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Evaluation of Hibiscus sabdariffa L. Extract's Antioxidant Activity and its Potential to Mitigate Chlorpyrifos-Induced Oxidative Stress in Adult Rats.

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Abstract: *Hibiscus sabdariffa L*. (H. sabdariffa) has long been recognized for its therapeutic properties, largely due to its abundant phytochemical content. This study investigated the antioxidant and anti-inflammatory potential of the calyces extract through both in vitro and in vivo approaches. In the laboratory, the extract was obtained by hot maceration with soaking durations ranging from 10 minutes to 24 hours. Results demonstrated that the extract yield did not vary significantly with extended soaking times, suggesting that a short brewing period is sufficient. GC-MS analysis of the brewed tea revealed several active compounds, while standard assays quantified the extract as containing 10.4 mg Gallic acid/g of phenolic acids and 1.562 mg Quercetin/g of flavonoids. Furthermore, the DPPH free radical scavenging assay indicated high antioxidant efficacy with an IC_{50} value of 0.503 ± 0.13. The in vivo segment involved thirty rats divided into five groups to evaluate the extract's protective effects against chlorpyrifos-induced oxidative stress, using vitamin C as a comparative reference. Serum markers, including (MDA and GSH), and liver enzymes (AST, ALT, and ALP), were measured along with the anti-inflammatory cytokine IL-10. Histological examination of liver tissue further supported the biochemical findings. Rats treated with the *H. sabdariffa* extract exhibited significant improvements in antioxidant status, reduced inflammatory indicators, and normalized liver enzyme activities, paralleling the effects of vitamin C.

Keywords: Antioxidant, Anthocyanin, Calyces extract, Chlorpyrifos, Hepatic Enzymes, *Hibiscus sabdariffa L*, Oxidative Stress, Vitamin C.

Introduction

Herbal medicine has been a respected healing practice for centuries and remains widely used today. Its natural compounds promote health, and due to their proven benefits, safety, affordability, and accessibility, herbal treatments are valuable in both traditional and modern healthcare systems (1). One of the most significant plants in this context is *H. sabdariffa*, a member of the Malvaceae family, which encompasses over 300 species (2). This remarkable plant has been utilized for over 3,000 years, both as a source of nourishment and as a therapeutic remedy (3). It is believed to be native to West Africa, particularly western Sudan, although some scholars argue that it originated from India. Today, this species is extensively cultivated in tropical regions worldwide, including Arab nations such as Saudi Arabia, Sudan, and Egypt, as well as in various other countries such as Mexico, India, the Philippines, China, Thailand, and many others (4). This plant is known by a variety of names across different regions, including Sour Tea, Gujarat, Karkade, Roselle, Bissap and Red Sorrel (5). Calyces, with their elevated bioactive compound concentration, are the most exploited plant component. Consequently, these calyces serve as the primary raw material in the formulation of the renowned tea (6). They are rich in phytochemicals, especially polyphenolic compounds such as flavonoids and phenolic acids. Additionally, they contain organic acids, vitamins, minerals, and essential nutrients (carbohydrates, proteins, and fats), providing approximately 49 calories per 100 grams (7).

Anthocyanidin, the main flavonoid in *H. sabdariffa* calyces, gives the extract its deep red color (8). This water-soluble pigment is packed with beneficial compounds and is safe even at high doses (9). They are primarily found as glycosides, notably delphinidin-3-O-sambubioside (hibiscin) and cyanidin-3-O-sambubioside (gossypicyanin) (10). Anthocyanins contribute to the potent antioxidant properties observed, effectively neutralizing free radicals, chelating metals, and other pro-oxidant agents. These capacities originate from the unique structural features of polyphenolic compounds, which facilitate the rapid donation of hydrogen atoms (electrons), thereby stabilizing reactive species (11).

A lot of research has dealt with the therapeutic uses of *H. sabdariffa*, including cardioprotective (12), prevention and treatment of high blood lipids (13), as a vasodilator (14), exhibits diuretic effects (15), hepatoprotective (16), antidiabetic (17), neuroprotective (18), improve hematological parameters (19), decreased risk of overweight and obesity, (20)stimulate insulin secretion and inhibit glucagon secretion (21), anti-inflammatory (22), and antioxidant properties (23). Wouldn't it be wonderful to enjoy some, if not all, of these benefits with a delicious, easy-to-prepare, affordable, safe, and readily available cup of tea?

