EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL

F

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci.* (F.Toxicology& Pest control) *Vol.14(1)pp189-200 (2022)*
DOI: 10.21608/EAJBSF.2022.242358

Egypt. Acad. J. Biolog. Sci., 14(1):189-200(2022)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

Assessment of the Potential Therapeutic Role of Methanolic Extract for *Cichorium intybus* and *Portulaca oleracea* against H_2O_2-Induced Toxicity

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ARTICLEINFO
Article History
Received: 19/3/2022
Accepted: 30/5/2022
Available:2/6/2022

***Keywords*:**
Cichorium intybus, *Portulaca oleracea*, hepatotoxicity, hydrogen peroxide

ABSTRACT

Cichorium intybus (*CI*) and *Portulaca oleracea* (*PO*) are considered rich sources of bioactive compounds. In the current study, the potential therapeutic effect of *CI* and *PO* was assessed by using two concentrations (150 and 300 mg/kg bw) of their methanolic extract against H_2O_2-induced toxicity in male albino rats. To this end, we carried out the liver function tests [Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Gama-glutamyl transferase (γ-GT), and Albumin], the renal function tests [Creatinine, Urea], the oxidative stress biomarkers [superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA)] in the plasma of the experimental animals. After the toxicity with H_2O_2, the two mentioned concentrations (with time and dose-dependent) of either *CI* or *PO* ameliorated the resulting damage significantly compared to the control group. For example, the levels of hepatic, renal biomarkers, and oxidative stress parameters showed a significant improvement. To conclude, the methanolic extracts of *CI* and *PO* form promising medical agents against the harmful effect induced by H_2O_2 toxicity.

INTRODUCTION

Aberrant accumulation of hydrogen peroxide (H_2O_2) causes oxidative stress, which indicates an excessive secretion of intracellular reactive oxygen species (ROS) levels, subsequently, resulting in impairment of the antioxidant defense system (Ajila & Prasada Rao, 2008; Cheong *et al.*, 2016; Abozid *et al.*, 2018). Although the recent advances in therapeutic and medical applications, hepatic diseases are considered a critical health problem worldwide (Hong *et al.*, 2015). As a result, treating liver diseases by finding novel therapeutic agents is still needed. Despite the powerful radical scavenging activity of multiple synthetic drugs, they still cause liver damage (Cheong *et al.*, 2016). So, new studies shall focus on protection against liver diseases in parallel with reducing drug-induced hepatotoxicity (Jiménez-Arellanes *et al.*, 2016).

Several plant extracts, with one or multiple bioactive compounds, showed hepatoprotective activity against chemically-induced liver damage (Pereira *et al.*, 2016). *Cichorium intybus L.* (Chicory) is one of the herbaceous chive plants belonging to the family Asteraceae, it is usually with bright blue flowers, rarely white or pink. Chicory is grown in different areas of the world and used as a leaf vegetable or salad green, especially in South Africa and India, and as a fructose crop in many parts of the world (Jamshidzadeh *et al.*, 2006; Ilaiyaraja, 2010; Thorat & Raut, n.d.). Chicory is rich in natural antioxidants, it contains many various chemical constituents such as polysaccharides (*e.g.*, inulin),

phenolic acids (*e.g.*, cichoric acid), aliphatic compounds & their derivatives, sesquiterpene lactones, coumarin derivatives, alkaloids, vitamins (*e.g.*, A, B and C), minerals (*e.g.*, K, Ca, Mn and Zn), and essential oils (Mulabagal *et al.*, 2009; Al-Snafi, 2016; Janda *et al.*, 2021). The by-products metabolites such as flavonoids, alkaloids, tannins, and coumarins present in chicory have some biological activities such as antioxidant, anticancer, anti-inflammatory, antiparasitic, antihepatotoxic, which impact positive health effects on humans and livestock (Hoste *et al.*, 2006; Das *et al.*, 2016). Many studies on rats have shown that the whole chicory extracts possess anti-diabetic (Pushparaj *et al.*, 2007), antioxidant (Mehmood *et al.*, 2012), antihepatotoxic (Ahmed *et al.*, 2008; Katiyar *et al.*, 2015)**,** immunotoxic (Kim *et al.*, 2002).

