Studying the Potential Effect of Centaurea cineraria and Centaurea cyanus Plants on Hepatotoxicity Induced in Rats

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Abstract

Centaurea species possess bioactive compounds with liver-protective and antioxidant potential. This study aimed to assess the hepatoprotective potential of *Centaurea cineraria* and *Centaurea cyanus* (cornflower), individually and in combination, against paracetamol-induced liver toxicity in rats. It also investigated their chemical composition, phenolic constituents, and the histopathological alterations in hepatic tissues. Forty-eight male albino rats were assigned to eight groups: a negative control, a paracetamol-induced hepatotoxic control, and six treatment groups fed diets containing *C. cineraria* or *C. cyanus* at 5% or 10%, either individually or in combined ratios of 2.5%+2.5% and 5%+5%. Biochemical parameters, including liver enzymes (AST, ALT, ALP), renal function markers (urea, creatinine, albumin) and oxidative stress indicators (MDA, CAT, SOD, GSH), were estimated, and histopathological examination of liver tissues was conducted to support the biochemical findings. Paracetamol markedly increased liver enzymes, MDA, urea, creatinine and while decreasing albumin and antioxidant markers (SOD, CAT, GSH). Supplementation with *C. cineraria* and *C. cyanus* notably improved the altered biochemical parameters, with the 5%+5% combination showing the strongest hepatoprotective effect by normalizing liver enzymes, lowering MDA, and restoring antioxidant markers (SOD, CAT, GSH) toward control levels. Histological examination supported biochemical results, revealing marked improvement in liver structure and a noticeable reduction in hepatic degeneration, most prominently in the groups receiving the combined supplementation.

Conclusion:

C. cineraria and *C. cyanus* demonstrated significant hepatoprotective and antioxidant effects against paracetamol-induced liver damage, with enhanced efficacy when used in combination. These findings suggest their potential as natural candidates for developing functional foods or nutraceuticals that support liver health.

Key words: *Centaurea cineraria*, *Centaurea cyanus* (Cornflower), Hepatoprotective, Antioxidant activities, Hepatotoxicity.

INTRODUCTION

The liver is a vital organ that regulates metabolism, synthesis, and detoxification processes essential for maintaining body homeostasis. It manages carbohydrate, protein, and lipid metabolism, nutrient storage, toxin elimination, and supports immune and endocrine functions. Consequently, liver dysfunction can cause systemic disturbances that compromise overall health and well-being **(Abdalla et al., 2016).**

Hepatic injury arises from an imbalance between damaging and protective mechanisms, often induced by environmental, dietary, chemical, or drug-related factors. Oxidative stress, inflammation, and immune dysfunction contribute to hepatocellular damage and functional loss, which may progress to fibrosis, cirrhosis, liver failure, or carcinoma if untreated. Understanding these mechanisms is crucial for developing preventive and therapeutic strategies **(Wolf et al., 2021; Alqasoumi et al., 2018).**

In experimental studies, hepatotoxicity is commonly induced using known hepatotoxins like paracetamol, carbon tetrachloride, or alcohol to simulate human liver disorders under controlled conditions. The resulting damage is assessed through physiological, biochemical, and histopathological markers, providing essential models for evaluating the hepatoprotective efficacy and safety of natural and synthetic agents before clinical use **(Younossi et al., 2019).**

increasing interest has focused on natural products and medicinal plants as alternative or complementary therapies for hepatotoxicity. Experimental and clinical evidence demonstrates that plant extracts and bioactive compounds possess antioxidant, anti-inflammatory, and cytoprotective properties, reinforcing the traditional use of herbs in liver health and guiding the development of new hepatoprotective agents **(Gulati et al., 2018).**

The genus *Centaurea* (family Asteraceae) is one of the largest within the family, comprising over 600 species distributed across Europe, Western Asia, and the Mediterranean region. It is characterized by a rich phytochemical profile that includes flavonoids, phenolic acids, sesquiterpene lactones, and essential oils with notable hepatoprotective and pharmacological properties. Traditionally, *Centaurea* species have been used to treat fever, diarrhea, and inflammatory disorders. In Egypt, around 17 species are naturally found along the Mediterranean coast, the Red Sea, and Sinai, underscoring their

ethnopharmacological and ecological importance **(Garcia-Jacas et al., 2000; Porusia & Septiyana, 2021 and Senosy et al., 2018).**

Centaurea cyanus L. (cornflower) is an annual herb recognized for its deep-blue flowers colored by the pigment protocyanin. Traditionally, it has been used for its diuretic, anti-inflammatory, and hepatoprotective properties. Likewise, *Centaurea cineraria* has gained interest for its bioactive compounds and potential role in reducing oxidative stress and liver damage **(Salih et al., 2022 and Hashim et al., 2023).**

Recent studies highlight the antioxidant potential of *C. cineraria* and *C. cyanus*, mainly linked to their high levels of flavonoids, phenolic acids, and related compounds. Nevertheless, detailed investigations, especially controlled animal experiments and clinical trials, are still scarce, underscoring the need for further research to clarify their mechanisms, confirm efficacy, and assess clinical relevance. Therefore, the aim of this study was to evaluate the potential effect of *Centaurea cineraria* and *Centaurea cyanus* plants on hepatotoxicity induced in rats.

Materials and Methods

Materials:

- **Paracetamol** was obtained from Gulf Pharmaceutical Industries (Julphar), while diethyl ether and other chemicals were supplied by El-Gomhoriya Company for Trading Drugs and Chemicals, Cairo, Egypt.
- **Casein, vitamins, minerals, cellulose, L-cystine, and choline chloride** were also purchased from El-Gomhoriya Company, Cairo, Egypt.
- **Sucrose, starch, and soybean oil** were sourced from local markets in Cairo.
- **Biochemical assay kits** were employed to evaluate Oxidative stress biomarkers such as glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation were also measured. Liver and kidney function indicators, including alkaline phosphatase, were analyzed as well. All kits were obtained from the Egyptian Company for Biotechnology–Spectrum Diagnostics, Obour City Industrial Area, Cairo, and from Bio-Diagnostic, Diagnostic and Research Reagents, Tahrir Street, Dokki, Giza, Egypt.

- ***Centuarea cineraria* and *Centuarea cyanus* (cornflower)** samples were collected from Zaranik Nature Reserve, North Sinai, Egypt.
- **Rats**: Forty-eight adult male albino rats (Sprague Dawley strain), weighing 180 ± 10 g, were obtained from the Animal Research Institute, Dokki, Egypt. The animals were housed in cages under controlled conditions (25 ± 4 °C) with free access to food and water. All experimental procedures followed standard ethical guidelines and were approved by the Agricultural Research Center's Animal Care and Use Committee (ARC-IACUC No. 108).

