. Group III were orally administered crocin (40 mg/kg bwt) in normal saline for 5 days. Animals in Group IV were pretreated with crocin for 3 days at the same dose as Group III before administered with 5-FU as in Group II. 5-FU and crocin were concurrently administered to rats in Group V, as in Groups II and III respectively while Group VI were treated as animals in Group II, at the end of which crocin was administered for 3 days as in Group III. At the end of the experimental period, the rats were sacrificed by cervical dislocation. Blood was collected by cardiac puncture into heparinzed tubes, centrifuged at 3, 000 g for 5 minutes and the plasma obtained was stored in Eppendoff tubes at -20^{o}C. The liver was excised, washed with ice cold saline, blotted dry and then frozen until used for analysis. The dose for crocin was selected based on previous studies [22]. Experiment protocols were conducted according to the guidelines of the LAUTECH Department of Biochemistry guidelines for the care and use of laboratory animals.

Biochemical analyses

Assay of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity

The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the method of Reitman and Frankel [23]. Alkaline phosphatase (ALP) was determined by the method of Bessey *et al*. [24].

Measurement of ferric reducing antioxidant power (FRAP) in plasma and tissues

Total antioxidant capacity (TAC) in plasma and rat tissues was determined using the ferric reducing/ antioxidant power (FRAP) assay, developed by Benzie and Strain [25]. Briefly, 1.5 ml of working FRAP reagent (300 mM acetate buffer, pH 3.6, 10 mM 2,4,6-tripyridyl-S-triazine in 40 mM HCl and 20 mM FeCl3 in ratio 10:1:1) pre-warmed at 37 ^{o}C was vortex mixed with 50 mL sample (plasma and in the case of the tissues, 10% homogenate of either liver, kidney or brain). The test was performed at 37 °C and the absorbance was read at 593 nm. Aqueous solutions of known Fe^{2+} concentration were used for calibration.

Determination of Advanced Oxidized Protein Products Concentration

Spectrophotometric determination of advanced oxidized protein products (AOPP) levels in plasma and

tissues was performed by the method described by Witko et al. [26]. Each sample (0.4 mL of plasma, or 10% tissue homogenate) in a tube, was treated with 1.6 ml of Phosphate Buffered Saline (PBS) solution and 0.1 ml 1.16 M potassium iodide (KI). This was followed 2 min later by 0.2 ml of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm against a blank containing 2 ml of PBS, 0.1 mL of KI, and 0.2 ml of acetic acid. Concentration of AOPP was calculated by using the extinction coefficient of 26, 000 M^{-1} cm^{-1}.

Measurement of lipid hydroperoxides

The concentrations of lipid hydroperoxides (LOOH) in plasma and tissue homogenates were estimated using the method described by Nourooz-Zadeh et al. [27]. Plasma or tissue homogenate (90 µl) was mixed with either 10 µl of 10 mM triphenylphosphine (TPP) in methanol or with 10 µl of methanol and incubated for 30 min at a room temperature. Then, 900 µl of FOX2 reagent (250 µM ammonium ferrous sulfate, 100 µM. xylenol orange, 25 mM H2SO4 and 4 mM butylated hydroxytoluene in 90 % methanol) was added and the mixture was incubated for another 30 min. The mixture was then centrifuged at 12 000 × g for 10 min to remove flocculated material and the absorbance was read at 560 nm. The absorbance of the sample with TPP was subtracted from the sample without TPP and lipid hydro¬peroxides concentration was calculated from the standard curve prepared using different concentrations (1–20 µM) of H_2O_2.

Determination of PON1 paraoxonase activity

Paraoxonase activity of PON1 was determined in plasma and tissue homogenates using paraoxon (O, O – diethyl-o-p-nitrophenylphosphate) as the substrate. The increase in absorbance at 405 nm due to the formation of 4-nitrophenol following the hydrolysis of paraoxon was measured as described by Furlong et al. [28]. Sample (20 µL) was added to 760 µL of the assay buffer containing 0.132 mol L-1 Tris-HCl, pH 8.5, and 1.32 mM CaCl2. The substrate, paraoxon in 50 mmol L-1 Tris-HCl (200 µl), was added to initiate the reacion. The change in absorbance was continuously monitored on the Genesys 10S UV-VIS spectrophotometer for 3 min. Molar extinction coefficient of 18,050 was used to calculate enzyme activity. One unit of paraoxonase activity was defined as enzyme quantity that disintegrated one micromole of paraoxon substrate in 1 min at 25 °C.

Estimation of lipid peroxidation product from liver homogenates.

Lipid peroxidation in terms of thiobarbituric acid reactive substances (TBARS) formation was measured using the method of Esterbauer and Cheeseman [29]. Sample containing 1 mg protein was mixed with 1 ml TCA (20 %), 2 ml TBA (0.67%) and heated for 1 h at 100^{o}C. After cooling, the precipitate was removed by centrifugation. The absorbance of the sample was measured at 535 nm using a blank containing all the reagents except the sample. TBARS concentration of the liver samples were calculated using the extinction coeffecient of MDA which is 1.56×10^{-5} $mmol^{-1}$ cm^{-1} since 99% of TBARS exists as MDA.

Estimation of CAT activity.

