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Ameliorative Effects of *Chlorella vulgaris* and *Saccharum officinarum* Against 5-Fluorouracil Hepatotoxicity in Rats: Roles of Oxidative Stress, Inflammation and Apoptosis

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ABSTRACT

5-fluorouracil (FU) is the most common drug used as chemotherapeutic, but its hepatotoxic side effect threats its clinical application use. In consequence, searching for hepatoprotective agents is urgent necessity to hepatic hazards prevention. The present study investigated the protective effect of *Chlorella vulgaris* (CV) and Sugarcane (*Saccharum officinarum L.,* SO) against FU-induced hepatotoxic effects. Sixty male Wister albino rats were used in our study that divided into 6 groups. The rats treated for 2 weeks as following: The control group rats were orally received distilled water daily, FU group rats received FU (150 mg/kg, IP, on 8th day), and CV group received CV (400 mg/kg, orally, daily). SO group received SO (15mL/kg, orally, daily), FU+CV, and FU+SO groups. Moreover, FU caused an elevation in serum ALT, AST activities, hepatic malondialdehyde (MDA) and nitric oxide (NO) contents with decrease of catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) content significantly. FU also caused severe histopathological alterations including degenerative, vascular, and inflammatory in the liver, along with activation of caspase-3, and COX-2 expression. Contrariwise, the concomitant treatment by CV and SO with FU ameliorated the previous biochemical, pathological, and histochemical adverse effects induced by FU. In conclusion, CV and SO have protective role against FU-induced hepatic damage, perhaps through their antioxidant, anti-apoptotic, and anti-inflammatory properties**.**

Keywords: Fluorouracil, *Chlorella vulgaris*, *Saccharum officinarum***,** hepatoxicity.

1. INTRODUCTION

Chemotherapy is a standard cancer therapy, antineoplastic agents as 5-fluorouracil, doxorubicin, and cisplatin (Akindele et al., 2017). They are used alone and/or with radiation therapy or

surgery (adjuvant and neoadjuvant therapy). The adverse clinical complications associated with chemotherapy such as secondary malignancy, resistance, and lack of selectiveness generating a bad impact on body normal cells that often require reduction of the dose or chemotherapeutic agent's withdrawal (Pujari and Bandawane (2021).

5-Fluorouracil, a fluorinated pyrimidine analog antimetabolite, is frequently used as a chemotherapeutic drug. It is used in the handling of various cancer types, as, breast, stomach colorectal, genitourinary, head, and neck cancers (Pujari and Bandawane (2021). Even though its many advantages, FU clinical application has been markedly restricted because drug resistance and organ toxicity (Akindele et al., 2017).

Common serious FU side effects include diarrhea, dermatitis, mucositis, cardiotoxicity myelosuppression, hepatorenal toxicity and toxicity of genital organ. FU main cytotoxicity mechanisms are overproduction of reactive oxygen species (ROS) beside inflammatory mediators production (Al-Asmari et al., 2016 and Khalaf et al., 2022) that result in restrain the RNA and DNA synthesis, initiating cell apoptosis and tissue damage (Focaccetti et al., 2015).

Sugarcane (SO, *Saccharum Officinarum* L.) is distinguished crop of Poaceae family (Singh et al., 2015). Saccharum is a Greek word 'Sakcharon,' means sugar, principally sucrose (Koh et al., 2009). It is a perennial grass, and is a vital crop in tropical and subtropical areas with some variations are consumed as fruits. Inaddition, it is a chief source of sugar used in food industry. Globally, it produces about 70 percent of the sugar (Sun et al., 2014).

Particularly, Sugarcane has attracted important consideration by worldwide researchers for its phytochemicals, including polyphenolic composits (phenolic acids, glycosides and flavonoids) in its juices and its unrefined derived products (Singh et al., 2015 and Gensini et al., 2022). Additionally presence of sterols, terpenoids, and lignins (Pinheiro et al., 2017). With the exception of its nutritive value, its biological rols assign to these constituents include, antihyperglycaemic, analgesic, diuretic (Ali et al., 2019), anti-inflammatory (Ledon et al., 2003), antihypercholesterolemic (Arruzazabala et al., 2000) antithrombotic effects (Molina et al., 2000, Gensini et al., 2022) and hepatoprotective effects (Koh et al., 2009, Karthikeyan and Simipillai, 2010). It is functioned as laxative, an aphrodisiac, cooling, antiseptic, demulcent and tonic, too (Khare, 2007).

Chlorella vulgaris (CV) is spherical small unicellular green colored algae that spontaneously exist in environments of freshwater (Bauer et al., 2017). A Greek word "chloros" is meaning green color, and Latin suffix "-ella" is meaning small (Phukan et al., 2011). Chlorella is belongs to Chlorophyta phylum and Trebouxiophyceae class. CV is the most studied species of this genus (Blinová et al., 2015).

It is considered a superfood including, 60% protein, 55% carbohydrates, and 40% lipid for each dry weight. Compellingly, this microscopic size alga has all essential fatty acids and all essential amino acids (Safi et al., 2014). It has many types of minerals and vitamins such as thiamine, niacin,

riboflavin, pantothenic acid, biotin, pyridoxine, folic acid, ascorbic acid, cobalamin, retinol, tocopherols, sodium, calcium, potassium, magnesium, copper, phosphorous, zinc, iodine,manganese, and iron (Tokuşoglu and üUnal, 2003, Yeh and Chang, 2011, Panahi et al., 2012). Furthermore, its contents of many vital antioxidants, e.g. chlorophyll, lutein, carotenoids, canthaxanthin, astaxanthin, phycobiliproteins, and violaxanthin (Plaza et al., 2009, Ahmed et al., 2014, Safi et al., 2014).