Portulaca oleracea L. (Purslane) is a highly variable herbaceous plant belonging to the family *Portulacaceae*, native to North Africa, the Middle East, India, and now its extensive distribution in many parts of the world (Zhou *et al.*, 2015). Purslane is considered highly nutritious because it is rich in many active substances such as β-carotene, ascorbic acid, and omega-3 fatty acids, which play an important role in human growth, development, and disease prevention (Alam *et al.*, 2014; Voynikov *et al.*, 2019). Moreover, it contains large amounts of vitamins A, C, E, and some complex-B (Filannino *et al.*, 2017), and contains many important minerals such as Ca, Mn, Fe, Mg, Se, and K, as well as proteins and carbohydrates (Chen *et al.*, 2012; Uddin *et al.*, 2014). Purslane has been used traditionally in several countries as an antiseptic, vermifuge, antipyretic, and so forth (Lee *et al.*, 2012). Recent pharmacological studies revealed that purslane has exhibited a large spectrum of pharmacological influences, such as antimicrobial (Dan Z., 2006), antiulcerogenic (Kumar *et al.*, 2010), anti-inflammatory (Chan *et al.*, 2000), antioxidant (Baradaran Rahimi *et al.*, 2019), and hepatoprotective (Eidi *et al.*, 2015) and cut healing (Rashed *et al.*, 2003) properties. For these reasons, World Health Organization listed it as one of the most important widely used medicinal plants (Xu *et al.*, 2006).

Thus, this research was designed to evaluate the potential therapeutic role of the methanolic extract of 80% of both *Cichorium intybus* and *Portulaca oleracea* against H_2O_2-induced toxicity

MATERIALS AND METHODS

1. Plant Collection and Identification:

Cichorium intybus and *Portulaca oleracea* were collected from the local field in Menoufia and the leaves of the plant were identified by botanical members of the Department of Botany, Faculty of Agriculture, Menoufia University. The leaves were washed and air-dried for 24 hours, then dried at 50 °C. The dried samples were ground into a powder using a commercial blender and kept in a refrigerator for analysis.

2. Preparation of Plant Extracts:

The specific weight of dried leaf powdered from each plant was extracted by methanol 80% at room temperature for 3 days. The resulting extracts were filtered using Whatman № 1 filter paper and the residues were re-extracted by the same process until plant materials were exhausted. The collected filtrates were pooled and evaporated to dryness under reduced pressure to give a semisolid residue, which was then lyophilized to get the powder and were stored at - 20 °C until used.

3. Experimental Animals and Study Design:

Thirty healthy adult male albino rats were obtained from the Memorial Institute of Ophthalmology in Giza Egypt. All rats were kept in plastic cages and placed in a well-ventilated rat house (temperature was 23 ± 2 °C and lighting conditions were natural light from large windows during the day and complete darkness during the night). They were fed

on commercial pellets (carbohydrate 80%; protein 10%; fats 5%; salt mixture 4%, and vitamins mixture 1%) and water *ad libitum* throughout the experimental period.

Two weeks later of the adaptation, rats' weight was (150-160 g) that were divided randomly into six groups each having five rats as follows: Group 1 served as a negative control, and groups from 2 to 6 were treated with 0.5 % H_2O_2 in drinking water daily for 15 consecutive days (until the level of ALT activity reached ≥ negative control with 3 folds), then group 2 was left as a positive control, groups 3 and 4 were given *PO* methanolic extract orally at a dose of 150 and 300 mg/kg bw respectively, while groups 5 and 6 were treated by *CI* methanolic extract orally at a dose of 150 and 300 mg/kg bw respectively, these treatments were continued for 30 consecutive days.

At zero time and after 15, 30, and 45 days after the beginning of the experiment blood samples were collected after overnight starvation by orbital sinus veins technique and then transferred to EDTA tubes. Samples were centrifuged at 4000 rpm for 10 min. to separate plasma. Plasma samples were kept in a deep freeze at (- 20 °C) till biochemical analysis.

4. Biochemical Assays:

The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured calorimetrically by using standard methods according to the method described by (Young, 2000), and Moss *et al*, (1987); the activity of γ-glutamyl transferase (GGT) was performed by the kinetic method according to (Shaw *et al.*, 1983). Plasma albumin (Alb) level was determined according to the method of Cannon et al., 1974). Plasma urea and creatinine were evaluated according to the method of (Young, D.S., 2001). Superoxide dismutase (SOD) activity was determined in plasma as (Nishikimi *et al.*, 1972) described. Catalase activity was determined in plasma as described by (Aebi, 1984). Lipid peroxidation in plasma was monitored by determining the concentration of malondialdehyde (MDA) as described by (Ohkawa *et al.*, 1979).