The activity was measured in different homogenates by the method of Bonaventura et al. [30]. The enzyme CAT converts H2O2 formed via the action of SOD on superoxide radical into water. Protein from each sample was mixed with 7.5 mM H2O2 and a time scan was performed for 10min at 240 nm at 25oC. The disappearance of peroxide depending on the CAT activity was observed. One unit of CAT activity is defined as the amount of enzyme, which reduces 1 µmol of H2O2 per minute.

Estimation of SOD activity.

The activity of SOD was assayed following the method originally developed by Nishikimi [31] and then modified by Kakkar et al. [32]. The sample was mixed with sodium pyrophosphate buffer, phenazine methosulphate (PMT) and nitro blue tetrazolium (NBT). The reaction was started by the addition of NADH. Reaction mixture was then incubated at 30^{o}C for 90 seconds and stopped by the addition of 1 ml of glacial acetic acid. The absorbance of the chromogen formed was measured at 560 nm. One unit of SOD activity is defined, as the enzyme concentration required inhibiting chromogen production by 50% in one minute under the assay condition.

Liver histopathology

Liver specimens from all the experimental groups were fixed in 10% buffered formalin and were processed for paraffin sectioning. The processed sections were stained with haematoxylin-eosin and observation of the general structure of the liver was performed under light microscopy.

Statistical analysis

Results are expressed as mean ± SD (n=7). One-way analysis of variance (ANOVA) followed by Tukey's test was used to analyze the results with $p < 0.05$ considered significant.

Results

Effect of treatment on body weight and relative liver weight.Table 1 depicts the effect of crocin on the body and liver weights of rats treated with 5-FU. Treatment with crocin alone resulted in about 6% weight gain, which was comparable and not significantly different to that gained by the control ($p>0.05$). All other groups administered with 5-FU exhibited significant reduction in weight ($p<0.05$). 5-FU alone resulted in 23% reduction in weight. Crocin treatment however, reversed some of the weight loss with a gain of about 14%, 12% and 10% more than the 5-FU only group for the pre-treated, concurrent and post-treatment groups respectively. The ratio of liver weight to 100 g body weight was significantly increased by 5-FU administration ($p<0.05$). However, crocin alone and the crocin-treated groups displayed no changes in the relative liver weight compared to the control.

Table 1: Effects of crocin on body and liver weights of 5-Fluorouracil treated rats

Group	Body weight (% of initial body weight)	Relative liver weight* (g/100 g body weight)
Control	108.14 ± 2.67[a]	3.32 ± 0.96[a]
5-FU alone	77.14 ± 5.58[b]	4.31 ± 0.61[b]
Crocin alone	106.23 ± 3.05[a]	3.57 ± 0.27[a]
Pretreatment	91.54 ± 5.71[c]	3.76 ± 0.60[d]
Concurrent	89.82 ± 6.72[c]	3.65 ± 0.27[c]
Post-treatment	87.51 ± 4.96[c]	3.85 ± 0.38[d]

Values are expressed as mean ± SD, n=7. Values in the same column bearing the same superscript letter are not significantly different from each other, at $p < 0.05$.

Effect of crocin on plasma activities of ALT, ALP and AST in 5-FU-treated rat

Activities of the enzymes ALT, ALP and AST are considered biochemical marker of hepatic injury. These parameters were determined to ascertain whether crocin can attenuate the liver damage induced by 5-FU treatment (Fig. 1). In 5-FU-treated rats, the activities of ALT, ALP and AST significantly increased from 28.90 ± 2.67, 140.30 ± 5.42, and 78.74 ± 2.62 U/L in control to 49.86 ± 4.15, 209.66 ± 4.98, and 116.68 ± 2.68 U/L, respectively. However, the 5-FU-induced increases were lowered by crocin treatment. Pretreatment with crocin attenuated 5-FU effect on these activities by 23%, 14% and 20% respectively while concurrent treatment yielded a reduction of 32%, 22% and 25% respectively. Post-treatment with crocin lowered the activities of these enzymes in 5-FU administration by 24%, 10% and 18% respectively.

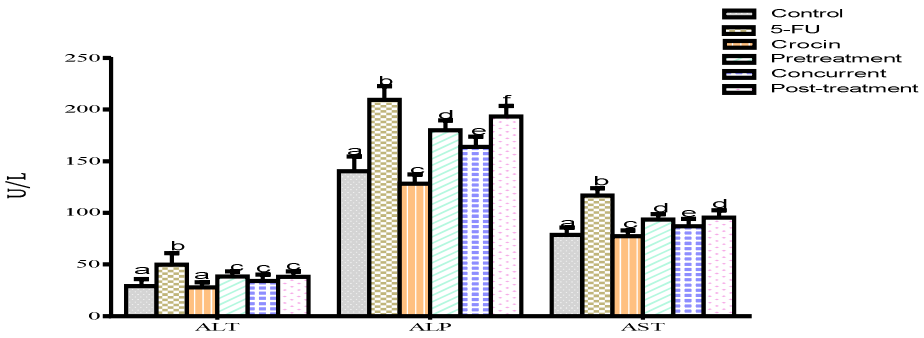


Fig 1: Effects of crocin treatment on plasma ALT, ALP and AST levels in 5-FU-treated rats. Each value is expressed as mean ± SD (n = 7). Different lowercase letters indicate a significant difference between groups at $p < 0.05$