This aforementioned nutritional composition makes CV have anti-inflammatory, antioxidant, immunomodulatory functions (Lee et al., 2010, Kwak et al., 2012, Haidari et al., 2018), antihypertensive (Sheih et al., 2009), and antitumor activities (Wang and Zhang, 2013). In addition, it has improved action on variable health conditions, e.g., hyperlipidemia, hyperglycemia, depression, obesity, cancer, and anxiety; thus, it is valued as a dietary multifunctional supplement (Panahi et al., 2016).

The use of antioxidant and/or anti-inflammatory medical substitutes has been recommended to diminish toxicity of chemotherapy (Akindele et al. 2018; Diba et al., 2021). Many agents were assessed to decline FU-related toxicities on body organs, until the present moment, there is no agreement concerning the maximum drug therapy (Agbarya et al. 2014). In light of the aforementioned, this experimental study was planned to estimate the protective and preventive role of sugarcane and CV against FU-induced hepatotoxicity in rats.

For this purpose, this study is designed to assess hematological and serum biochemical and spotlight the mechanistic probable role of oxidative stress by measuring lipid peroxidation (MDA) and antioxidant markers of CV and SO against hepatodamage induced by FU. In addition, trying to clarify the importance of Cox2 and Caspase-3 as hepatic inflammation and apoptotic markers. This may open the door to the capabilities use of them for therapeutic purposes.

2. MATERIALS AND METHODS

2.1. Chemicals:

5-Fluorouracil used as Utoral® one vial has fluorouracil 500mg (Hikma Company, Badr city, Cairo, A.R.E. (M.O.H. Reg. No. : 26001/2009). *Chlorella vulgaris* obtained as pure powder from Animal Health Research Institute. The diagnostic kits used for measuring of tissue and serum biochemical markers were obtained from Biodiagnostic Company, Dokki

2.2. Preparation of sugarcane juice:

Sugarcane stems were available commercially at local markets at Sadat City, Egypt. Sugarcane juice was processed according to Khan et al., (2015) and Yasmin et al., (2010) method. The stems of sugarcane (*Saccharum Officinarum L*) were cut into pieces that equal in the lengths, washed, and extract the juice by a three-roller power crusher then filtered through a muslin cloth in a sterilized container. The produced juice was pasteurized at 85–90 °C for 5 min. with adjusted pH to ≤4.00, removed the waxy material. Use the sterilized air tight glass bottles to store the juice without any food preservative at 3–4 °C until use.

2.3. Animals and Experimental Design:

The experimental outline was previously published by El-Gendy et al. (2024). In brief, sixty healthy male albino Wistar

rats (100–120 g) were purchased from Laboratory Animal Colony, Giza, Egypt. The animals were housed in a laboratory animal house. Rats were lodged in polypropylene cages in basic sterile conditions. It is given an adequate amount of water and diet. The rats were kept under natural daily light/dark cycle, ventilation, at a temperature of 20–25°C. Rats were adapted for 14 days before the start of the experiment. All experimental measures and techniques were approved (Ethical approval number: VUSC-025-1-24) by the Research Ethics Committee of the Faculty of Veterinary Medicine, University of Sadat City, Egypt.

Animals were randomly allocated into 6 groups, 10 animals of each as follows:

Control group: It received distilled water orally.

Fluorouracil (FU) group: Rats intoxicated with FU (150 mg/kg b.w.) (Al-Asmari et al., 2016) on the 8th day i.p.

***Chlorella Vulgaris* (CV) group:** Rats received CV (400 mg/kg b.w) (Sikiru et al., 2019), daily for two weeks orally.

***Saccharum Officinarum* (SO) group:** Rats received sugarcane juice (15 ml/kg b.w) (Khan, 2015; Khan et al., 2018; Hussein and EL-Shafey 2019) daily for two weeks orally.

Fluorouracil and Chlorella Vulgaris (FU+CV) group: Rats received FU, on the 8th day i.p. and *Chlorella Vulgaris*, daily for two weeks orally

Fluorouracil and Sugarcane juice (FU+SO) group: Rats received FU, on the 8th day i.p. and sugarcane juice daily for two weeks orally.

2.4. Sample collection:

At the experiment end, rats were fastened overnight and anesthetized by NEW-FLOTAN for the collection of samples. Two blood samples were taken from the middle eye canthus of rat. The blood samples (5mL) were collected without anticoagulant and centrifuged at 3000 rpm for 10 min. The separated serum samples were stored at −20°C. Immediately, after sacrificing the rats, the liver tissue was rapidly dissected and splited into two parts; the first used for tissue biochemical analyses, was washed in physiological saline then stored at −80°C, while the 2nd was used for histopathology and immunohistochemistry investigations, was fixed in 10% neutral buffered formalin.

2.5. Estimation of Serum hepatic Biomarkers:

Serum liver enzyme activities, alanine aminotransferase (ALT), aspartate aminotransferase (AST), were estimated by Reitman and Frankel (1957) method, total protein (TP) and Albumin levels were evaluated by rendering to the methods described by Doumas et al., (1981) and Beng and Lim (1973) respectively, following the manufacturer's instructions of commercial kits.

2.6. Estimation of Hepatic Oxidant/Antioxidant Biomarkers:

Liver oxidant markers, malondialdehyde and nitric oxide contents were estimated according to Ohkawa et al. (1979) and Montgomery and Dymock (1961) respectively while the antioxidant biomarkers, reduced glutathione contents, superoxide dismutase and catalase activity, were estimated rendering to Beutler (1963), Nishikimi, et al., (1972) and Aebi (1984), respectively, following the manufacturer's instructions of commercial kits.