Chlorpyrifos (CPF), an organophosphorus compound, is one of the most extensively utilized broad-spectrum chlorinated pesticides globally (24). Its application is not confined to agricultural settings; it is also commonly used in residential environments, gardens and ornamental plants (25). According to the World Health Organization's classification of pesticides, CPF is categorized as moderately toxic (26).

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Chlorpyrifos (CPF) irreversibly inhibits acetylcholinesterase, thereby leading to the accumulation of acetylcholine at nerve endings and neuromuscular junctions, which is associated with neurotoxicity (27). Moreover, CPF inflicts cellular damage through the induction of oxidative stress (OS) (28). This OS may result from either an increased prod uction of Reactive Oxygen Species (ROS) (29) or a diminished Antioxidant System Capacity (30). In either case, the intracellular accumulation of ROS adversely affects cellular function and can ultimately lead to carcinogenesis or cell death (31).

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and the superoxide anion ($O_2^{\bullet-}$), are inherently unstable compounds that seek stability by reacting with neighboring substances. Cell membranes, which are particularly rich in polyunsaturated fatty acids (PUFAs), are especially prone to oxidative damage (32). This leads to lipid peroxidation, compromising membrane integrity and potentially resulting in cell death. Moreover, OS activates pro-inflammatory cytokines and initiates inflammatory responses that further disrupt cellular function (33).

The antioxidant system (AOS) removes excess ROS from various sources and plays a crucial role in preserving cellular vitality against potential damage induced by OS (34). Therefore, it has become essential to enhance this system and support its functions. In this context, natural extracts rich in antioxidants play a crucial role (35), with *H. sabdariffa* extract being one of the most significant.

The objective of this study was to assess the antioxidant properties of *H. sabdariffa* extract prepared using traditional methods, with particular emphasis on the effect of steeping time on tea concentration. The study further analyzed the extract's chemical composition, evaluated its antioxidant efficacy both in vitro and in vivo, and examined its potential to shield various organs, especially the liver, from CPF-induced oxidative damage. Moreover, the mitigation effects were compared with those of vitamin C.

Materials and Methods

Materials:

Vitamin C, Chlorpyrifos and Dried calyces of *H. sabdariffa* were bought from the local Ashar market in Basrah state, Iraq. The origin of the *H. sabdariffa* is Nigeria. Chlorpyrifos is made in France under the trade name Ciko Span, and pure vitamin C powder is made in India.

The *H. sabdariffa* were identified by the College of Agriculture at the University of Basra, Iraq. This identification employed the Global Biodiversity Information Facility (GBIF), which has documented the plant's record from Iraq since 1962. The specimen is archived at the Royal Botanic Gardens, Kew under the catalogue code K006188855 (36).

The chemicals used, such as DPPH, Total phenolic acid and flavonoid content, along with solvents and other tools, were used in the laboratories of the College of Pharmacy / Al-Basra University/Iraq

The kit that was used to examine the MDI, GSH, ALT, AST, ALP, and IL10 in the rat serum was purchased from Sunlong Biotech CO., Ltd., China.

Extraction Process and Residue Formation.

The aqueous extract of *H. sabdariffa* was prepared using a hot soaking method, with water as the solvent. Specifically, 100 ml of boiling water was added to 10 grams of *H. sabdariffa* calyces, maintaining a fixed ratio of water to calyces (10:1). The mixture was allowed to steep for various durations, including 10, 30 minutes, and 1, 3, 12, and 24 hours. This process, as described by Pacheco-Coello et al. (2021), was modified slightly (37). After each steeping period, the solution was filtered, and the solvent was evaporated in a water bath at 70-80°C, resulting in a dry, dark red precipitate (38).

The parameters measured include the weight (w) of the residue (H. sabdariffa extract), the volume (v) of the resulting solution, and the concentration of the produced solutions (w/v). Additionally, the yield percentage of crude calyces is calculated by applying the equation (39):

$$Percentage\ Yield = \frac{Mass\ of\ Crude\ Extract}{Initial\ Mass\ of\ Sample} \times 100$$

All these measurements were taken six times for each soaking duration and are presented as mean values ± standard error of the mean (SEM) in Table 2.

Gas Chromatography-Mass Spectrometry (GC-MS)

A sample of *H. sabdariffa* solution was analyzed by Gas Chromatography-Mass Spectrometry (GCMS 5977), the data processing and analysis by Mass Hunter GC/MS software, and compared with the NIST Mass Spectral Library (National Institute of Standards and Technology).