Methods:

- **Dried of *Centaurea cineraria* and *Centaurea cyanus* plants:**

  The samples were dried using solar energy at 40 °C – National Research Center, Cairo, Egypt.

- **Chemical Composition Analysis:**

The chemical composition of *Centaurea cineraria* and *Centaurea cyanus* was determined using the standard procedures recommended by the Association of Official Analytical Chemists **(AOAC, 2020).** The analyzed parameters included moisture, protein, fat, ash, and crude fiber. The total carbohydrate content was calculated by difference using the following equation:

Total carbohydrate (**%**)=100-
(Moisture**%**+Protein**%**+Fat**%**+Ash**%**+Fiber**%**)

The phenolic constituents of *Centaurea cineraria* and *Centaurea cyanus* extracts were analyzed using Gas Chromatography–Tandem Mass Spectrometry (GC–MS/MS) to achieve precise identification and profiling. **(Santana et al., 2013).**

- **Experimental plan and procedures**

The experiment was conducted using forty-eight (48) male Sprague Dawley rats with an average body weight of 180 ± 10 g. The animals were housed in stainless-steel metabolic cages under hygienic and controlled environmental conditions, including a 12/12-hour light/dark cycle, a

temperature of 25 ± 4°C, and relative humidity ranging from 46% to 52%. Food and water were provided ad libitum. The basal diet was nutritionally adequate according to the AIN-93G formulation, with vitamin and mineral mixtures prepared as described by **Reeves et al. (1993).**

The rats were randomly assigned into two main groups. The first group (6 rats) served as the negative control, while the second group (42 rats) received a single oral dose of paracetamol (750 mg/kg) to induce liver toxicity, following the procedures described by **Plaa and Hewitt (1982)** and **Dash et al. (2007)**. After induction, the second group was further divided into seven subgroups (n = 6) as follows: a positive control group (paracetamol-induced hepatotoxicity) and six treated groups receiving paracetamol along with diets supplemented with *C. cineraria* (5% or 10%), *C. cyanus* (5% or 10%), or their combinations (2.5% + 2.5% and 5% + 5%). All experimental diets were standardized for vitamin, mineral, and fiber content and administered for 8 weeks. Body weight and feed intake were monitored weekly. Nutritional assessment was performed according to the method described by **Chapman et al. (1959)**.

At the end of the experimental period, Feed Intake was estimated, Body weight gain % and liver-to-body weight % were calculated. Blood samples were centrifuged at 4000 ×g for 15 minutes, and the separated serum and plasma were stored at -20°C for analysis. Liver tissues were homogenized and analyzed for antioxidant enzyme activities, including superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT), expressed as units per milligram of protein.

The activities of SOD, CAT, and glutathione (GSH) were determined according to the methods of **Nishikimi et al. (1972)**, **Aebi (1984)**, and **Paglia and Valentine (1967)**, respectively. Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) using the thiobarbituric acid (TBA) assay as described by **Draper and Hadley (1990)**. For liver function, the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured colorimetrically following **Reitman and Frankel (1957)**. Kidney function was assessed by determining urea levels according to **Fawcett and Scott (1960)** and creatinine levels following **Bartels et al. (1972)**.

Pathological Alterations and Liver Index

At the time of sacrifice, the entire liver was excised and photographed to document gross pathological alterations. The livers were then carefully washed with phosphate-buffered saline (PBS), blotted dry using filter paper, and weighed. The liver index was subsequently calculated using the following formula:

Liver index=Liver weight (g)/Body weight (g)×100 as described by **Chapman (1959)**.

Histopathological Examination

Liver tissue specimens were collected and immediately fixed in 10% neutral buffered formalin for 24 hours. Samples were then dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections of 5 µm thickness were prepared and stained with hematoxylin and eosin (H&E) for routine histological examination **(Suvarna et al., 2018)**. All sections were microscopically evaluated for histopathological alterations, and representative images were captured using a Swift microscope equipped with a Swift digital camera **(Gibson-Corley et al., 2013)**.

Statistical Analysis:

Data were expressed as mean ± standard deviation (SD). Statistical evaluations were conducted using SPSS software (GraphPad Software Inc., San Diego, CA, USA). A one-way analysis of variance (ANOVA) was applied, followed by Duncan's multiple range test for post hoc comparisons. A P-value of ≤ 0.05 was considered statistically significant, following the methodology of **Sendecor and Cochran (1979).**

Results and Discussion:

Chemical composition of *Centaurea cineraria and Centaurea cyanus* plants:

The nutritional composition of medicinal and wild edible plants reflects their health benefits and functional value. Variations in nutrient content influence metabolism and overall well-being. In this context, *Centaurea cineraria* and *Centaurea cyanus* (cornflower) show promise for nutritional and therapeutic use due to their traditional medicinal roles and rich phytochemical content. Table (1) compares the chemical composition of *Centaurea cineraria and Centaurea cyanus.*

Table (1): Chemical composition of *Centaurea cineraria and Centaurea cyanus* plants

Items of plant / Nutrients	*Centaurea*	
	Cineraria	***Cyanus***
Protein (g/100g)	5.18	10.33
Fat (g/100g)	0.94	1.18
Ash (g/100g)	0.94	1.18
Moisture (g/100g)	7.74	6.81
Fiber(g/100g)	38.6	29.8
Carbohydrate (g/100g)	46.6	50.7
Total energy (kcal/100g)	221.179	263.134

Each value listed in the table represents the meaning of two determinations.

An analysis of their chemical composition shows distinct differences between the two species. The significantly higher protein content of *Centaurea cyanus* (10.33 g/100g) compared to *Centaurea cineraria* (5.18 g/100g) reflects an increase of nearly 99.4%. Similarly suggests that the former may serve as a better source of amino acids for metabolic and structural functions. Increased protein intake from plant sources is associated with improved tissue repair and enzymatic activity, both of which are essential during hepatic recovery from oxidative stress and toxic injury **(Skrajda-Brdak et al., 2020**).

On the other hand, *Centaurea cineraria* was found to be richer in dietary fiber (38.6 g/100g), which is almost 29.53% higher than that of

Centaurea cyanus. Dietary fiber is well established as a regulator of gut health, bile acid metabolism, and cholesterol absorption, thereby contributing indirectly to hepatoprotection and cardiovascular health (**Sowbhagya et al., 2019**). This suggests that *Centaurea cineraria* may exert protective effects by modulating lipid metabolism and maintaining intestinal-liver axis balance.