2.7. Histopathological Examination:

The formalin-fixed hepatic tissue were trimmed, washed then dehydrated by ascending grades of alcohol, cleared in xylene, embedded in paraffin and sectioned at 4-6 µ thickness by a microtome (LEICA RM 2135) finally stained by using hematoxylin and eosin stain (H&E) following Carleton, (1976) method. Histopathological photographing and examination were done by a digital Leica photomicroscope (LEICA DMLB, Germany). A semi-quantitative scoring of lesions was evaluated as follows: (0): no change, (1): mild < 25%, (2): moderate < 50%, and (3): severe > 50% of examined field sections.

2.8. Immunohistochemical (IHC) Investigation:

Hepatic tissue sections were submitted to IHC examination using Caspase-3 with 1:200 dilutions and Cox2 with 1:100 dilutions (Abcam, Cambridge, USA). At room temperature, 5-µm sections were routinely proceeded into different xylene and alcohol solutions, blocked with hydrogen peroxide 3% (H_2O_2) for 10 min., passed to citrate buffer (Ph 5.4) for antigen retrieval for 15 min., incubated with horseradish peroxidase secondary antibody (Abcam, UK) for 30 min. Immune signals were detected using diaminobenzidine reagent (Sigma Company, USA) for 2 min, followed by counterstaining with hematoxylin stain. Immune-expression of Caspase-3 and Cox2 were semi-quantitative scored with some modifications as follows; 30 high-power fields were counted and their average was taken and scored as no change: (0), mild: (1); ≤ 25%, moderate: (2); ≤ 50%, severe: (3); ≤ 75%, and very severe: (4); > 75% of all examined fields (Tahoun et al., 2021).

2.9. Statistical Analysis:

Statistical analyses of all results were done by one-way ANOVA then Duncan's Multiple Range test to apply post hoc analysis, performed using SPSS analytical software SPSS (Statistical Package for Social Sciences), Version 8 according to Snedecor and Cochran (1986). Values were expressed as mean ± SE. Significant differences were statistically at $P<0.05$.

3. RESULTS

3.1. Chlorella Vulgaris and sugarcane juice ameliorated 5-Fluorouracil-induced incretion in serum hepatic functions of rats:

Table 1 showed that there were no variation in the activities of serum ALT and AST between the control, CV and SO groups. Exposure of rats to FU at 150 mg/kg b.w. on the 8th day i.p. significantly ($P< 0.05$) elevated the serum ALT and AST activities, compared to control values. Conversely, pretreatment with either CV (400 mg/kg b.w.) or SO (15 ml/kg) before and after FU injection normalized the elevated enzyme activities. Total protein and albumin in the intoxicated group were non-significant increase.

Table (1): The effect of FU, CV and/or SO administration on serum hepatic functions biomarkers of rats

Parameters	Experimental groups					
	Control	**FU**	**CV**	**SO**	**FU+CV**	**FU+SO**
ALT (U/ml)	48±12.51^{b}	1.2000E2±9.08^{a}	53±11.24^{b}	67±15.29^{b}	73±9.69^{b}	60±3.16^{b}
AST (U/ml)	319 ±17.05^{b,c}	480±37.41^{a}	250± 27.24^{c}	361± 27.54^{b}	314± 31.^{2b,c}	260± 26.5^{c}
TP (g/dl)	7.64±.17	7.7±.28	8.21±.21	7.68±.21	8.32±.15	8.9±.9
Albumin (g%)	3.61±.06	3.61±.03	3.75±.08	3.45±.04	3.05±.09	3.71±.2

Data are expresses as means±SE (n=8). (^{a,b}) Different letters in the same row means significant differences at p<0.05. FU: 5-Fluorouracil, CV: *Chlorella Vulgaris*, SO: Sugarcane juice, FU+CV: 5-Fluorouracil and *Chlorella Vulgaris*, FU+SO, 5-Fluorouracil, and Sugarcane juice, ALT: alanine aminotransferase, AST: aspartate aminotransferase and TP: total protein.

3.2. Chlorella Vulgaris and sugarcane juice improved 5-Fluorouracil-induced hepatic oxidant/antioxidant biomarkers alterations of rats.

Referring to the alterations in the hepatic oxidant/ antioxidant status, no significant ($p < 0.05$) modification in hepatic oxidant/antioxidant biomarkers between the control, CV and SO groups. However, significant increase of MDA and NO levels along with significant decrease of GSH content, SOD and CAT activity were recorded in the liver tissue of 5-Fluorouracil-exposed rats, compared to those of control group. On the other hand, the significant amelioration in the hepatic oxidant/antioxidant status was recorded with pretreatment with either CV or SO alone or with 5-Fluorouracil that appeared in restoring the normal control values of hepatic MDA, NO, GSH, SOD, and CAT.

Table (2): The effect of administration of 5-Fluorouracil, Chlorella Vulgaris and/or sugarcane juice on hepatic oxidant/antioxidant biomarkers of rats

Parameters	Experimental groups					
	Control	**FU**	**CV**	**SO**	**FU+CV**	**FU+SO**
MDA(nmol/g)	6.58±0.5^{b}	16.04±0.58^{a}	6.64±0.18^{b}	7.24±0.54^{b}	6.56±0.35^{b}	7.8±1.41^{b}
NO (µmol/L)	5.25±0.64^{c}	12.71±0.65^{a}	5.09±0.36^{c}	5.56±0.24^{c}	6.05±0.36^{b,c}	7.2±0.59^{b}
CAT (U/g)	191±3.12^{a}	68.77±4.23^{d}	178±2.37^{a,b}	192±7.75^{a}	162±6.72^{b,c}	148±17.19^{c}
SOD(U/g)	38.75±2.36^{a,b,c}	13.51±1.81^{d}	43.47±1.54^{a}	40.19±1.46^{a,b}	33.66±1.08^{c}	35.94±1.67^{b,c}
GSH(mmol/g)	71.56±1.3^{a,b,c}	27.4±2.97^{d}	79.17±3.43^{a}	73.38±2.34^{a,b}	67.49±3.37$^{b.c}$	62.82 ± 5.93^{c}

Data are expresses as means±SE (n=8). ([a,b]) Different letters in the same row means significant differences at $p<0.05$. FU: 5-Fluorouracil, CV: *Chlorella Vulgaris*, SO: Sugarcane juice, FU+CV: 5-Fluorouracil and *Chlorella Vulgaris*, FU+SO, 5-Fluorouracil and Sugarcane juice, MDA: malondialdehyde, NO: nitric oxide, CAT: catalase, SOD, superoxide dismutase, and GSH: reduced glutathione.