Effects of crocin on levels of MDA, LOOH, AOPP and FRAP

5-FU produced significant increase in MDA levels in the liver. Its treatment generated a 45% increase in lipid peroxidation products, from 1.24 ± 0.12 nmol/mg protein in control to 1.89 ± o.31 nmol/mg protein in 5-FU treated group (Fig. 2A). The increase was accompanied be elevation of plasma lipid hydroperoxide concentration of about 126% (Fig. 3A). Crocin displayed significant lipid peroxidation lowering ability and was able to ameliorate the 5-FU induced lipid peroxidation observed in the study. Pretreatment with crocin reduced the 5-FU generated hepatic MDA concentration to 1.47 ± 0.13 nmol/L while lowering the level of LOOH from 27.24 ± 0.45 µmol/L to 19.57 ± 0.34 µmol/L. Concurrent treatment with crocin further reduced LOOH level to 17.24 ± 0.22 µmol/L while post-treatment lowered it to 19.07 ± 0.20 µmol/L. MDA concentrations were similarly reduced by the three crocin treatments. The results revealed significant elevation of both liver and plasma AOPP concentrations, as induced by 5-FU (Fig. 2C and 3C). 5-FU administration resulted in significant 118% and 84% increases in liver and plasma AOPP levels respectively, in the rats ($p<0.05$). Concurrent treatment with crocin produced the largest reduction of these oxidant modified proteins. The treatment reduced the levels in the 5-FU-treated rats by 39% and 34%, respectively. Hepatic concentration of the modified proteins reduced by 26% and 34% after post- and pre-treatment with crocin respectively, while plasma level were lowered by 24% and 23% respectively (Figs. 2c and 3C). 5-FU caused a significant reduction in hepatic total antioxidant capacity measured as FRAP, lowering the antioxidant potential by 2.5 fold, when compared to the control group (Fig. 2B.). Crocin alone significantly elevated the antioxidant power of the liver from 16.04 ± 0.88 µmol/mg protein in the control group to 19.13 ± 0.46 µmol/mg protein, a significant 19% increase. Similarly, crocin improved the FRAP value from 6.53 ± 0.51 µmol/mg protein obtained in 5-FU treatment to 11.43 ± 0.46 µmol/mg protein, 15.37 ± 0.51 µmol/mg protein and 12.76 ±0.65 µmol/mg protein in pre-, concurrent and post-treatment respectively. Plasma total antioxidant capacity was also equally improved by crocin treatments (Fig. 3B). Pre-, concurrent and post-treatment with crocin enhanced antioxidant capacity in 5-FU treatment by 40%, 57% and 61% respectively.

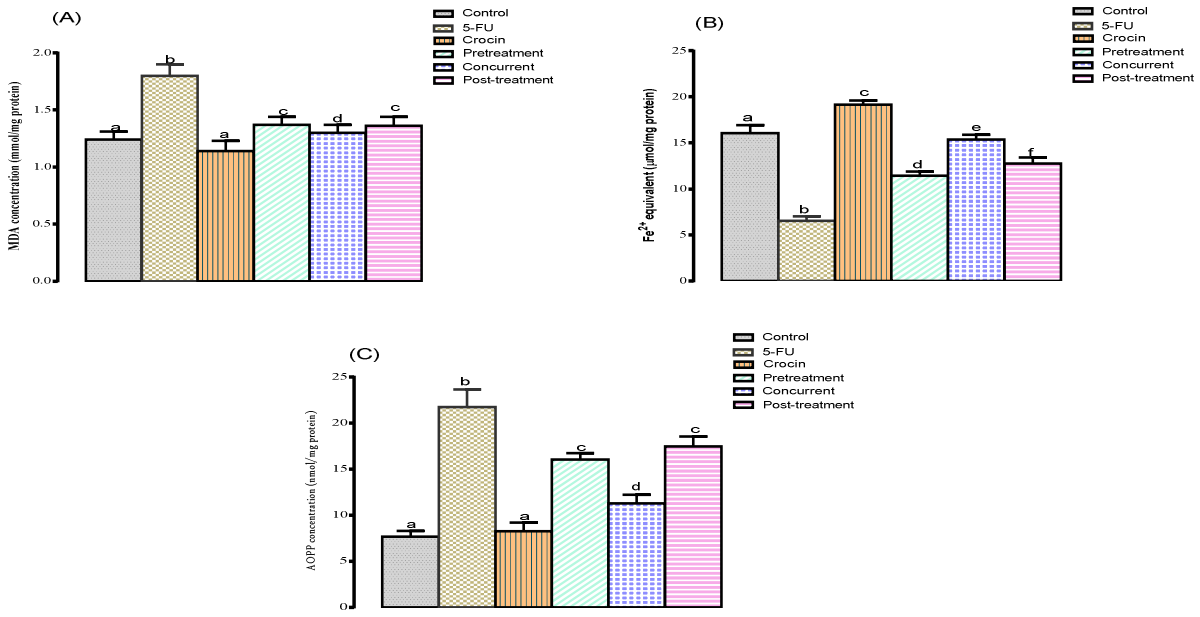


Fig 2: Effects of crocin on hepatic levels of MDA (A), FRAP (B) and AOPP (C) in 5-FU-treated rats. Each value is expressed as mean ± SD (n = 7). Different lowercase letters indicate a significant difference between groups at $p < 0.05$.