The higher carbohydrate and energy values observed in *Centaurea cyanus* (50.7 g/100g and 263.13 kcal/100g, respectively) further support its role as an energy-dense plant food. Such attributes may be beneficial in conditions where energy requirements are elevated but may also require moderation in individuals with metabolic disorders. Conversely, the greater moisture content of *Centaurea cineraria* (7.74 g/100g) may enhance digestibility and contribute to hydration. These findings are consistent with previous reports that highlight the variability in proximate composition among wild edible plants, where species-specific differences in carbohydrate, fiber, and moisture content reflect adaptive and ecological variations (**Khalil et al., 2021**).

The results demonstrated that both the fat and ash contents of *Centaurea cyanus* (1.18 g/100 g) were 25.5% higher than those of *Centaurea cineraria* (0.94 g/100 g). Although the fat content in both plants was relatively low, the higher level in *C. cyanus* may contribute to improved energy density and facilitate the absorption of fat-soluble vitamins. Similarly, the greater ash content reflects higher mineral availability, which is consistent with the role of *Centaurea* species as potential sources of essential micronutrients. These findings align with reports that indicate members of the *Centaurea* genus are rich in bioactive compounds and minerals that support metabolic and physiological functions (**Aktumsek et al., 2013**). The comparative differences suggest that *C. cyanus* may offer advantages in terms of energy and micronutrient supply, whereas *C. cineraria* remains valuable for its higher fiber and moisture levels, which may benefit digestive health.

Overall, the variation in nutrient composition between these species highlights their complementary nutritional roles. *Centaurea cyanus* may serve as a superior source of energy and protein enrichment, while *Centaurea cineraria* could contribute significantly to dietary fiber intake and hydration,

underscoring their potential applications in functional foods and health-promoting diets.

Phenolic compounds of *Centaurea cineraria* and *Centaurea cyanus*:

The comparative analysis of phenolic compounds in *Centaurea cineraria* and *Centaurea cyanus* highlights the biochemical diversity and potential therapeutic significance of these species. Both plants demonstrated a complex mixture of phenolics, flavonoids, chalcones, and lipid derivatives, many of which are well-documented for their antioxidant, anti-inflammatory, and hepatoprotective activities.

In *C. cineraria*, the predominance of 2′,4,4′,6′-tetramethylether-3′-prenylchalcone (52.78%) is particularly noteworthy. Chalcones are recognized for their strong free radical scavenging capacity and ability to modulate oxidative stress pathways, which may explain the traditional use of Centaurea species in managing liver-related disorders (**Çelik et al., 2019**). The presence of other bioactive compounds such as 3′,4′,7-trimethylquercetin (3.73%), 9,12,15-octadecatrienoic acid (3.72%), and glycerol monooleate (5.47%) further supports the hepatoprotective potential of this plant. These molecules are associated with stabilizing hepatocyte membranes, modulating lipid metabolism, and reducing inflammatory responses. The relatively high proportion of phospholipid-like molecules, such as 1-hexadecyl-2(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (27.68%), may also contribute to preserving liver cell integrity under conditions of oxidative stress.

By contrast, *C. cyanus* exhibited a distinct phenolic profile, dominated by 2′-methoxy-β-naphthoflavone (77.59%). This compound, while structurally different from typical flavonoids, is reported to act as a modulator of hepatic detoxification enzymes, thereby enhancing the liver's capacity to neutralize toxic insults. Additionally, chalcone derivatives such as 3,4-dimethoxy-2′-(acetyl)oxy-5′-methylchalcone (7.89%) and flavonoid glycosides like luteolin 6,8-di-C-glucoside (1.06%) indicate that C. cyanus also contributes to antioxidant defense and hepatocyte protection. The detection of β-sitosterol (1.24%) further adds nutritional and pharmacological relevance, given its lipid-lowering and anti-inflammatory effects.

Table 2: Total Phenolics in dried *Centaurea Cineraria* and *Centaurea cyanus* plants.

	NO.	RT	Name	Area Sum%
Centaurea Cineraria	1	15.54	2-Hexadecanol	1.84
	2	16.54	3′, 4′, 7 −Trimethylquercetin	3.73
	3	16.66	9,12,15-Octadecatrienoic acid	3.72
	4	18.82	2′, 4,4′, 6′ −Tetramethylether -3'-prenylchalcone	52.78
	5	19.26	5,7-Dihydroxy-3',4'-dimethoxyflavone	1.10
	6	20.90	1,3-Diolein	0.97
	7	21.25	Luteolin 6,8-c-diglucoside	0.58
	8	22.00	Glycerol monooleate	5.47
	9	22.44	3′, 4′, 5′, 5,6,7 −Hexamethoxyflavone	2.13
	10	23.38	1-Hexadecyl-2(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine	27.68
Centaurea Cyanus				
	1	12.13	2'-Methoxy-β-naphthoflavone	77.59
	2	12.62	4'-Methoxy-α-naphthoflavone	2.21
	3	12.89	4'-Methoxy-β-naphthoflavone	1.00
	4	13.12	4',5,7-Trihydroxy 3,6,8-trimethoxyflavone	3.02
	5	17.00	Jaceidin	0.92
	6	17.35	9-Octadecenoic acid (Z)-	1.33
	7	18.46	β-Sitosterol	1.24
	8	20.33	6,7,3',4'-Tetramethoxyflavone	1.36
	9	20.84	3,4-Dimethoxy-2'-(acetyl) oxy-5'-methylchalcone	7.89
	10	21.98	2',4,4',6'-Tetramethylether-3'-prenylchalcone	1.40
	11	22.42	3',4',7-Trimethylquercetin	0.99
	12	23.92	Luteolin 6,8-di-c-glucoside	1.06

The observed phenolic diversity in both species aligns with previous studies that reported significant phenolic content and antioxidant activity in Centaurea extracts, with values ranging between 30 and 50 mg gallic acid equivalents (GAE)/g dry weight, depending on the extraction method and plant part analyzed. Such variation is consistent with environmental factors, harvest timing, and extraction solvents, all of which influence the phytochemical profile (**Al-Kateb et al. 2020** and **Salachna and Kaczmarek, 2021)**.

Collectively, these findings suggest that *C. cineraria* and *C. cyanus* possess complementary phenolic profiles: *C. cineraria* is rich in chalcones and phospholipid derivatives that stabilize hepatocyte function, whereas *C. cyanus* is characterized by methoxylated flavones and phytosterols that enhance detoxification and antioxidant defense. The therapeutic implications are significant, indicating potential applications of both species in the development of functional foods or herbal formulations aimed at protecting the liver from chemically induced injury.

Biological evaluation:

This study evaluated the effects of dietary supplementation with *Centaurea cineraria* (C.C.), *Centaurea cyanus* (cornflower, C), and their combinations on feed intake, body weight gain, and the liver-to-body weight ratio in rats with paracetamol-induced hepatotoxicity. Table (3) presents the effects of these supplements, at different inclusion levels, on the nutritional parameters of the experimental groups.