3.3. Chlorella Vulgaris and sugarcane juice improved 5-Fluorouracil-induced alterations in hepatic architectures of rats:

Microscopical results are reported in Figure 1 and Table 3. Control, CV, and SO groups had the normal histological appearance of liver central veins, sinusoids, hepatic cords, and Kupffer cells. In contrast to previous groups, the liver of the FU group had massive damage including congested central veins and sinusoids, hemorrhage, loss of hepatic cord organization, and hepatic cell necrosis with inflammation. CV or SO treatments with 5-fluorouracil succeed in reversing the damage induced by fluorouracil.

Table (3): Semi-quantitative scoring of liver pathological lesions in different treated groups.

	Experimental groups					
Histopathological lesion	**Control**	**FU**	**CV**	**SO**	**FU+CV**	**FU+SO**
CV congestion	0	3	0	0	0	1
SU congestion	0	2	0	0	1	0
Vacuolation	0	2	0	0	1	1
Necrosis	0	3	0	0	0	0
HC disorganization	0	2	0	0	0	0

The histopathological changes are scoring as follows: (0): no change, (1): mild changes < 25%, (2): moderate changes < 50%, (3), severe changes > 50%. CV: central vein, SU: sinusoid, HC: hepatic cord, FU: 5-Fluorouracil, CV: Chlorella Vulgaris, SO: Sugarcane juice, FU+CV: 5-Fluorouracil and Chlorella Vulgaris, FU+SO, 5-Fluorouracil and Sugarcane juice.