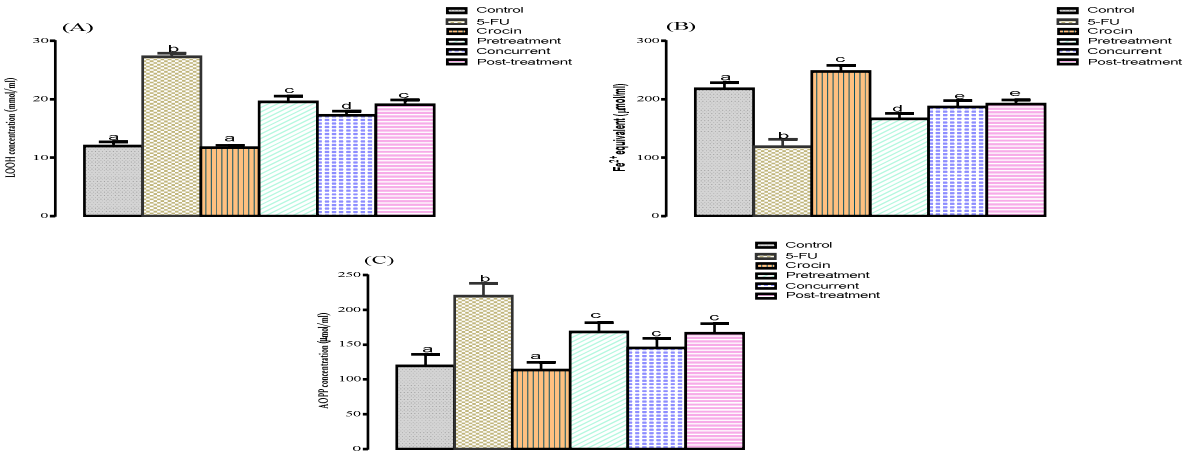


Fig. 3: Effects of crocin on plasma levels of LOOH (A), FRAP (B) and AOPP (C) in 5-FU-treated rats. Each value is expressed as mean ± SD (n = 7). Different lowercase letters indicate a significant difference between groups at p < 0.05

Effects of crocin on activities of antioxidant enzymes

Figures 4 and 5 depict the activities of enzymatic antioxidants, plasma PONase in addition to plasma and liver SOD and CAT activities. A significant decrease ($p<0.05$) in the plasma activities (Fig. 4A, B and C) of these enzymes was observed after 5-FU administration (24.67 ± 2.65 U/ml, 30.98 ± 3.53 U/ml, and 0.45 ± 0.04 U/ml from 47.07 ± 2.07 U/ml, 53.07 ± 3.01 U/ml, and 0.94 ± 0.05 U/ml in control respectively). Activities of CAT and SOD in the liver (Fig. 5A and B) were also marked reduced by 5-FU administration. The drug caused a depression of these enzymes' activities from 74.06 ± 3.62 U/mg protein and 12.58 ± 0.65 U/mg protein to 43.38 ± 4.68 U/mg protein and 6.76 ± 0.67 U/mg protein respectively, a loss of more than 40% for either protein. Crocin treatments significantly enhanced the enzymes' activities by reversing some of the inhibitory effect of 5-FU. Pre-, concurrent and post-treatment with crocin stimulated hepatic CAT by 63%, 66% and 60% respectively and SOD by 30%, 44% and 22% respectively.

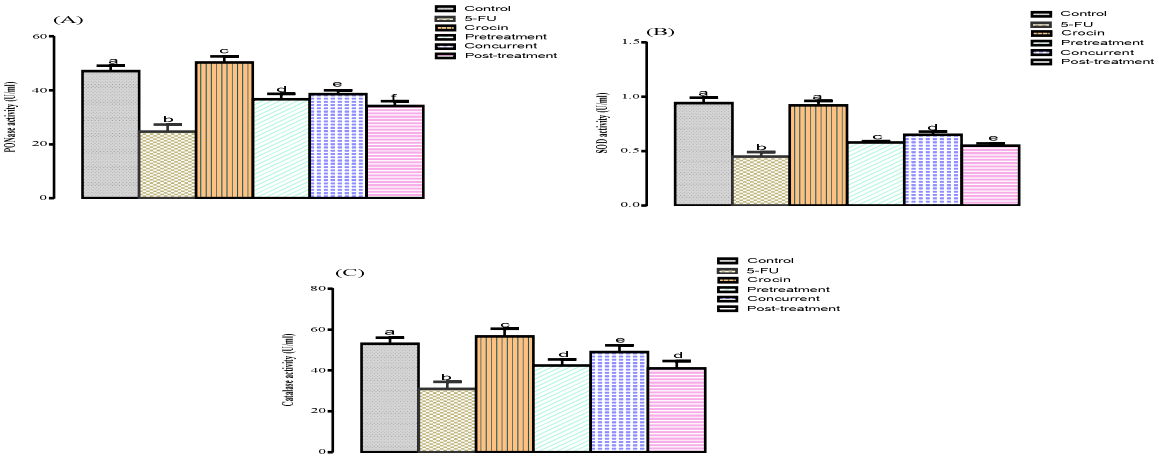


Fig 4: Effect of crocin on plasma PONase (A), SOD (B) and CAT (C) activities in 5-FU-treated rats. Each value is expressed as mean ± SD (n = 7). Different lowercase letters indicate a significant difference between groups at p < 0.05.