No significant differences in feed intake were observed among all experimental groups ($p > 0.05$), suggesting that supplementation with either plant or their combination did not negatively impact palatability or appetite. This finding aligns with previous studies reporting that herbal supplements often do not interfere with voluntary feed consumption in experimental animals (**Chougule et al., 2014[a],; Chougule et al., 2014[b]**). Maintaining feed intake is crucial to avoid confounding factors in studies assessing hepatoprotective effects.

Body weight gain was significantly reduced in hepatotoxic rats compared to healthy controls, reflecting the systemic impact of liver damage on metabolism and nutrient utilization (**Gan et al., 2025**). Rats which are treated

with *C. cineraria* at 5% and 10% exhibited further reductions in body weight gain, which may be related to its higher fiber content (38.6 g/100 g) and lower caloric density compared to *C. cyanus* (**Teixeira et al., 2017**). In contrast, *C. cyanus*-supplemented groups showed non-significant differences in weight gain relative to hepatotoxic controls, likely due to their higher protein (10.33 g/100 g) and carbohydrate content (50.7 g/100 g), providing sufficient energy to maintain body mass (**Tong et al., 2019**). Combined supplementation appeared to mitigate weight loss to some extent, possibly reflecting a synergistic effect of both plants in supporting metabolic homeostasis during hepatotoxic stress.

Table (3): Effect of *Centaurea cineraria* and *Centaurea cyanus* Plants on feed intake, body weight gain%, and liver weight/body weight% in rats with hepatotoxicity.

Parameters / Groups	Feed Intake (g/day/each rat)	Body Weight Gain%	Liver Weight/Body Weight%
Control (-ve)	17.753 ^{a} ± 0.366	55.714 ^{a} ± 4.938	2.878 ^{c} ± 0.301
Control (+ve)	17.556 ^{a} ± 0.577	50.030 ^{b} ± 2.949	3.552 ^{a} ± 0.081
***Centaurea Cineraria* (5% C.C)**	17.960 ^{a} ± 0.230	40.388 ^{c} ± 5.348	3.023 ^{c} ± 0.309
***Centaurea Cineraria* (10 % C.C)**	17.640 ^{a} ± 0.207	39.903 ^{c} ± 2.344	2.904 ^{c} ± 0.448
***Centaurea cyanus* (Cornflower 5%C.)**	17.930 ^{a} ± 0.277	47.877 ^{b} ± 2.991	3.124 $^{b\,c}$ ± 0.093
***Centaurea cyanus* (Cornflower 10 %C.)**	17.660 ^{a} ± 0.207	46.169 ^{b} ± 4.147	3.123 $^{b\,c}$ ± 0.127
(2.5% C.C) plus (2.5% (Cornflower C.)	17.920 ^{a} ± 0.303	48.897 ^{b} ± 5.849	3.427 $^{a\,b}$ ± 0.242
(5% C.C) plus (Cornflower 5% C.)	17.680 ^{a} ± 0.342	45.439 $^{b\,c}$ ± 3.986	2.880 ^{c} ± 0.180

C.C: *Centaurea Cineraria*, ***C.:*** *Centaurea Cornflower*

All parameters are represented as a means of replicates ± standard Dev.

Means with different small superscript letters in the same row are significantly different at $p \leq .05$.

The liver index, which reflects organ hypertrophy or edema associated with hepatotoxicity, was significantly elevated in the positive control group (3.552 ± 0.081) compared to the negative control (2.878 ± 0.301). This increase is consistent with paracetamol-induced hepatic injury, characterized by

hepatocellular swelling and inflammatory infiltration (**Younossi et al., 2019**). Treatment with *C. cineraria* significantly reduced the liver index, indicating a protective effect that likely stems from its antioxidant phenolic compounds, such as 2',4,4',6'-Tetramethylether-3'-prenylchalcone, which have been shown to stabilize hepatocyte membranes and reduce oxidative stress (**Al-Snafi , 2020**).

Centaurea cyanus given independently demonstrated moderate reductions in liver index, which may be attributed to its high content of 2'-Methoxy-β-naphthoflavone and other flavonoids capable of scavenging free radicals and modulating detoxification pathways (**Al-Kateb et al., 2020**). Interestingly, the combination treatment at higher inclusion levels (5% C.C plus 5% C) resulted in the lowest liver index (2.880 ± 0.180), comparable to the healthy control. This suggests a synergistic hepatoprotective effect, where bioactive compounds from both plants may act additively or complementarily to reduce hepatocellular edema and restore liver morphology. In contrast, the lower combined level (2.5% C.C plus 2.5% C.) yielded a less pronounced effect, indicating that both concentration and compound interactions play a critical role in hepatoprotection.

Overall, these findings demonstrate that *C. cineraria* and *C. cyanus*, individually and in combination, can modulate liver injury markers and maintain metabolic balance in hepatotoxic rats. The observed effects are likely mediated through antioxidant, anti-inflammatory, and membrane-stabilizing activities of the phenolic and flavonoid compounds present in both plants (**Sariburun et al., 2010; Akhtar et al., 2021**).

Liver Enzymes:

The liver enzymes AST, ALT, and ALP are critical biomarkers of liver function, with elevated levels signaling liver damage or impairment. The data in Table (4) demonstrate the effects of two levels of *Centaurea cineraria* (C.C.) and *Centaurea cyanus* (*Cornflower, C*) on liver enzyme levels-Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP)-in hepatotoxic rats.

In the present study, hepatotoxicity induced by paracetamol in rats caused a significant increase ($P \leq 0.05$) in AST (50.000 ± 5.185 U/l), ALT (28.000 ± 2.645 U/l), and ALP (336.000 ± 5.567 U/l) compared to the negative control group, confirming liver injury (**McGill et al., 2012**; **Nakagawa et al.,**

2008). Elevated levels of these enzymes indicate leakage from hepatocytes due to oxidative stress and membrane damage (**Li et al., 2019**). Supplementation with *Centaurea cineraria* and *Centaurea cyanus*, individually or in combination, significantly reduced these liver enzymes in hepatotoxic rats, suggesting hepatoprotective activity. Notably, 10% C.C. treatment decreased ALT to 12.333 ± 1.527 U/l and AST to 36.000 ± 2.00 U/l, approaching levels observed in healthy rats. Similarly, combined high-dose treatments (5% C.C + 5% C) reduced AST to 33.000 ± 3.605 U/l and ALP to 184.666 ± 19.857 U/l. These results indicate a synergistic effect when both plants are combined (**Poprac et al., 2017**). The hepatoprotective effect of these plants may be attributed to their high phenolic and flavonoid content, which exerts antioxidant and free radical scavenging activity, stabilizing hepatocyte membranes and reducing enzyme leakage (**Alkadi, 2020; Cristani et al., 2016**). Previous studies on plant-derived anthocyanins and chalcone derivatives have demonstrated similar reductions in ALT, AST, and ALP in drug-induced hepatotoxicity models (**Korga et al., 2017**).