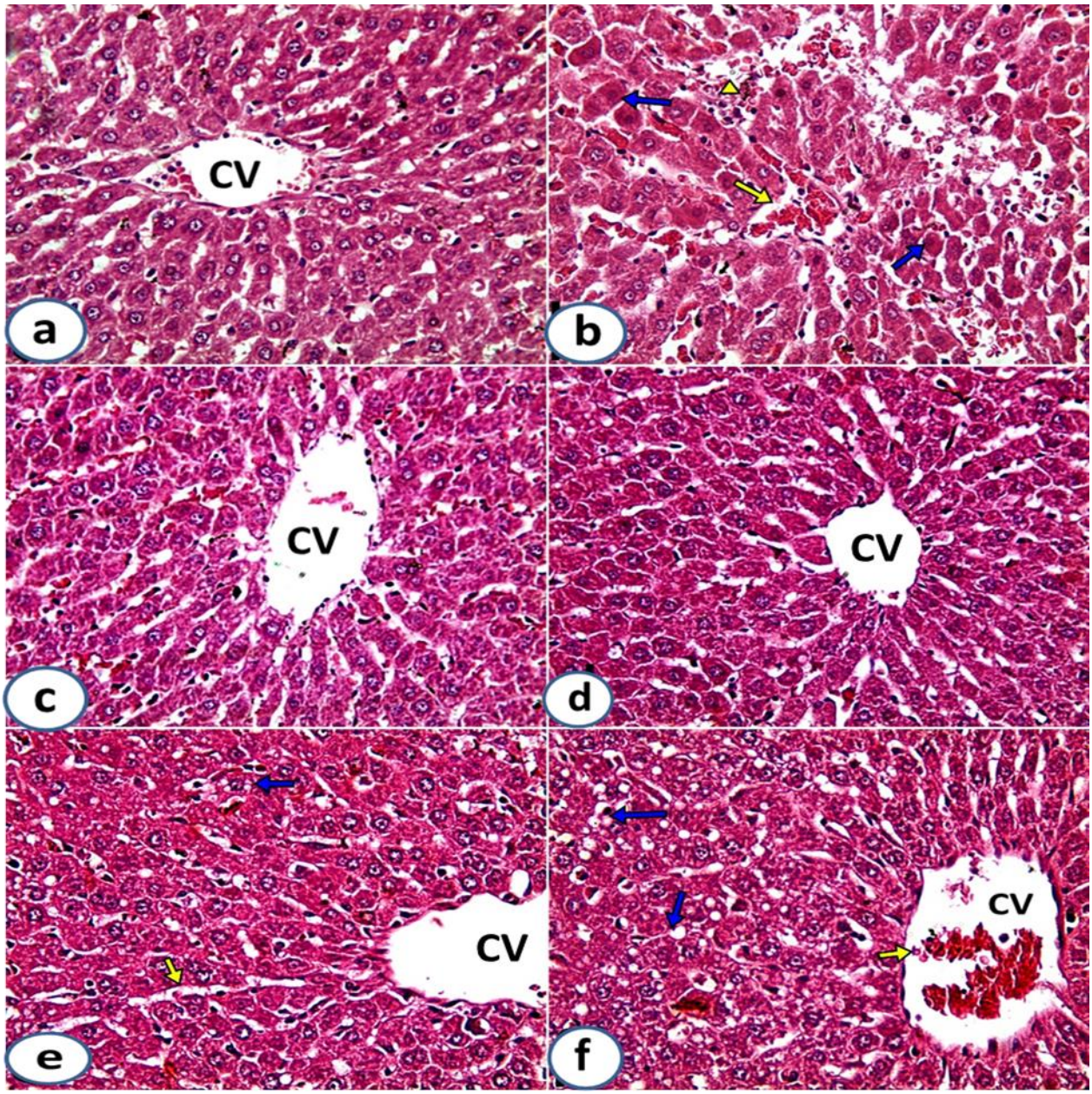

Fig. (1): Representative photomicrographs of hepatic histopathological changes of experimental groups (H&E stain X_{20}, Scale bar 50 μm): (a) Control group, (c) CV, *Chlorella vulgaris*, group and d) SO, *Saccharum Officinarum*, showing normal liver cell architectures, (b) FU, 5-Fluorouracil, group showing congested central veins (yellow arrow) and sinusoids, hemorrhage (arrowhead), loss of hepatic cord organization, and hepatic cells necrosis (blue arrows), (e) FU+CV, *Chlorella vulgaris*+Fluorouracil group showing mild dilated hepatic sinusoids (yellow arrow) and slight vacuolation of hepatocytes (blue arrow), (f) FU+SO, *Saccharum officinarum*+Fluorouracil, group showing congested central veins (yellow arrow) and slight vacuolation of hepatocytes (blue arrows).

3.4. Chlorella Vulgaris and sugarcane juice modulated 5-Fluorouracil-elevated Caspase-3 and Cox2.

Immunohistochemistry results are cleared in Figures 2 & 3 and Table 4. The control, CV and SO groups were negative for Caspase-3 and Cox2 immune expression. The liver of the FU-treated group had strong immune signals of Caspase-3 and Cox2. Treatment with *Chlorella Vulgaris* and *Saccharum Officinarum* with fluorouracil succeeds in decreasing the expression of Caspase-3 and Cox2 as shown in Fig. 2 and 3.

Table (4): Semi-quantitative scoring of Caspase-3 and Cox2 immunostaining of liver of different experimental groups.

IHC	**Experimental groups**					
	Control	**FU**	**CV**	**SO**	**FU+CV**	**FU+SO**
Caspase-3	0	3	0	0	1	1
Cox2	0	3	0	0	1	1

Immunohistochemistry scoring: no change: (0), mild: (1); ≤ 25%, moderate: (2); ≤ 50%, severe: (3); ≤ 75%, and very severe: (4); > 75% of all examined fields. IHC: Immunohistochemistry, FU: 5-fluorouracil, CV: *Chlorella Vulgaris*, SO: *Saccharum Officinarum*.