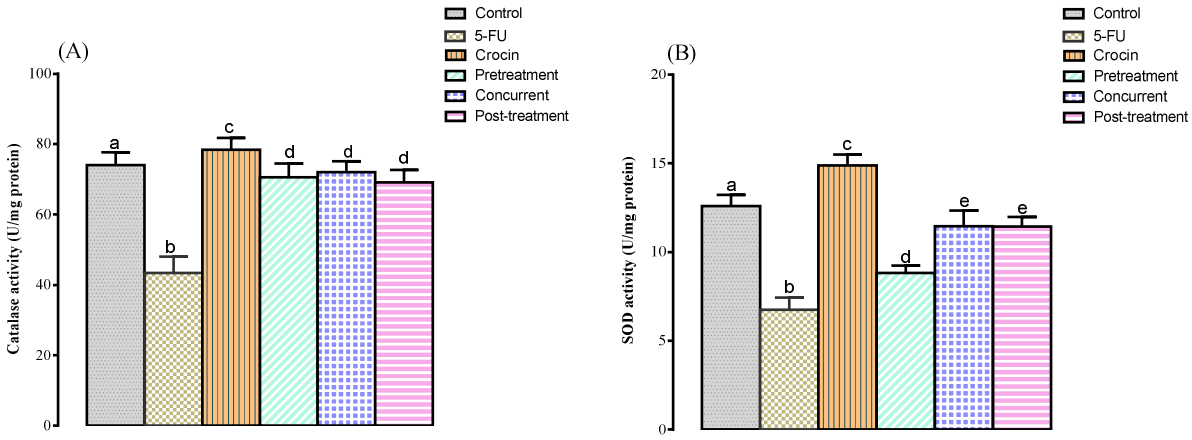
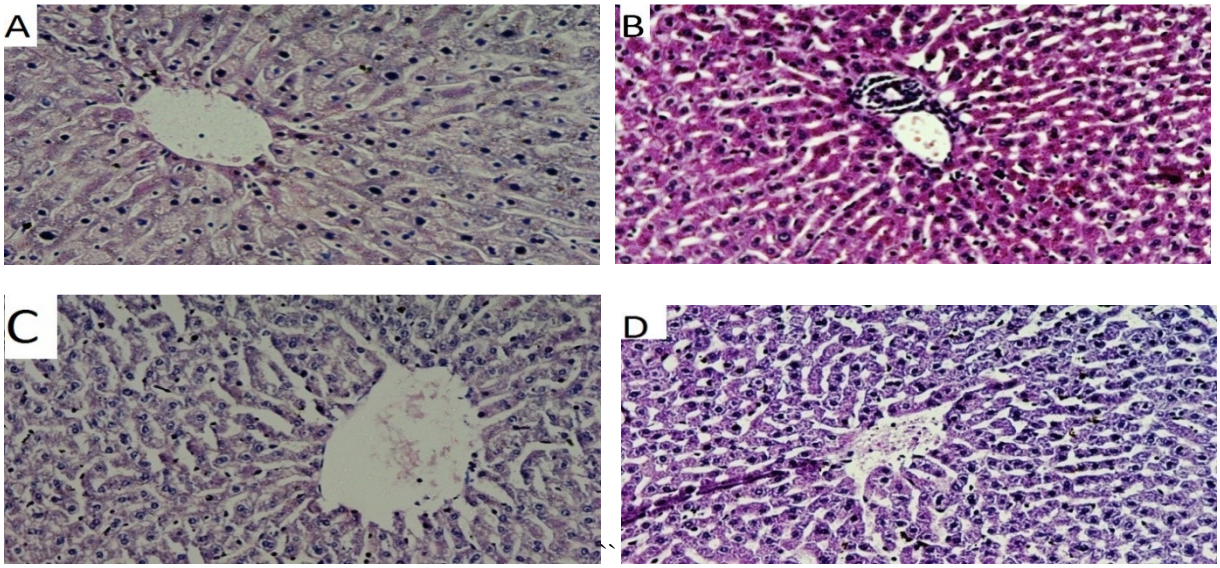


Fig. 5: Effect of crocin on liver CAT (A) and SOD (B) activities in 5-FU-treated rats. Each value is expressed as mean ± SD (n = 7). Different lowercase letters indicate a significant difference between groups at $p < 0.05$.

Histopathological changes

Light microscopy observation showed that the control liver tissue displayed normal hepatic lobules and plates radiating outward from a central vein (Fig. 6A). Animals administered with 5-FU showed signs of visible histology changes in the liver including structure damage, hepatocellular degeneration and disruption of cord-like pattern, changes that are not present in the liver of normal rats (Fig. 6B). Liver tissue from rats administered crocin alone exhibited normal histological structure similar to the control (Fig. 6C). Liver sections of rats treated with crocin prior (Fig. 6D), along with (Fig. 6E) and after (Fig. 6F) 5-FU administration showed improvement on the pattern of hepatic lesions displayed by 5-FU alone, with the severity of lesions reduced significantly.