5. Statistical Analysis:

All data were expressed as mean ± SD and statistical analysis was made using the Statistical Package for Social Sciences (SPSS 25.0 software and Microsoft Excel). For tests, analysis of differences between groups consisted of a one-way analysis of variance (ANOVA) with repeated measures, followed by post-hoc comparisons (LSD test). Differences were considered statistically significant at $P < 0.05$ (Landau & Everitt, 2004).

RESULTS

Effect of H_2O_2, *PO*, and *CI* Extract on Liver Functions Parameters:

Figure 1 and Table 1 show a significant ($p<0.001$) rise in the levels of plasma hepatic marker enzymes ALT, AST, ALP, and γ-GT activities of rats intoxicated with H_2O_2 by 317.9%, 352.2%, 162.1%, and 278.5% respectively, and concomitant significant ($p<0.001$) decrease in the albumin level by 50.1% compared to negative control after 45 days from the beginning.

The two concentrations (150 and 300 mg/kg bw) of both plant *PO* and *CI* extracts were significant ($p<0.001$) at mediating effective reduction of ALT by (52.6 and 58.6%; 57.9 and 67% respectively), AST (51.2 and 58.7%; 58.4 and 66.7% respectively), ALP (31.6 and 38.3%; 41.7 and 47% respectively), and γ-GT activities by (44.3 and 51.7%; 49.6 and 60.5% respectively), and concomitant significant ($p<0.001$) induction in the albumin level by (86 and 92%; 91 and 96% respectively) compared to the positive control (H_2O_2) after 30 days from the curing period. The *CI* extract provided higher hepato-protectivity than the *PO* for the improvement of previously mentioned hepatic parameters and the effect of both extracts was time and dose-dependent.

Effect of H_2O_2, *PO*, and *CI* Extract on Kidney Function Tests:

A significant ($p \leq 0.001$) elevation of 213.86 and 163.27% in plasma urea and creatinine levels were observed in the H_2O_2-treated group when compared to the control group after 45 days of the exposure period (Fig. 2). Co-treatment with *PO* extract at 150 and 300 mg/kg significantly ($p \leq 0.001$) reduced the levels of plasma urea and creatinine by (49.2 and 51.1%; 38.3 and 43.9% respectively). While co-treatment with *CI* extract at the same concentration significantly ($p \leq 0.001$) reduced their levels by (54.6 and 59.6%; 48.2 and 54.4% respectively), when compared to the H_2O_2-treated group after 30 days from the curing period.

Table 1: The effect of *CI* and *PO* on the plasma albumin level in male albino rats.

Treatments \ Exposure period		At the beginning	After 15 days	After 30 days	After 45 days
Negative control		4.12 ± 0.084	4.08 ± 0.084[a]	4.06 ± 0.055 [a]	4.01 ± 0.089 [a]
H_2O_2 treatment	**Positive control**	4.11 ± 0.074	3.50 ± 0.071[b]	2.42 ± 0.084 [c]	2.00 ± 0.071 [d]
	PO_1	4.10 ± 0.071	3.52 ± 0.084 [b]	3.67 ± 0.097 [b]	3.72 ± 0.084 [c]
	PO_2	4.12 ± 0.091	3.54 ± 0.055 [b]	3.74 ± 0.089 [b]	3.84 ± 0.089 [bc]
	CI_1	4.08 ± 0.084	3.48 ± 0.084 [b]	3.76 ± 0.089 [b]	3.82 ± 0.084 [bc]
	CI_2	4.10 ± 0.100	3.50 ± 0.100 [b]	3.81 ± 0.089 [b]	3.92 ± 0.084 [ab]

Values represent means ± SD (n = 5). Results are displayed related to negative control values and $P < 0.05$; Statistical analysis was done using one way ANOVA test followed by the Tukey's B.