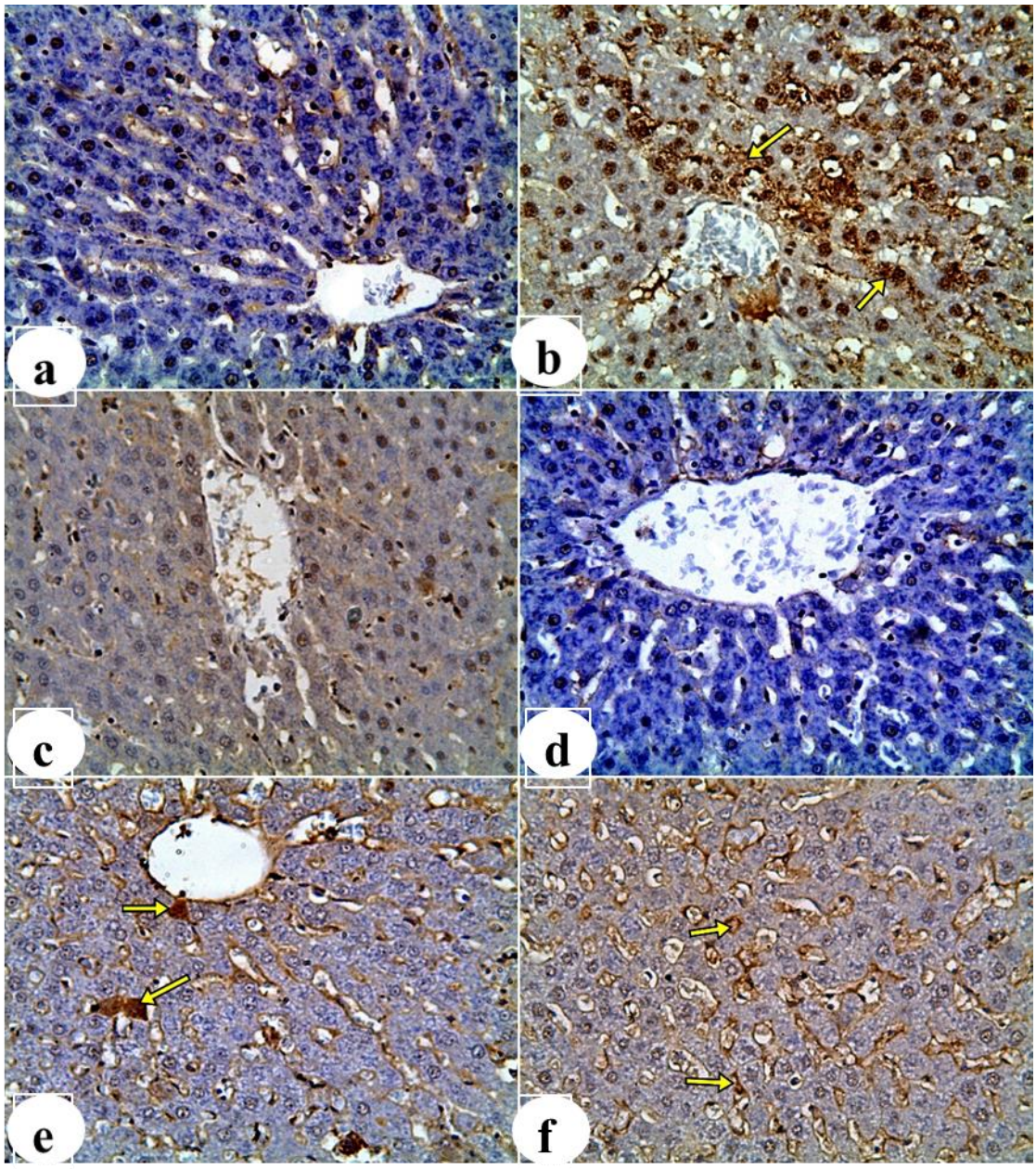

Fig. (2): Immuno-histochemical staining photomicrographs of caspase-3 in liver of different groups (Caspase-3 IHC; scale bar = 50 μm): (a) Control group sections showing no brown signals of Caspase-3 in normal liver. (b) FU, 5-Fluorouracil-treated group showing severe brown signals of Caspase-3 in hepatocytes cytoplasm (yellow arrows). (c) CV, *Chlorella Vulgaris*, and (d) SO, *Saccharum Officinarum*, group showing absence of Caspase-3 in normal liver. (e) FU+CV, *Chlorella vulgaris*+5-Fluorouracil, group and (f) FU+SO, *Saccharum officinarum+5-Fluorouracil*, group showing mild expression of Caspase-3 (yellow arrows). Hematoxylin counter stained X20.

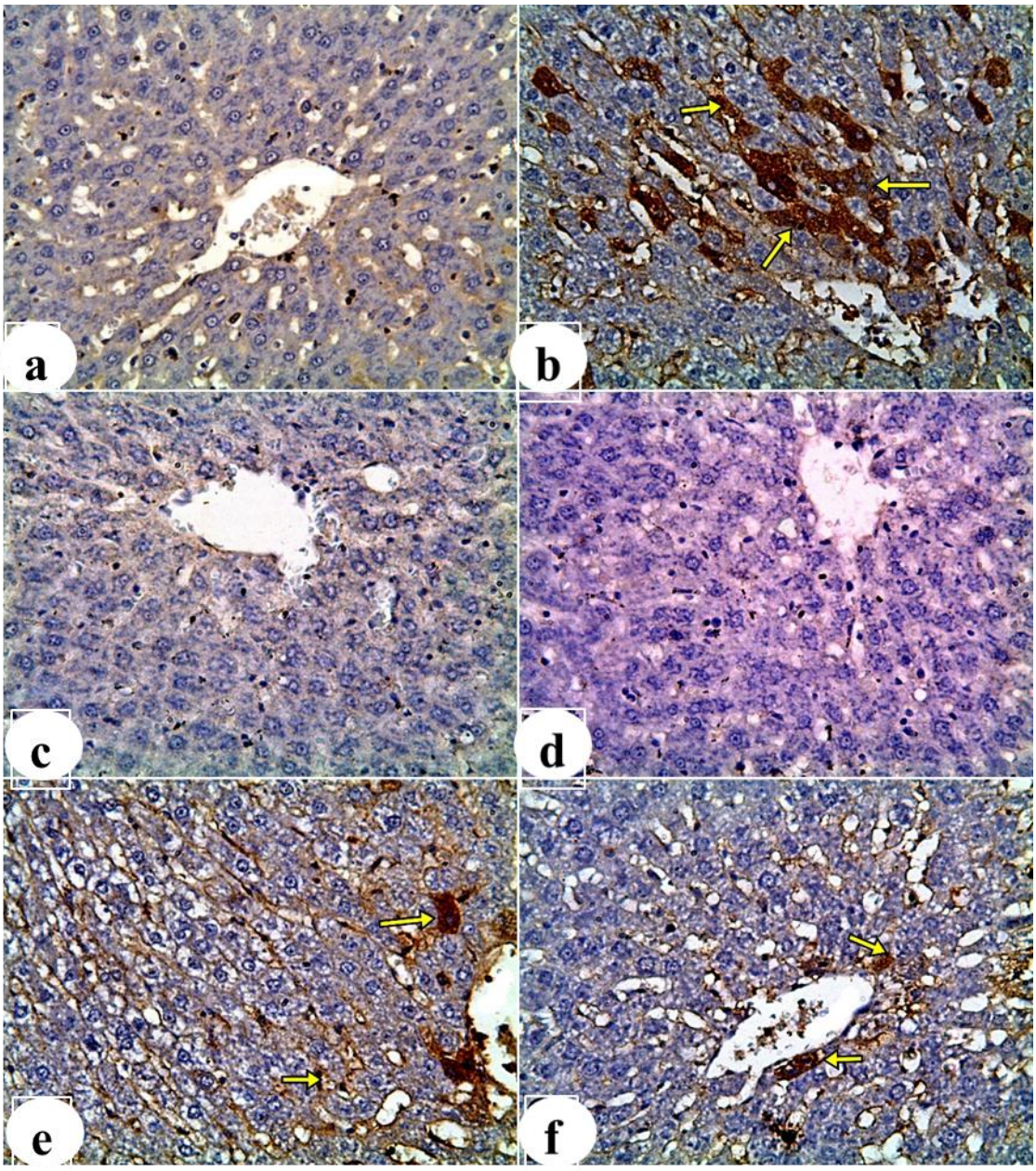

Fig.(3): Photomicrographs of immuno-histochemical staining of Cox2 in different groups liver (Cox2 IHC; scale bar = 50 μm): (a) Control (c) CV, *Chlorella Vulgaris*, and (d) SO, *Saccharum Officinarum*, group showing absence of Cox2 in normal liver. (b) FU, 5-Fluorouracil-treated group showing severe brown signals of Caspase-3 in cytoplasm of hepatocytes (yellow arrows). (e) FU+CV, *Chlorella vulgaris*+5-Fluorouracil, group and (f) FU+SO, *Saccharum officinarum+5-Fluorouracil*, group showing mild expression of Cox2 in cytoplasm of hepatocytes (yellow arrows). Hematoxylin counterstained X20.