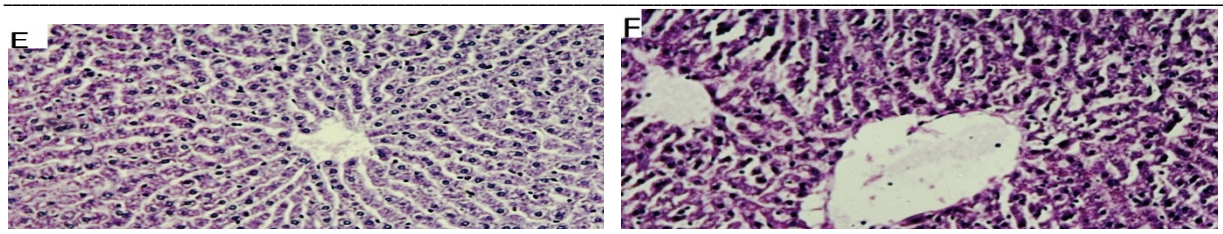


Fig 6: Photomicrographs of control and treated rat liver, H&E 100x. (A) Control rat showing normal hepatocytes, central canal and sinusoids. (B) Section of rat liver treated with 5-FU alone showing massive loss of tissue structural pattern, signs of fat droplets and fibrous appearance. (C) Crocin alone had no marked histological alteration except enlargement of the central vein and the sinusoids. (D) Pre-treatment with crocin restored partial histology with enlargement of the sinusoids, accompanied by shrinkage of hepatocytes and hemorrhage of the central vein. (E) Concurrent application of crocin with 5-FU showed a more improved restoration of the hepatic histology but with contracted central vein and increased presence of leukocytes. (F) Post-treatment with crocin after 5-FU administration displayed enlargement of both sinusoids and the central vein but with distortion of the structural histology.

Discussion

Although a number of studies have been carried out on the protective effect of natural agents against chemotherapeutic drugs, this present study, to the best of our knowledge, would be the first to investigate the potential protective effect of crocin against 5-fluorouracil induced liver damage. 5-FU is a potent anti-neoplastic drug, however, its hepatotoxic side effects potentially decreases its therapeutic value in the treatment of cancer. On the other hand, crocin has demonstrated hepatoprotective properties [21]. This study therefore, evaluated the ability of crocin to protect against 5-FU induced hepatic injury. The results of the present study suggest a beneficial role of crocin in reversing some hepatotoxic effects of 5-FU in rats by improving a number of biochemical variables and parameters indicative of hepatic injury and oxidative stress. Rats administered 5-FU were pre-, concurrently and post-treated with crocin and the treatments were compared. Overall, the data showed that concurrent crocin administration proved more effective compared with either pretreatment or post-treatment.Oxidative stress results when generation of excess reactive oxygen species exceeds the capacity of the antioxidant system [33]. These reactive species mediate cell damage in a variety of pathophysiological conditions and are responsible for severe damage to macromolecules, tissues and organs through lipid peroxidation (LPO), protein modification and DNA strand breaks [33, 34]. The effect of 5-FU administration on lipid peroxidation was assessed by estimating the hepatic MDA and plasma hydroperoxides concentrations. 5-FU significantly elevated both indices in the two compartments. The increased hepatic lipid peroxidation evidenced by the elevated MDA concentration is an indication that 5-FU-induced hepatic damage is mediated in part, by the generation of free radicals. Supporting the generation of reactive oxygen species by 5-FU is the elevation of advanced oxidative protein products (AOPP) in these animals. AOPP are dityrosine-containing crosslinked protein products and are important markers of degree of oxidant-mediated protein damage [35]. Increased production of ROS is known to be accompanied with the generation of oxidized proteins [36]. Crocin treatments attenuated both the increased lipid peroxidation and the oxidant mediated protein damage caused by the increased oxidative stress, indicative of the antioxidant property of crocin. Increased oxidative stress typified by the high peroxidation levels, also signifies a 5-FU-induced depletion of the antioxidant system. Furthermore, 5-FU reduced intracellular antioxidant power, as manifested by the lowered FRAP level in the 5-FU group. The antioxidant capacity of the liver was determined by the FRAP assay and it provides a wider representation of the relative antioxidant activity of all antioxidants present in the tissue. The reduction in total antioxidant capacity by 5-FU is reflective of antioxidant imbalance, an indication that the capacity of the antioxidant system has been exceeded. All these changes were significantly improved by treatment with crocin, prior, with and after 5-FU administration. This suggests that crocin performed an antioxidant role in 5-FU induced generation of ROS, probably by stabilizing the antioxidant system in the rats. Furthermore, plasma and hepatic activity of the antioxidant enzymes CAT, SOD and plasma PONase, an enzyme with hepatic origin, were inhibited by 5-FU treatment in the rats. The