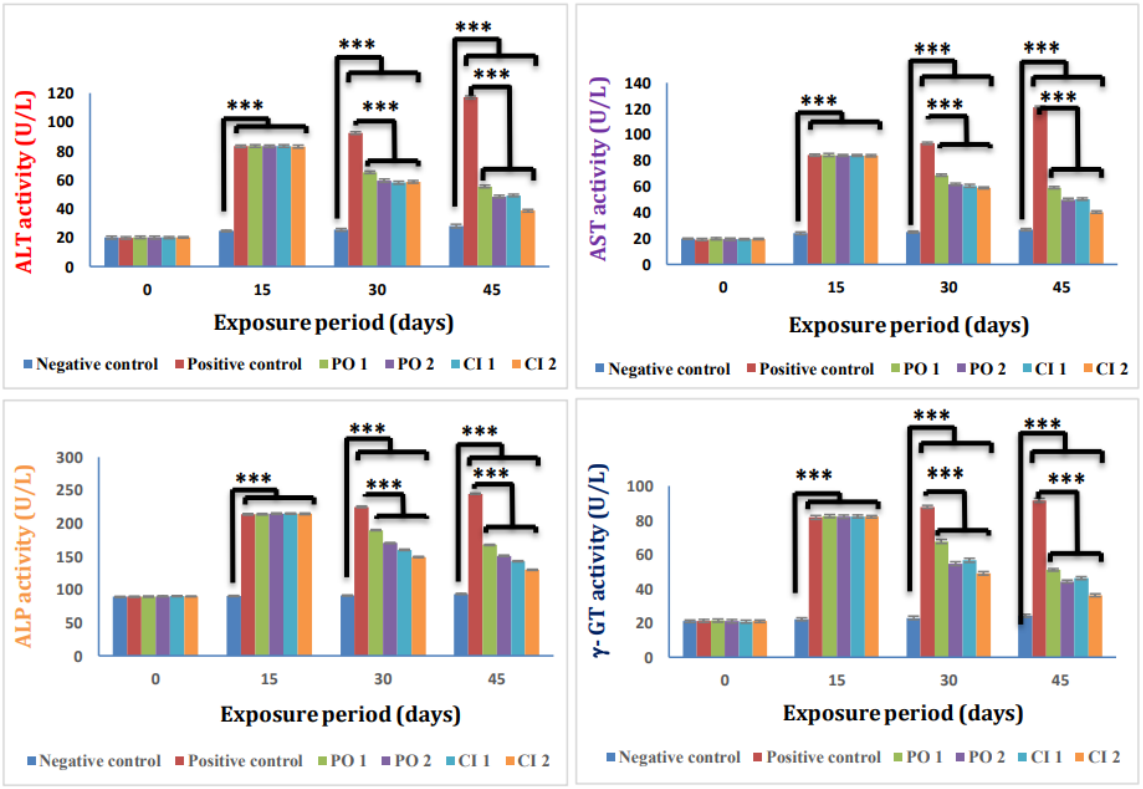

Fig.1: Effect of H_2O_2, *PO*, and *CI* on plasma activities of ALT, AST, ALP, and γ-GT in rats. Each value is representative of means ± SD (n = 5). Data were analyzed by a one-way ANOVA test followed by Tukey's B. * Significantly different from the control and H_2O_2 groups (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Effect of H_2O_2, *PO*, and *CI* Extract on Oxidative Stress Parameters:

The data presented in Figure 3 and Table 2, revealed that animals treated with H_2O_2 induced a significant ($p \leq 0.001$) decrease by 52.42 and 36.51% in plasma SOD and CAT activities, and a concomitant significant ($p<0.001$) increase in the MDA level by (192.3%) compared to negative control after 45 days from the beginning. Co-treatment with *PO* extract at 150 and 300 mg/kg significantly ($p \leq 0.001$) induced the levels of plasma SOD and CAT activities by (61.9 and 68.6%; 19.4 and 21.9% respectively). While co-treatment with *CI* extract at the same level as previous doses of *PO* significantly ($p \leq 0.001$) induced their levels by (74.6 and 86.4%; 23.8 and 35.3% respectively), when compared to the H_2O_2-treated group after 30 days from the curing period.