Table (4): Effect of *Centaurea cineraria* and *Centaurea cyanus* Plants on liver enzymes in rats with hepatotoxicity

Parameters / Groups	AST	ALT	ALP
	U/l		
Control (-ve)	30.000 ^{c} ± 3.00	14.333 $^{d e}$ ± 1.527	206.000 ^{d} ± 29.597
Control (+ve)	50.000 ^{a} ± 5.185	28.000 ^{a} ± 2.645	336.000 ^{a} ± 5.567
5% Centaurea Cineraria (C.C)	38.666 ^{b} ± 1.527	22.000 $^{b c}$ ± 3.605	262.333 $^{b c}$ ± 25.967
10 % Centaurea Cineraria (C.C)	36.000 $^{b c}$ ± 2.00	12.333 ^{e} ± 1.527	218.666 $^{c d}$ ± 35.019
5% Centaurea cyanus (Cornflower C)	37.333 $^{b c}$ ± 2.081	18.666 $^{c d}$ ± 2.081	230.000 $^{c d}$ ± 18.681
10 % Centaurea cyanus (Cornflower C)	39.333 ^{b} ± 4.725	20.000 $^{b c}$ ± 3.605	277.000 ^{b} ± 14.000
2.5% (C.C) + 2.5% (Cornflower C)	34.333 $^{b c}$ ± 3.214	24.333 $^{a b}$ ± 2.516	277.000 ^{b} ± 34.698
5% (C.C) + 5% (Cornflower C)	33.000 $^{b c}$ ± 3.605	20.333 $^{b c}$ ± 2.516	184.666 ^{d} ± 19.857

C.C: *Centaurea Cineraria*, ***C.:*** *Centaurea Cornflower*

All parameters are represented as a means of replicates ± standard Dev.

Means with different small superscript letters in the same row are significantly different at $p \leq .05$.

Renal Function Markers

Hepatotoxicity also affected renal function markers. The data presented in Table (5) it show the effect of two levels of *Centaurea cineraria*, *Centaurea cyanus* Plants and their combination on kidney functions including (albumin, creatinine and urea**)** in rats with hepatotoxicity.

The positive control group showed increased urea (50.133 ± 3.308 mg/dl) and creatinine (0.920 ± 0.052 mg/dl), alongside reduced serum albumin (2.303 ± 0.094 g/l), indicating secondary kidney stress due to systemic toxicity (**Xu et al., 2017**).

Table (5): Effect of *Centaurea cineraria* and *Centaurea cyanus* Plants on kidney functions in rats with hepatotoxicity.

Parameters / Groups	Albumin g/l	Urea mg/dl	Creatinine mg/dl
Control (-ve)	3.286 ^{a} ± 0.145	36.733 ^{d} ± 3.3650	0.816 $^{b\,c\,d}$ ± 0.041
Control (+ve)	2.303 ^{b} ± 0.094	50.133 ^{a} ± 3.308	0.920 ^{a} ± 0.052
5% *Centaurea Cineraria (C.C)*	3.330 ^{a} ± 0.043	47.133 $^{a\,b\,c}$ ± 4.747	0.883 $^{a\,b}$ ± 0.032
10 % *Centaurea Cineraria (C.C)*	3.360 ^{a} ± 0.149	40.800 ^{d} ± 1.135	0.810 $^{b\,c\,d}$ ± 0.026
5% Centaurea cyanus (Cornflower C)	3.370 ^{a} ± 0.235	39.066 ^{d} ± 4.437	0.750 ^{d} ± 0.070
10 % Centaurea cyanus (Cornflower C)	3.253 ^{a} ± 0.320	41.900 $^{c\,d}$ ± 2.690	0.756 ^{d} ± 0.032
2.5% *(C.C) + 2.5% (Cornflower C)*	3.253 ^{a} ± 0.232	47.966 $^{a\,b\,c}$ ± 2.055	0.860 $^{a\,b\,c}$ ± 0.036
5% *(C.C) + 5% (Cornflower C)*	3.470 ^{a} ± 0.494	42.166 $^{b\,c\,d}$ ± 2.358	0.773 $^{c\,d}$ ± 0.061

C.C: *Centaurea Cineraria*, ***C.:*** *Centaurea Cornflower*

All parameters are represented as a means of replicates ± standard Dev.

Means with different small superscript letters in the same row are significantly different at $p \leq .05$.

Centaurea supplementation restored these parameters toward normal levels. For instance, 10% C.C. reduced urea to 40.800 ± 1.135 mg/dl and creatinine to 0.810 ± 0.026 mg/dl, while maintaining albumin at 3.360 ± 0.149 g/l. Combined treatment (5% C.C + 5% C) also normalized kidney markers (urea: 42.166 ± 2.358 mg/dl; creatinine: 0.773 ± 0.061 mg/dl), highlighting potential nephroprotective effects alongside hepatoprotection. These improvements may result from reduced oxidative stress and enhanced detoxification, consistent with findings in traditional hepatoprotective plant studies **(Attah et al., 2020)**.

Oxidative Stress Markers and Antioxidant Enzyme Activities

Oxidative stress is a major contributor to paracetamol-induced hepatotoxicity and nephrotoxicity, resulting from excessive reactive oxygen species (ROS) production, lipid peroxidation, and depletion of endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) (**Dhouibi et al., 2020; Fattaheian-Dehkordi et al., 2021**). Table (6) illustrates the effects of *Centaurea cineraria* and *Centaurea cyanus* on malondialdehyde (MDA) and antioxidant enzymes in rats with hepatotoxicity.

In the positive control group, elevated MDA levels (7.866 ± 0.381 nmol/g liver) confirmed enhanced lipid peroxidation and cellular membrane injury, while the reduced activities of CAT (15.136 ± 0.622 U/g liver), SOD (23.890 ± 0.808 U/g liver), and GSH (18.236 ± 1.190 mmol/g liver) reflected severe impairment of antioxidant defenses.

Treatment with *C. cineraria* and *C. cyanus* significantly reversed these effects in a dose-dependent manner. Administration of 10% *C. cineraria* lowered MDA to 4.716 ± 0.539 nmol/g and enhanced CAT, SOD, and GSH to 18.506 ± 0.304, 49.760 ± 1.537, and 26.026 ± 1.794, respectively. The combination (5% *C. cineraria* plus 5% *C. cyanus*) produced even significant improvements, reducing MDA to 4.700 ± 0.259 nmol/g and elevating CAT, SOD, and GSH to 19.350 ± 0.825, 95.332 ± 3.031, and 28.643 ± 1.216, respectively.