4. DISCUSSION

The liver is considered the main organ in body involved in the waste products clearance (detoxification). Liver damage is one of critical side effect related to neoplasm treatment based on chemotherapy (King and Perry, 2001; Maor and Malnick, 2013). FU, a vital

drug which has been commonly used for treatment of cancer, is metabolized mainly in liver tissue. The FU toxic metabolites cause hepatic injury creating severe liver toxicity, which affecting on the utility of FU as an efficacious chemotherapeutic anticancer agent (Pujari and Bandawane, 2021). The oxidative stress role is well documented as a toxic action of FU because of the tempering of the ROS in the different signaling pathways (Rashid et al., 2014; Yoshino et al., 2013).

Algae are gaining great popularity in the cosmetic and pharmaceutical sectors due to their strong capability to produce a many of biologically active compounds (Hussein et al., 2023). Therefore, this experimental study is designed to estimate the efficacy of CV and sugarcane juice against FU-induced hepatotoxicity, focusing on the recognition of the associated mechanisms of their protective effects.

The current findings indicated the exposure to FU (150 mg/kg) on the 8th day i.p. leading to disturbance in hepatic functions, Revealed by a significant increment in the activities of serum ALT and AST in addition non-significant changes in albumin and total protein levels. Transaminases enzymes are specific hepatic labels for the cellular damage and/or toxicity (Zeashan et al. 2009). The cell membrane's permeability can be changed by the liver injury leading to release of these enzymes into blood circulation (Rahim et al., 2014, Ozer et al., 2008). This elevation in transaminases activities, indicating the ability of 5-Fluorouracil to induce hepatocellular injury was confirmed by the histopathological findings. In consistent with our results, various investigators recorded 5-Fluorouracil-induced increase in activities of serum ALT and AST (Khalaf et al., (2022) and Gelen et al. 2018).

Importantly, our recorded results showed enhancement of lipid peroxidation (MDA) and increase of liver oxidative damage in FU exposed rats, revealed by significant increment in hepatic MDA and NO in addition to a decrease the content of GSH, CAT and SOD activities, which considered one of the potential explanations behind 5-FU induced hepatic damage causing rise of ALT and AST activities.

Our study findings dealing with the hepatic oxidant/antioxidant status were coinciding with previously by many studies, (Khalaf et al., 2022, Arab et al., 2018; Gelen et al., 2018). In addition, there is little of evidence demonstrating that the FU-induced organ toxicities are mediated by oxidative stress (Guo et al. 2015). It should be referred to that oxidative stress arises by an unbalance between pro-oxidants and antioxidants, which are very toxic to cells (Zaidi et al., 2014).

The production of free radicals by act of oxidative stress overwhelm antioxidant enzymes, leading to exhaustion of the antioxidant defenses and consecration of lipid peroxidation manifested by the MDA elevation (Akindele et al., 2010, Awodele et al., 2015). This give rise to the progression of liver damage in a different of hepatic disorders (Girish et al., 2009). To achieving living organism’s stability, it is essential to achieving a balance between the oxidative and antioxidant defense (Blokhina et al., 2003).

Here, an elevation in MDA levels and reduction of antioxidants (SOD, CAT, and GSH) reflect the free radicals’ over-generation and antioxidant defense depletion, which leads to cytostasis,

cytotoxicity, and finally, causes hepatocellular dysfunction.

The present histopathological findings are in agreement with our biochemical outcome. Different morphological lesions were approved in FU group as congested central veins and sinusoids, hemorrhage, loss of hepatic cord organization, and hepatic cell necrosis with inflammation. These findings in parallel to Alessandrino et al. (2019) and Khalaf et al. (2022) who stated that FU sever affected the liver histological structure.

Moreover, our hepatic immunohistochemistry results suggested the stimulation of the cell death pathway in FU-treated rats, indicated by a significant increment in the liver caspase-3 immune expression. Regulation of cell survival and elimination of deteriorated or diseased cells is biologically complex process defined as Apoptosis (Orabi et al., 2020; Kuranaga, 2012). Caspase-3, a vital regulator of apoptosis, demonstrates irreversible cell death (Yu et al., 2014). It is activated in response to leakage of cytochrome C from out the mitochondria (Ahmed et al. 2019; Rana, 2008). It is well known that the mitochondria-mediated signaling pathway of apoptosis is strongly caused by oxidative stress (Yiran et al., 2013; Green, 2000). Additionally, the accelerating generation of NO, resulting from FU- intoxication, mediates apoptosis through altering the balance of pro- and anti-apoptotic proteins, also enhances the release of the mitochondrial cytochrome c mediates the subsequent stimulation of caspase-3, (Sizova et al., 2012, Ghosh et al., 2014). Herein, the over expression of caspase-3 in this study confirmed the hepatic damage in the FU group.

Furthermore, matching other earlier research of Al-Asmari et al. (2016), Horrillo et al. (2007) and Hu (2003), our results reported that FU- induced a significant elevation of the of Cox-2 immune expression in liver tissue. Cox-2, proteins of stress response, is plays an important participation in toxicity and progression of disease during chemotherapy (Yeoh et al., 2007). It has been stated that pro-inflammatory cytokines e.g. IL-1ß, IL-6, and TNF-α, are increased in the serum following the FU administration in rats (Khalaf et al., 2022; Arab et al. 2018; Chang et al. 2017). These pro-inflammatory signals have a vital role in the stimulation of Cox-2 transcription by stimulation of mitogen activated protein kinase (MEKK, MAPKK, MAPK, and nuclear factor κB (NF-κB), (Desai et al., 2018).

Importantly, several studies have demonstrated that ROS have participated in the activation of extracellular signal regulated kinase (ERK) that improves transcription factors as NF-κB (Arab et al. 2018) one of the Cox-2 regulatory pathways.