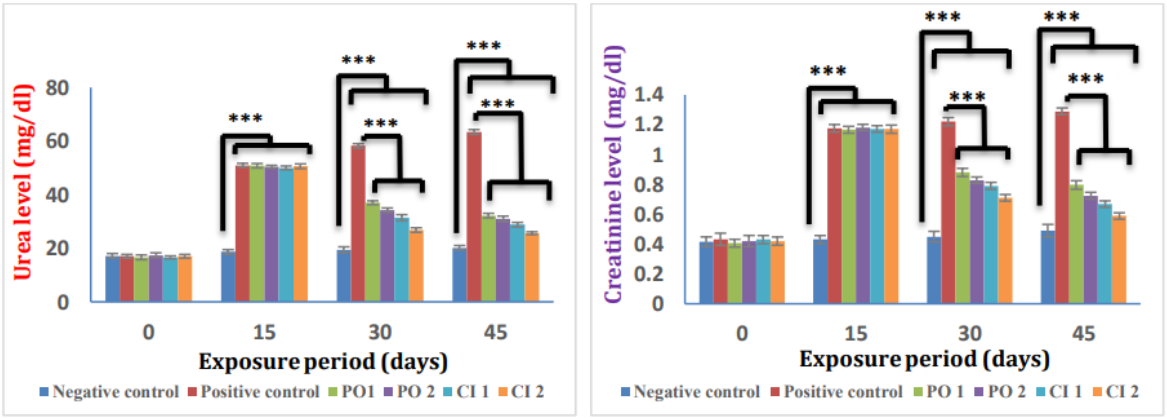

Fig. 2: The effect of H_2O_2, *CI*, and *PO* on the plasma levels of urea and creatinine in rats. Each value is representative of means ± SD (n = 5). Data were analyzed by a one-way ANOVA test followed by Tukey's Post Hoc test. * Significantly different from the control and H_2O_2 groups (* P < 0.05, ** P < 0.01, *** P < 0.001).

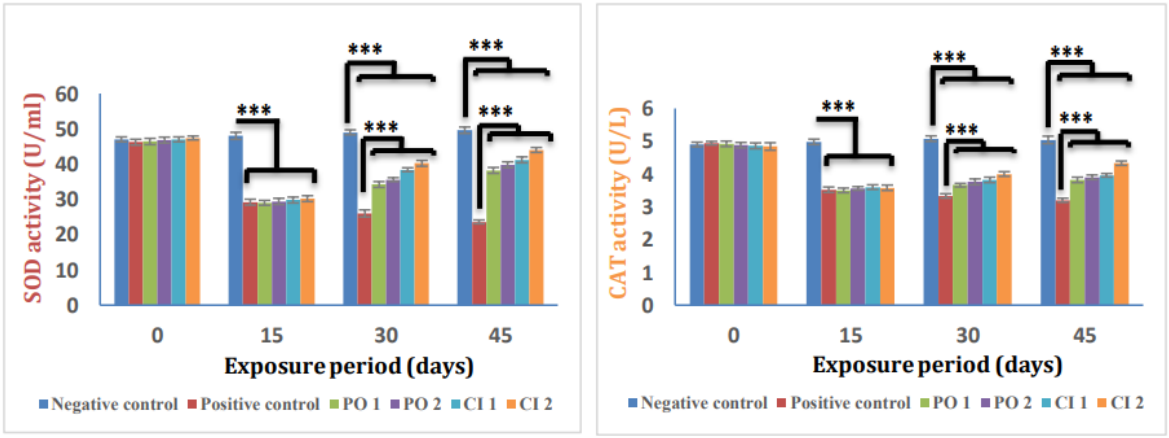

Fig. 3: Effect of H_2O_2, *CI*, and *PO* on the plasma activities of ALT, AST, ALP, and γ-GT in rats. Each value is representative of means ± SD (n = 5). Data were analyzed by a one-way ANOVA test followed by Tukey's Post Hoc test. * Significantly different from the control and H_2O_2 groups (* P < 0.05, ** P < 0.01, *** P < 0.001).