These findings demonstrate that both *Centaurea* species scavenge ROS, restore endogenous antioxidant defenses, and inhibit lipid peroxidation. The enhanced effect of the combination treatment suggests synergistic interactions between their phytochemicals particularly flavonoids, phenolics, and anthocyanins which collectively boost antioxidative capacity. Previous studies

have similarly highlighted the hepatoprotective potential of phenolic-rich extracts and chalcones from *Centaurea* spp., which enhance CAT and SOD activity while reducing MDA levels (**Pires et al., 2018**).

Table (6): Effect of *Centaurea cineraria* and *Centaurea cyanus* Plants on malondialdehyde and antioxidant enzymes in rats with hepatotoxicity

Parameters / Groups	MDA nmol/g liver	CAT U/g liver	SOD U/g liver	GSH mmol/g liver
Control (-ve)	5.050 $^{b\,c\,d}$ ± 0.400	21.280 ^{a} ± 2.644	97.603 ^{a} ± 2.932	29.483 ^{a} ± 1.221
Control (+ve)	7.866 ^{a} ± 0.381	15.136 ^{c} ± 0.622	23.890 ^{f} ± 0.808	18.236 ^{d} ± 1.190
5% *Centaurea Cineraria (C.C)*	5.816 ^{b} ± 0.575	18.180 ^{b} ± 1.275	43.883 ^{e} ± 1.950	26.830 $^{a\,b\,c}$ ± 1.507
10 % *Centaurea Cineraria (C.C)*	4.716 $^{c\,d}$ ± 0.539	18.506 ^{b} ± 0.304	49.760 ^{d} ± 1.537	26.026 $^{b\,c}$ ± 1.794
5% Cornflower (C)	4.900 $^{b\,c\,d}$ ± 0.655	18.213 ^{b} ± 0.504	41.013 ^{e} ± 1.869	27.270 $^{a\,b\,c}$ ± 1.825
10 % Cornflower (C)	4.100 ^{d} ± 0.694	19.556 $^{a\,b}$ ± 1.002	71.830 ^{b} ± 0.303	26.016 $^{b\,c}$ ± 1.589
2.5% *(C.C) + 2.5% (C)*	5.333 $^{b\,c}$ ± 0.453	18.486 ^{b} ± 0.739	68.076 ^{c} ± 2.202	24.533 ^{c} ± 1.401
5% *(C.C) + 5% (C)*	4.700 $^{c\,d}$ ± 0.259	19.350 $^{a\,b}$ ± 0.825	95.332 ^{a} ± 3.031	28.643 $^{a\,b}$ ± 1.216

C.C: *Centaurea Cineraria*, ***C.:*** *Centaurea Cornflower*

MDA: Malondialdehyde, **CAT:** catalase, **SOD:** Superoxide Dismutase, **GSH:** glutathione

All parameters are represented as mean of replicates ± standard Dev.

Means with different small superscript letters in the same row are significantly different at $p \leq .05$.

Sharonova et al. (2021), observed similar hepatoprotective activities, the hepatoprotective effects of *C. cineraria* and *C. cyanus* appear to involve multiple complementary mechanisms. ROS scavenging, whereby phytochemicals directly neutralize radicals, preventing lipid, protein, and DNA oxidation. Upregulation of antioxidant defenses, possibly via Nrf2-mediated activation of antioxidant response elements (**Lockowandt et al., 2019**), thereby

increasing SOD, CAT, and GSH activities; and synergistic phytochemical interactions, as shown by the pronounced SOD restoration (95.332 ± 3.031 U/g) in the combination therapy group, suggesting complementary radical-stabilizing and antioxidant-regenerating effects (**Albayrak et al., 2017**).

Furthermore, by reducing ROS and restoring antioxidant enzymes, *Centaurea* treatment likely prevents mitochondrial dysfunction, lipid peroxidation of membranes, and subsequent apoptosis or necrosis of hepatocytes and renal cells (**Özcan et al., 2019**). This protective action is reflected in the concurrent normalization of serum liver enzymes (AST, ALT, ALP) and kidney markers (urea, creatinine).

In summary, *C. cineraria* and *C. cyanus* exert potent hepatoprotective and renoprotective effects through ROS scavenging, activation of endogenous antioxidants, and attenuation of oxidative damage at cellular and mitochondrial levels. Combination therapy provides superior benefits, highlighting a synergistic mechanism of protection.

Histopathology Examination of Liver:

The histopathological examination of liver sections in this study highlights the damaging effects of paracetamol and the protective potential of *Centaurea* species *cineraria* and *cyanus* treatments. Examined sections from liver of Control –ve group fed on basal diet (photo 1) showed normal histological structures of hepatic cords, kupffur cells, sinusoids, portal triads and central veins. However, control + ve group fed on basal diet which treated with paracetamol to induce hepatotoxicity (photo 2) exhibited focal areas of hydropic degenerated hepatocytes with unicellular necrosis beside engorged hepatic vasculatures were also observed. In the other hand, apparently normal hepatic cords and congested central vein, sinusoids were seen in Group of rats that fed on diet containing 5% of *Centaurea Cineraria* plant (photo 3). Moreover, Group of rats that fed on diet containing with 10% of *Centaurea Cineraria* plant (photo 4) showed mild degree of degenerative changes within hepatocytes beside patent central vein and other vasculatures. Group of rats that fed on diet containing 5% of *Centaurea Cyanus* plant (photo 5) revealed moderate degree of degeneration particularly vacuolar or fatty degeneration and dilatation of portal vein, central vein. Most of the hepatic parenchyma were histologically normal at group of rats that fed on diet containing 10 % of *Centaurea Cyanus* plant (photo 6) with presence of minute perivascular leukocytic infiltrates. Group of rats that fed on diet containing 2.5% of *Centaurea Cineraria* and 2.5% of *Centaurea Cyanus* plant (photo 7) showed

number of apoptotic cells that characterized by shrinked hepatocytes with pyknotic nuclei, and more eosinophilic cytoplasm. Group of rats that fed on diet containing 5% of *Centaurea Cineraria* and 5% of *Centaurea Cyanus* plant (photo 8) revealed high ameliorations of hepatic parenchyma, blood vessels with mild degree of degeneration.

Paracetamol caused significant liver injury, as evidenced by elevated serum AST, ALT, and ALP levels, reduced albumin, and histopathological alterations such as hydropic degeneration, necrosis, and vascular congestion, consistent with previous reports on acetaminophen-induced hepatic damage (**Fernandes et al., 2019**). Treatment with C.C. and Cornflower, individually or in combination, significantly ameliorated these biochemical and histological changes. *Centaurea cineraria* at 10% and Cornflower at 10% restored liver enzyme levels and improved hepatic architecture, whereas the 5% C.C + 5% Cornflower combination demonstrated the most pronounced effect, suggesting a synergistic hepatoprotective mechanism.