reduced activity of these enzymes could be due to enhanced lipid peroxidation or inactivation of these proteins by the anti-neoplastic drug. Data from this study demonstrated that crocin has a positive effect in reversing the effect of 5-FU on these enzymes. Concurrent and post treatment with crocin restored hepatic SOD activity to near the control range while concurrent treatment completely reversed hepatic CAT activity back to normal. Treatment with 5-FU caused pronounced increase in plasma ALT, AST and ALP activities of the rats, indicating severe hepatotoxicity. This is in agreement with previous study showing elevation of these enzymes in 5-FU treatment [37]. The excess production of oxidants resulting in oxidative stress and the accumulation of oxidation products in the liver, as observed in this study apparently has damage biological membranes and the endothelium lining of the liver. This likely contribute to the hepatic injury leading to the high flux of the transaminases found in the blood. Transaminases are considered the most important biomarkers directly associated with the extent of cellular damage and toxicity [38]. Significant elevation of plasma AST, ALT and ALP activities have been employed as reliable markers of acute liver injury [39, 40]. ALT, a cytosolic enzyme is more specific for the liver, while AST is located in mitochondria and is mainly found in the liver, skeletal muscles and kidneys. ALP is an enzyme which activity is increased in the plasma as a result of obstruction or inflammation of the biliary tract. Increase in their activities in the blood is majorly a result of leakage of these transaminases from the hepatocytes into the circulation, indicating liver damage or dysfunction. Crocin application prior, with or after 5-FU administration induced significant recovery in these variables, causing significant lowered activities of these enzymes which is suggestive of reduced hepatotoxicity in the animals exposed to 5-FU. However, rats concurrently treated with 5-FU and crocin showed significantly better reduction in plasma ALT, AST and ALP values compared to both pre and post treatment with the carotenoid.Histological examination of the liver reveals that 5-FU treatment caused abnormal ultrastructural changes in the organ. In crocin treated animals, the changes were less compared to the 5-FU group, showing that crocin administration could improve the changes brought about by the drug on the liver. Crocin has been reported to display antioxidant effects and the present study provides further evidence for this as reflected by its effectiveness at the tested dose, in reducing 5-FU-induced elevated tissue oxidative damage, typified by high lipid hydroperoxides, MDA and AOPP concentrations and restoring the antioxidant system through the improvement of hepatic antioxidant power, in addition to stimulating the antioxidant enzymes. It was also able to reduce 5-FU-induced hepatotoxicity depicted by its correction of the altered liver function indices and improve the histopathological damage inflicted by the drug. On the whole, the study demonstrated that crocin has considerable protective potential against 5-fluorouracil induced oxidative stress and hepatic damage and at a higher dose, could effectively protect against the toxicity.

References

1. Grem JL. 5-Fluorouracil: Forty-Plus and Still Ticking. A Review of its Preclinical and Clinical Development, *Investigational New Drugs* 2000, 18(4): 299-313
2. Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies, *Nature Reviews Cancer* 2003; 3: 330-338
3. Zhang N, Yin Y, Xu S-J, Chen W-S. 5-Fluorouracil: mechanisms of resistance and reversal strategies, *Molecules* (Basel, Switzerland) 2008;13(8):1551-1569
4. Arias JL, Ruiz MAA, López-Viota M, Delgado AV. Poly(alkylcyanoacrylate) colloidal particles as vehicles for antitumour drug delivery:a comparative study, *Colloids and surfaces B, Biointerfaces* 2008;62(1):64-70
5. Cabellos, R., Garcia-Carbonero R, Garcia-Lacalle C, Gomez P, Tercero A, Sanchez D, Paz-Ares L. Fluorouracil-based chemotherapy in patients with gastrointestinal malignancies: influence of nutritional folate status on toxicity, *J Chemother* 2007; 19:744-749
6. Summya R, Nemat A, Sana N, Syed KH, Sarwat S. Mitigation of 5-Fluorouracil induced renal toxicity by chrysin via targeting oxidative stress and apoptosis in wistar rats. *Food and Chemical Toxicology* 2014; 66:185–193
7. Alavizadeh, S.H. Hosseinzadeh H. Bioactivity assessment and toxicity of crocin: A comprehensive review, *Food and Chemical Toxicology* 2014; 64: 65–80
8. Chen, Y., Yang, T., Huang, J., Tian, X., Zhao, C., Cai, L., Feng, L.J., Zhang, H. Comparative evaluation of the antioxidant capacity of crocetin

and crocin in vivo. *Chinese Pharmacol. Bull.* 2010;, 248–251

9. Hosseinzadeh H, Modaghegh MH, Saffari Z. Crocus sativus L. (Saffron) extract and its active constituents (crocin and safranal) on ischemia-reperfusion in rat skeletal muscle, *Evid. Based Complement. Alternat. Med*. 2009; 6: 343–350

10. Zheng, Y.Q., Liu, J.X., Wang, J.N., Xu, L. Effects of crocin on reperfusion induced oxidative/nitrative injury to cerebral microvessels after global cerebral ischemia. *Brain Res.* 1138, 2007; 86–94

11. He, S.Y., Qian, Z.Y., Tang, F.T., Wen, N., Xu, G.L., Sheng, L., 2005. Effect of crocin on experimental atherosclerosis in quails and its mechanisms. Life Sci. 77, 907– 921

12. Sheng L, Qian Z, Zheng S, Xi L. Mechanism of hypolipidemic effect of crocin in rats: crocin inhibits pancreatic lipase. *Eur. J. Pharmacol*. 2006; 543: 116–122

13. Imenshahidi, M., Hosseinzadeh, H., Javadpour, Y. Hypotensive effect of aqueous saffron extract (Crocus sativus L.) and its constituents, safranal and crocin, in normotensive and hypertensive rats, *Phytother. Res*. 2010; 24: 990–994