Table 2: The effect of *CI* and *PO* on the plasma MDA level in male albino rats**.**

Treatments \ Exposure period		At the beginning	After 15 days	After 30 days	After 45 days
Negative control		0.107 ± 0.0011	0.116 ± 0.0013 [a]	0.118 ± 0.0021 [a]	0.122 ± 0.0020 [a]
H_2O_2 treatment	**Positive control**	0.106 ± 0.0010	0.333 ± 0.0057 [b]	0.345 ± 0.0071 [f]	0.356 ± 0.0055 [f]
	***PO*$_1$**	0.106 ± 0.0011	0.336 ± 0.0038 [b]	0.296 ± 0.0055 [e]	0.245 ± 0.0035 [e]
	***PO*$_2$**	0.106 ± 0.0015	0.337 ± 0.0045 [b]	0.275 ± 0.0050 [d]	0.228 ± 0.0027 [d]
	***CI*$_1$**	0.106 ± 0.0018	0.333 ± 0.0045 [b]	0.252 ± 0.0027 [c]	0.216 ± 0.0042 [c]
	***CI*$_2$**	0.106 ± 0.0025	0.336 ± 0.0055 [b]	0.224 ± 0.0042 [b]	0.185 ± 0.0050 [b]

Values represent means ± SD (n = 5). Results are displayed related to negative control values and $P < 0.05$; Statistical analysis was done using one way ANOVA test followed by the Duncan test.

DISCUSSION

Hydrogen peroxide (H_2O_2) is unique among general toxins due to its stability in abiotic environments at ambient temperature and neutral pH, yet rapidly kills all the types of cells by producing highly reactive hydroxyl radical ($OH^{\bullet}$). Moreover, the half-life of H_2O_2 is comparatively longer than that of other reactive oxygen species (ROS) (Clarkson & Thompson, 2000). H_2O_2 uses aquaporins to cross cell membranes rapidly (Henzler & Steudle, 2000) and therefore, could diffuse in and out through the cell membrane (Gamaley & Klyubin, 1999). The hepatoprotective effect of some plants was investigated in rats with oxidative stress induced by H_2O_2, which is commonly used in animal models (Al-Malki & Abo-Golayel, 2013; Abozid *et al.*, 2018).

As a result of being the site of basic biochemical reactions, the liver is considered the most important organ in the body. It detoxifies toxic substances and synthesizes beneficial biomolecules. Thus, any injury to it leads to serious consequences (Liu *et al.*, 2010; Subramaniam *et al.*, 2015). Liver damage is associated with cellular necrosis, elevation in the oxidative stress marker, and depletion in the antioxidant indicators. Furthermore, levels of many biochemical parameters such as aminotransferases (ALT, and AST), ALP, γ-GT, bilirubin, triglycerides, and cholesterol are elevated in liver disease (Abozid *et al.*, 2018). It is increasingly believed that ROS and free radicals play an important role in the initiation and progression of liver diseases, regardless of the original causative agent (Siegel *et al.*, 2014).

In the current study methanolic extract of 80% of *PO* and *CI* was tested to overcome H_2O_2-toxicity in the liver and kidneys of rats. As shown in figure 1 and table 1, Obvious changes occurred in H_2O_2-treated rats (positive control group). Severe damage occurred compared to the control group, these damages were manifested in the detection of significant ($p \leq 0.001$) elevations in the activities of hepatic indicators (ALT, AST, ALP, and γ-GT activities) and a significant ($p \leq 0.001$) decrease in the level of albumin, which comes in agreement with the findings of (Rahim *et al.*, 2014; Abozid *et al.*, 2018). The changes in the albumin level resulted from an imbalance between the rate of protein synthesis and the rate of its degradation in the liver. In contrast, treatment with methanolic extract of *PI* and *CI* after 15 days of exposure rat to H_2O_2 restored the albumin level to within the normal range after 30 days of treatment. On the other hand, a significant improvement in the activities of hepatic parameters under study was observed compared with the positive control, but the reduction in their activities was still significant compared to the normal control. The protective effect of *PO* and *CI* extract may be due to its active ingredient components such as omega-3-fatty acids, terpenoids, saponins, flavonoids, polyphenols, and alkaloids (Mulabagal *et al.*, 2009; Eidi *et al.*, 2015; El-Sayed *et al.*, 2015; Elgengaihi *et al.*, 2016; Zlatić & Stanković, 2017; Baradaran Rahimi *et al.*, 2019; Mostafa *et al.*, 2021).

Polyphenols and flavonoids have been reported to possess a membrane-stabilizing activity by inhibiting the ROS generation induced by H_2O_2 and maintaining the cell membrane structural integrity (Abozid *et al.,* 2018). Moreover, it was observed that the therapeutic effect of the methanolic extract of *PO* and *CI* was dose- and time-dependent.