These improvements were supported by enhanced antioxidant defenses: reduced malondialdehyde (MDA) levels and increased CAT, SOD, and GSH activities, indicating mitigation of oxidative stress and lipid peroxidation. In the hepatotoxic group, elevated MDA (7.866 ± 0.381 nmol/g liver) and depleted antioxidant enzyme activities (CAT: 15.136 ± 0.622 U/g liver, SOD: 23.890 ± 0.808 U/g liver, GSH: 18.236 ± 1.190 mmol/g liver) confirmed oxidative stress-induced exhaustion of cellular defense mechanisms (**Dhouibi et al., 2020; Al-Shurait and Al-Ali ,2022**). Treatment with C.C. and Cornflower effectively reversed these alterations, with the combination therapy providing superior outcomes by significantly reducing MDA and restoring antioxidant enzyme activities toward or above baseline values.

From a biochemical perspective, the hepatoprotective effects of *Centaurea* extracts may be attributed to their phenolic and flavonoid constituents, which scavenge reactive oxygen species, inhibit lipid peroxidation, stabilize hepatocyte membranes, and modulate apoptotic pathways. The observed synergistic effect of combined administration likely arises from complementary phytochemicals (flavonoids, phenolics, anthocyanins) working in concert to enhance antioxidant defense mechanisms.

Additionally, kidney function markers (urea, creatinine) and serum albumin were also improved, indicating systemic protective effects of the extracts, consistent with reports on the traditional use of *Centaurea* species for organ protection (**Al-Asmari et al., 2014; Attah et al., 2020**).

Overall, these findings suggest that *Centaurea cineraria* and *Centaurea cyanus* are potent natural hepatoprotective agents, with combined administration providing superior efficacy against drug-induced oxidative liver injury by scavenging ROS, upregulating endogenous antioxidants, and preventing oxidative damage at the cellular and mitochondrial levels.

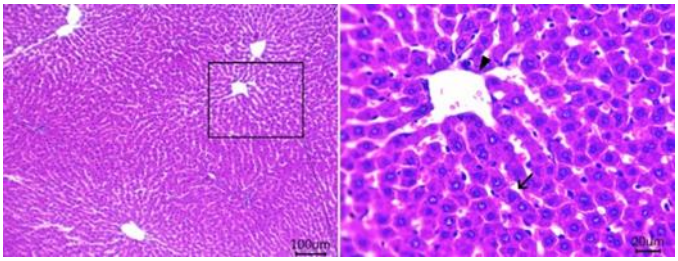

Photo 1. Representative photomicrograph of H&E-stained liver section from rats fed on a basal diet (Control –ve group), showing normal histological architecture of hepatic cords (black arrow), Kupffer cells, sinusoids, portal triads, and central vein (arrowhead). *Scale bars: 100 µm, 20 µm.*

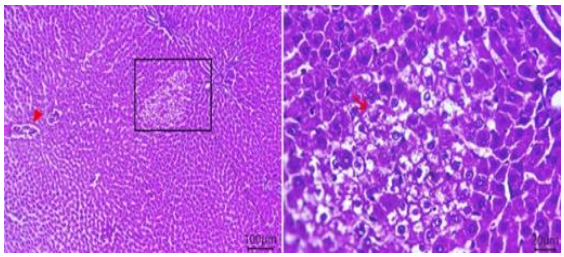

Photo 2. Representative photomicrograph of H&E-stained liver section from rats in the Control +ve group (fed on basal diet and treated with paracetamol to induce hepatotoxicity), showing focal areas of hydropically degenerated hepatocytes (red arrow) with unicellular necrosis, alongside engorged hepatic vasculature (red arrowhead). *Scale bars: 100 µm, 20 µm*

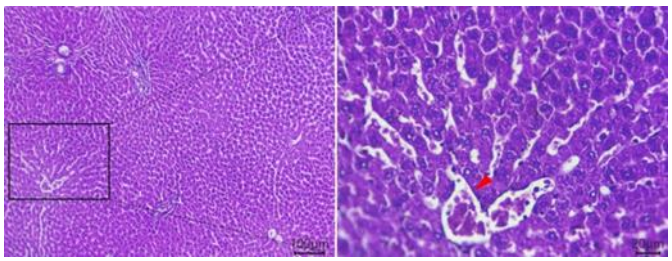

Photo 3. Representative photomicrograph of H&E-stained liver section from rats in Group 3 (fed on diet containing 5% *Centaurea cineraria*), showing apparently normal hepatic cords with congested central vein (red arrowhead) and sinusoids. *Scale bars: 100 µm, 20 µm.*

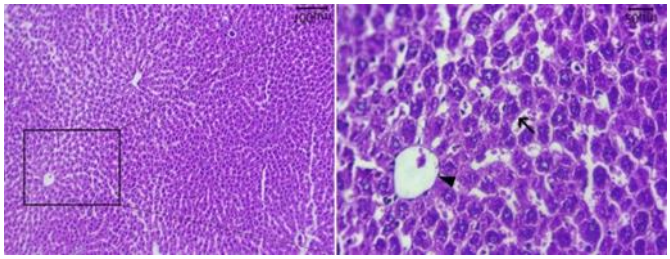

Photo 4. Representative photomicrograph of H&E-stained liver section from rats in Group 4 (fed on diet containing 10% *Centaurea cineraria*), showing mild degenerative changes within hepatocytes (arrow) with patent central vein (arrowhead) and other vasculatures. *Scale bars: 100 µm, 20 µm.*

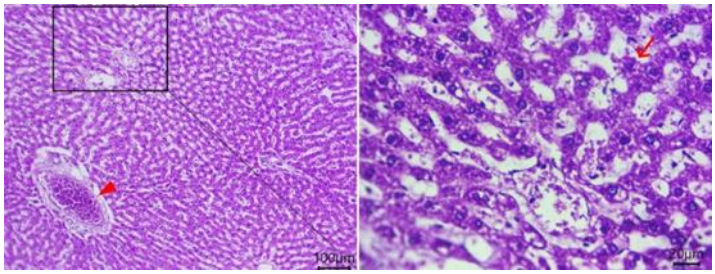

Photo 5. Representative photomicrograph of H&E-stained liver section from rats in Group 5 (fed on diet containing 5% *Centaurea cyanus*), showing moderate degeneration, particularly vacuolar (fatty) degeneration (arrow), with dilatation of the portal vein (arrowhead) and central vein. *Scale bars: 100 µm, 20 µm***.**