14. Goyal, S.N., Arora, S., Sharma, A.K., Joshi, S., Ray, R., Bhatia, J., Kumari, S., Arya, D.S. Preventive effect of crocin of Crocus sativus on hemodynamic, biochemical, histopathological and ultrastuctural alterations in isoproterenol induced cardiotoxicity in rats. *Phytomedicine* 2010; 17: 227–232

15. Rastgoo, M., Hosseinzadeh, H., Alavizadeh, H., Abbasi, A., Ayati, Z., Jaafari, M.R. Antitumor activity of PEGylated nanoliposomes containing crocin in mice bearing C26 colon carcinoma. *Planta Med.* 2013; 79 447–451

16. Garc-Olmo DC, Riese, H.H., Escribano, J., Onta, J., Fernandez, J.A., Atiénzar, M., Garcí-Olmo, D. Effects of long-term treatment of colon adenocarcinoma with crocin, a carotenoid from saffron (Crocus sativus L.): an experimental study in the rat, *Nutr. Cance* r1999; 35: 120–126

17. Xu, G.L., Li, G., Ma, H.P., Zhong, H., Liu, F., Ao, G.Z. Preventive effect of crocin in inflamed animals and in LPS-challenged RAW 264.7 cells, *J. Agric. Food Chem*. 2009; 57, 8325–8330

18. Deslauriers, A.M., Afkhami-Goli, A., Paul, A.M., Bhat, R.K., Acharjee, S., Ellestad, K.K., Noorbakhsh, F., Michalak, M., Power, C. Neuroinflammation and endoplasmic reticulum stress are coregulated by crocin to prevent demyelination and neurodegeneration. *J. Immunol*. 2011; 187: 4788–4799

19. Naghizadeh, B., Boroushaki, M., Vahdati, M.N., Mansouri, M. Protective effects of crocin against cisplatin- induced acute renal failure and oxidative stress in rats. *Iran. Biomed. J.* 2008; 12: 93

20. Lin JK, Wang CJ. Protection of crocin dyes on the acute hepatic damage induced by aflatoxin B1 and dimethylnitrosamine in rats, *Carcinogenesis* 1986; 7(4): 595-599

21. El-Maraghy, S.A., Rizk, S.M., El-Sawalhi, M.M. Hepatoprotective potential of crocin and curcumin against iron overload-induced biochemical alterations in rat. *Afr. J. Biochem. Res*. 2009; 3, 215–221

22. Dianat M, Esmaeilizadeh M, Badavi M, Samarbaf-zadeh AR, and Naghizadeh B. Protective Effects of Crocin on Ischemia-reperfusion Induced Oxidative Stress in Comparison With Vitamin E in Isolated Rat Hearts, *Jundishapur J Nat Pharm Prod*. 2014 May; 9(2): e17187

23. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate–oxaloacetate and pyruvate transaminases, *Am. J. Clin. Pathol*. 1957; 28, 56–63

24. Bessey, O.H., Lowry, M.D., Brock, A. A method for the rapid determination of alkaline phosphatase with 5 mm3 of serum. J. Biol. Chem. 1946; 164: 321–329

25. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay, *Anal Biochem* 1996; 239: 70-76

26. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* 1996; 49: 1304–1313

27. Nourooz-Zadeh J, Tajaddini-Sarmadi J, Wolff SP. Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine. *Anal Biochem* 1994; 220: 403-409

28. Furlong CE, Richter RJ, Seidel SL, et al. Spectrophotometric assay for the enzymatic hydrolysis of active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase. *Anal Biochem* 1989; 180: 242-247

29. Esterbauer, H., Cheeseman, K.H. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Meth. Enzymol*. 1990; 186: 407–421

30. Bonaventura J, Schroeder WA, Fang S. Human erythrocyte catalase: an improved method of isolation and revaluation of reported properties. *Arch Biochem Biophys*. 1972; 150:606–617

31. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun*. 1972;46: 849–854

32. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys*. 1984; 21:130–132

33. Halliwell B, Gutteridge JMC. Oxidative stress: adaptation, damage, repair and death. In: Halliwell B, Gutteridge JMC, editors. Free radicals in biology and medicine. Oxford, UK: Oxford University Press; 1999, pp. 284–330

34. Zaidi S. M. and Banu N. Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. *Clin. Chim. Acta* 2004; 340: 229–233

35. Alderman C, Shah S, Foreman JC, Chain BM, Katz DR. The role of advanced oxidation protein products in regulation of dendritic cell function. *Free Radic Biol Med* 2002; 32: 377–385

36. Penna C, Mancardi D, Rastaldo R, et al., Cardioprotection: a radical view: free radicals in pre and postconditioning. *Biochim Biophys Acta* 2009, 1787: 781-793

37. Dimitriu D, Lupusoru C, Cojocaru I, Gafitanu C, Palade L, Lupusoru R. Assessing biochemical andoxidative stress parameters after vaginal and oral administration of 5-fluorouracil in laboratory animals. *Farmacia*. 2015; 63:72

38. Stockham, S.L., Scott, M.A. Fundamentals of Veterinary Clinical Pathology. Ames, Iowa State University Press. 2002; 434–459

39. Zeashan H, Amresh G, Singh S, Rao CV. Hepatoprotective and antioxidant activity of Amaranthus spinosus against CCl4 induced toxicity. *J Ethnopharmacol*. 2009;125(2):364–366

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

Source of Support: Nil
Conflict of Interest: None