The kidney is one of the target organs of experimental animals attacked by toxins (Lentini *et al.,* 2017; Satarug 2018). A disorder of kidney function reduces the excretion of creatinine, resulting in increased blood creatinine levels. Thus, creatinine levels give an approximation of the glomerular filtration rate. However, it is known that an increase in creatinine occurred with renal failure (Hounkpatin *et al.,* 2019). The functional renal toxicity in this study was evaluated by measuring the urea and creatinine levels which were raised ($p \leq 0.001$) significantly in the H_2O_2-treated group as compared to the control one. These findings come in the same line with previous studies by Salahudeen *et al.,* 1991; Li *et al*., 2016; Abozid *et al.,* 2018; Yalçın et al., 2020 which revealed; that H_2O_2 and other toxins causing renal dysfunction by a significant ($p \leq 0.05$) enhancement of blood creatinine, urea, and uric acid levels, and renal histological changes in experimental animals.

The functional renal toxicity in this study was indexed through urea and creatinine levels which were raised in the H_2O_2-treated group as compared to the control one (Abozid *et al.,* 2018). Salient amelioration in kidney function was detected after being treated with methanolic extract of *PO* and *CI*, which supports the protective effect of each one against H_2O_2 renal toxicity. The results of this study are consistent with previous studies that supported the positive effect of purslane on kidney functions in rats. In different treatments, purslane showed a significant lowering effect on urea and creatinine levels in the blood compared to the group treated with renal toxic agents (Abozid *et al.,* 2018; **Seif et al., 2019**). The kidney-protective effect of methanolic extract of *PO* and *CI* can be explained by the high content of these extracts of phenolic compounds and flavonoids, which are known to be strong antioxidants capable of resisting the oxidative stress that causes kidney cell damage (Panuganti *et al.,* 2006; Lee *et al*., 2008; Farid HEA, *et al.*, 2019).

It is known that the organism's body is enclosed by a complex antioxidant defense lattice based on endogenous enzymatic and non-enzymatic antioxidant molecules. These molecules act collectively against free radicals to ultimately counteract their harmful effects on vital biomolecules and body tissues (Ziad Moussa, 2019). The SOD and CAT Indicators are important and indispensable, they represent the first line of defense in the complete antioxidant defense strategy, especially regarding the superoxide anion radical that is permanently generated in the body's normal metabolism, particularly through the mitochondrial energy production pathway (MEPP). Determination of enzyme markers CAT, SOD, GPx and MDA are widely accepted and used in antioxidant evaluation studies (Celik, 2007; Ighodaro & Akinloye 2018).

To evaluate the effect of methanolic extract of *PO* and *CI* on H_2O_2-induced oxidative stress, the antioxidant indices such as CAT, SOD activities, and MDA levels were measured. The present study showed that H_2O_2 caused a significant decrease in the SOD and CAT activity, while it caused a significant increase in MDA levels compared with a control group. The treatment with methanolic extract of *PO* and *CI* led to a significant decrease in levels of MDA and a significant increase in the activity of both CAT and SOD compared to the positive control (Eidi *et al.,* 2015; El-Sayed *et al.,* 2015; Asadi *et al.,* 2018; Rahimi *et al.,* 2019). The ability of the AER to restore the activities of SOD and CAT enzymes and MDA levels to normal levels in the H_2O_2-treated animals might be attributed to the antioxidant and the free radical scavenging properties of plant extracts as antioxidants appear to counteract disease by increasing the antioxidant enzymes SOD and CAT activities and decreasing the lipid peroxidation indicator MDA (Bansal *et al.,* 2005).

The results strongly further suggest the significant role of methanolic extract *PO* and *CI* in protecting liver and kidney cells and rebalancing the antioxidant defense system in rats against strong oxidizing agent H_2O_2. This protective effect can be clearly attributed to the high content of these extracts of natural antioxidants, which are mainly responsible for combating free radicals that cause cell damage, such as H_2O_2.

Conclusions

Collectively, these results demonstrate the possibility of using both *CI* and *PO* extracts to improve the harmful effect of H_2O_2 on biological systems, because it has a hepatorenal protective effect as it can protect tissues from free radicals, and the use of such extracts may provide a way for a prolonged therapeutic option against kidneys and liver diseases without harmful side effects. Higher doses of both extracts produced higher effects on all variables under study than lower doses, and the therapeutic effect of *CI* was more than that of *PO*.

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