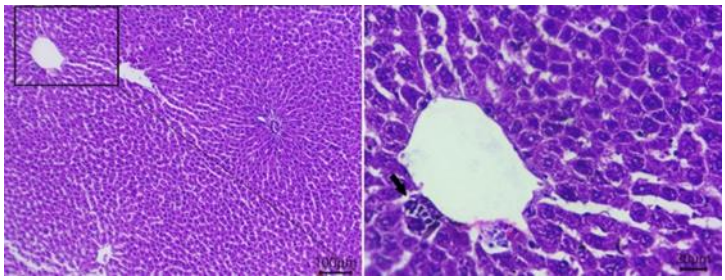

Photo 6. Representative photomicrograph of H&E-stained liver section from rats in Group 6 (fed on diet containing 10% *Centaurea cyanus*), showing largely normal hepatic parenchyma with minute perivascular leukocytic infiltrates (thick arrow). *Scale bars: 100 µm, 20 µm.*

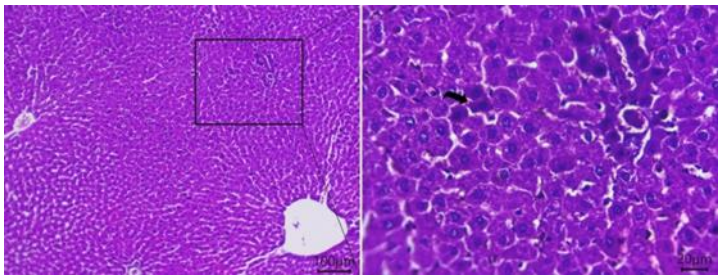

Photo 7. Representative photomicrograph of H&E-stained liver section from rats in Group 7 (fed on diet containing 2.5% *Centaurea cineraria* + 2.5% *Centaurea cyanus*), showing several apoptotic cells (curved arrow) characterized by shrunken hepatocytes with pyknotic nuclei. *Scale bars: 100 µm, 20 µm.*

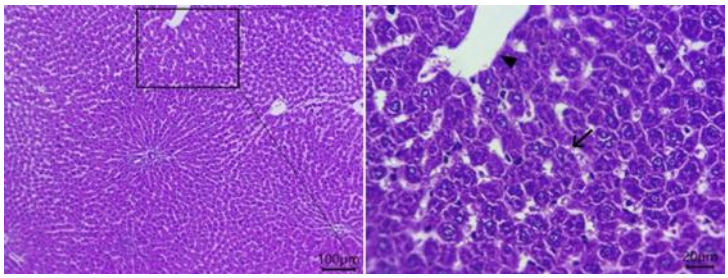

Photo 8. Representative photomicrograph of H&E-stained liver section from rats in Group 8 (fed on diet containing 5% *Centaurea cineraria* + 5% *Centaurea cyanus*), showing marked amelioration of hepatic parenchyma and blood vessels (arrowhead), with only mild degenerative changes (arrow). *Scale bars: 100 µm, 20 µm.*

Conclusion

Centaurea cineraria and Centaurea cyanus protect against paracetamol-induced liver and kidney damage by reducing oxidative stress, restoring antioxidant enzymes, and normalizing liver and kidney function markers. Their combined use shows a synergistic effect, highlighting their potential as natural hepatoprotective and renoprotective agents.

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دراسة التأثير المحتمل لنباتي *Centaurea cineraria* و *Centaurea cyanus* على السمية الكبدية المستحدثة في الفئران

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المستخلص العربي

تحتوي أنواع *Centaurea* على مركبات حيوية فعالة تمتلك خصائص واقية للكبد ومضادة للأكسدة. هدفت هذه الدراسة إلى تقييم التأثير الوقائي لكل من *Centaurea cineraria* و *Centaurea cyanus* (cornflower)، كل على حدة وخليطهم، ضد السمية الكبدية الناتجة عن تناول الباراسيتامول في الفئران. كما تناولت الدراسة تحليل التركيب الكيميائي والمكونات الفينولية لهذين النباتين، بالإضافة إلى دراسة التغيرات النسيجية المرضية في أنسجة الكبد. تم تقسيم ثمانية وأربعين فأرا أبيض ذكرا إلى ثماني مجموعات: مجموعة ضابطة سالبة، ومجموعة ضابطة مصابة بالسمية الكبدية المستحثة بالباراسيتامول، وعدد ست مجموعات معاملة تم تغذيتها على وجبات غذائية تحتوي على *C. cineraria* أو *C. cyanus* بنسبة ٥٪ أو ١٠٪، إما منفردتين أو بنسب مختلطة قدرها ٢,٥٪+٢,٥٪ و٥٪+٥٪. تم تقدير المؤشرات البيوكيميائية التي شملت إنزيمات الكبد (AST ، ALT، ALP)، ومؤشرات وظائف الكلى (اليوريا، الكرياتينين، الألبومين) بالإضافة إلى مؤشرات الإجهاد التأكسدي (MDA، CAT، SOD، GSH.) كما أُجري الفحص النسيجي لأنسجة الكبد لدعم النتائج البيوكيميائية. أدى تناول الباراسيتامول إلى ارتفاع ملحوظ في إنزيمات الكبد ومستويات MDA واليوريا والكرياتينين ومعظم مكونات دهون الدم، في حين تسبب في انخفاض الألبومين و HDL-c ومؤشرات مضادات الأكسدة (SOD ، CAT، GSH) أما تناول *C. cineraria* و *C. cyanus* فقد حسن بشكل واضح المؤشرات البيوكيميائية المتأثرة، حيث أظهرت مجموعة الفئران التى تم معاملتها بنسبة 5%+5% أقوى تأثير وقائي للكبد من خلال إعادة إنزيمات الكبد إلى مستوياتها الطبيعية وخفض MDA، واستعادة مستويات مضادات الأكسدة (SOD، CAT، GSH) إلى قيمها القريبة من المجموعة الضابطة. أكد الفحص النسيجي نتائج التحاليل البيوكيميائية، حيث أظهر تحسنا واضحا في بنية الكبد وانخفاضا ملحوظا في درجة التدهور الخلوي الكبدي، وكان هذا التحسن أكثر وضوحا في المجموعات التي بخليط النباتيين. الاستنتاج: أظهرت نباتات Centaurea cineraria و Centaurea cyanus تأثيرات واقية للكبد ومضادة للأكسدة بشكل ملحوظ ضد تلف الكبد الناتج عن الباراسيتامول، مع فعالية أعلى عند استخدامهما معا. وتشير هذه النتائج إلى إمكانية الاستفادة منهما كمصادر طبيعية واعدة في تطوير الأغذية الوظيفية أو المكملات الغذائية الداعمة لصحة الكبد.

الكلمات المفتاحية: *Centaurea cineraria*، *Centaurea cyanus*، الوقاية الكبدية، النشاطات المضادة للأكسدة، السمية الكبدية.