Contrarily, regarding the ameliorative effects of CV and SO against FU-hazards, our findings revealed that *Chlorella Vulgaris* and *Saccharum Officinarum L* normalized the altered hematological markers in rats. This improvement may result to high iron content in SO (Cavalcante *et al.,* 2016). Our study results were parallel to those of Xu *et al.*, (2014) and Abd El Latif *et al.*, (2021) who stated that CV improves hematological parameters alongside enhancements in RBCs, WBC count, PCV, Hb, and lymphocytes. Interestingly, WBCs consider as a most competent immune cells against infectious and non-infectious illnesses (Khani *et al*., 2017). The different doses

of CV in fish fed led to an increment of the fish WBC population size (Magnadottir, 2006)

The findings of this study proved the ameliorative role of CV, and SO against FU-induced liver toxicity.

Daily oral co-administration of CV (400 mg/kg) or SO (15 ml/kg) for two weeks with 5-FU at the 8^{th} day significantly reduce the elevated serum activities of transaminases (ALT and AST) with marked regeneration of hepatic histoarchitecture, reflecting the cytoprotective impact of both CV and SO by improving the hepatocyte integrity and metabolic function. In the same line, Abd El Latif et al. (2021) and El-sheikh *et al.,* (2018) reported that CV reduced serum ALT and AST activities in paracetamol- Deltamethrin intoxicated rats respectively. This reduction and ameliorated effect of SO is evidenced by Singh and Shukla, (2021) who reported that SO decreased serum transaminase activities in paracetamol-intoxicated rats. Interestingly, *Chlorella Vulgaris*, and *Saccharum Officinarum* exert potent nutritional values and immense health benefits because of the high polyphenolic compounds (flavonoids, glycosides, and phenolic acids) content in SO juices ((Singh et al., 2015; Gensini et al., 2022), chlorophyll, carotenoids, tocopherols, flavonoids, ubiquinone, and polyphenols in *Chlorella Vulgaris* (Plaza et al., 2009; Safi et al., 2014; Coulombier et al., 2021) which are extremely potent antioxidants. In addition, their contents of minerals and vitamins. Depending on the fact that hypothesizes the role of oxidative stress in the hepatotoxic effect of FU, thus the hepatoprotective effect of CV and SO recorded in current study may be attributed to their potent antioxidant effect as clarified in the decrease of MDA, NO levels with increase of hepatic SOD, CAT activity and GSH content in this study and earlier studies (Li et al., 2013; Mohammed *et al.*, 2021; Yu *et al.*, 2023).

There is a strong positive correlation between the antioxidant activities and quantity of these compounds due to their redox activities that play a central role in scavenging and capturing of free radicals, peroxide decomposition, and oxygen suppression (Ramirez-Anaya et al., 2015; Martins et al., 2016; Renugadevi et al., 2018). Furthermore, it is well known that the Nrf2–ARE pathway activation could protect the cells from the death mediated by oxidative stress. Going in line with this hypothesis, it was evinced that flavonoids could activate the Nrf2–ARE pathway that is responsible for increasing the endogenous antioxidant enzymes and, consequently, enhances the antioxidant capacity (Saw et al., 2014).

There is no doubt that oxidative stress has an important role in the aggravation and triggering of inflammation and apoptosis as a response to various stimuli exposure. Based on this fact, therefore, the strong relationship between oxidative stress damage and inflammatory/apoptotic responses was considered in this investigation. Our immunohistochemical results findings revealed that CV and SO caused down regulation of caspase-3 and Cox-2 in liver tissue, which could be interpreted by the anti-apoptotic and anti-inflammatory role of CV and SO, possibly via its potent antioxidant properties (Morvaridzadeh et al., 2020).

Furthermore, Saberbaghi et al. (2013) also showed that CV is capable of reducing DNA damage and apoptosis due to its antioxidant properties that

diminish free radicals and ROS preventing DNA damage. Additionally, our results indicate that CV administration resulted in a reduction in caspase-3 expression, showing that *Chlorella vulgaris* reduces FU-induced hepatotoxicity through suppression of apoptosis. This outcome is in agreement with (Ibrahim et al., 2021) who tested the anti-apoptotic properties of Chlorella vulgaris in illness models.

It is known that COX-2 is the major isoform of inflammatory cells (Lorenz *et al.*, 1999). Its Excessive expression is accompanied by tissue injury and inflammation (Aslan et al., 2022) and its molecular levels are correlated with the tumors size and their tendency to underlying tissue invading (Prescott and Fitzpatrick, 2000). Therefore, blocking COX-2 early on prevents the growth of malignant tumors, and retreats pre-malignant tumors confirming the ant-inflammatory effect of CV and SO.

Similarly, Khadrawy et al., (2023) reported the anti-inflammatory activity of CV through an elevation of peroxisome proliferator-activated receptors (PPAR) gene expression. That transcription factor is activated by carotene, which is one of bioactive components in CV (Dembinska-Kiec, 2005). Moreover, the inhibition of COX-2 expression by CV supplementation blocks the formation of the inflammatory mediator's by COX-2 inhibitors as reported and discussed by Cheng et al. (2009).

5. CONCLUSION

In conclusion, the FU administration, a drug used in cancer treatment, induced liver damage indicated by raising the inflammatory biomarkers, oxidative stress, and histopathological alterations. Concurrent administration of CV or SO reduced these effects. By suppressing the inflammatory marker COX-2, which was raised by FU, the therapy of CV or SO reduced inflammation in liver tissue. When FU was used in conjunction with CV or SO, the livers of the treatment group showed greater immunological signals for Caspase-3, indicating a reduction in apoptosis. The present study suggests that CV or SO exhibits a noticeable hepatoprotective action against FU-induced hepatotoxicity in rats. Possibly through antioxidant and antiinflammatory activities. These results proposed that intake of CV or SO may be useful for liver diseases patients.

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