DPPH Free Radical Scavenging Capacities

The antioxidant properties of *H. sabdariffa* extract were evaluated by applying the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay. A stock solution was prepared by dissolving 24 mg of DPPH in 100 ml of methanol. Subsequently, various concentrations of the *H. sabdariffa* extract (4, 2, 1, 0,5, and 0,2 mg/ml) were formulated and combined with the DPPH solution. The mixtures were incubated in darkness for 30 minutes, ensuring minimal light exposure. Following incubation, the color variation was analyzed at a wavelength of 517 nm using a spectrophotometer

Vitamin C (given to positive control group G5) was evaluated using the same method and concentrations as the *H. sabdariffa* extract, enabling a direct comparison of their results.

Inhibition concentration obtained by:

$$\%\ \mathbf{of}\ \text{inhibition} = (\boldsymbol{A}^{\circ} - \boldsymbol{A1}/\boldsymbol{A}^{\circ}) * 100$$

In which Aº is the DPPH absorbance, and A1 is the absorbance of the tested samples (40).

Total Phenolic Content

One milliliter of *H. sabdariffa* extract at concentrations of 2, 1, and 0.5 mg/mL was combined with 1 mL of Folin-Ciocalteu phenol reagent and 10 mL of a 7% sodium carbonate (Na_2CO_3) solution. The mixtures were allowed to react at room temperature for 30 minutes. Subsequent absorbance measurements were taken at a wavelength of 550 nm using UV/VIS spectrophotometry. Gallic acid was used as the calibration standard, and the results were expressed as mg of Gallic Acid Equivalents (GAE) per gram of *H. sabdariffa* extract (41).

Total Flavonoids Content

H. sabdariffa extract solutions at concentrations of 4, 2, 1, 0.5, and 0.25 mg/mL (0.5 mL each) were combined with 0.3 mL of a 5% sodium nitrite ($NaNO_2$) solution and allowed to react for 5 minutes. Subsequently, 0.3 mL of a 10% aluminum chloride ($AlCl_3$) solution was added, and the mixture was incubated for an additional 5 minutes. Next, 2 mL of sodium hydroxide (NaOH) was incorporated, and distilled water was added to adjust the final volume to 5 mL. The solution was then maintained in the

dark for 15 minutes. Quercetin was employed as a calibration standard, and the results were expressed as milligrams of quercetin per gram of *H. sabdariffa* extract.(42)

Animal Handling.

The experiment commenced with the 30 rats (180-210 g) purchased from an animal house farm in Tikrit-Iraq. Following a two-week adaptation period, rats were divided into five groups, each consisting of six rats, as shown in Table 1. The experiment was designed to last 28 days ((30)). All treatments are diluted with water to achieve the desired concentration in 1 ml/dose, even the CPF emulsion (43) and given orally via Gavage.

The animals are handled according to ethical principles of International Ethical Guidance for Health-related research involving humans or animals prepared by the Council International Organization of Medical Science (CIOMS) in collaboration with the World Health Organization (WHO), and following the framework of the Office International des Epizootic (OIE) principles. Authorized by the University of Basra/College of Pharmacy in Approval number EC50 on 1/9/2025.

Preparation of H. sabdariffa Extract for Experimental Rat Treatment.

The same procedure utilized for preparing the dry extract was applied. After obtaining the dry precipitate, it was carefully weighed, and water was added to create a solution with the desired dose in a volume of 1 ml.

Collection of Blood Samples and Biomarker Analysis.

After the 28-day trial, blood samples were collected from sacrificed rats, centrifuged, and the serum stored at -20 °C. Oxidative biomarkers (MDA and GSH), liver function enzymes (ALP, AST, and ALT), and the anti-inflammatory marker IL-10 were analysed using the ELISA method.

Tissue Sampling and Histological Analysis.

Liver tissue samples were collected, washed with normal saline, and fixed in a 10% formaldehyde solution. The tissues were then dehydrated using ethanol and cleared with xylene to facilitate paraffin infiltration at 60°C. Once cooled to 20°C, the Paraffin blocks were sectioned into thin slices (4-6 µm) and mounted on glass slides for Hematoxylin and Eosin staining (44).

Statistical analysis

The analysis of the results was conducted employing IBM's SPSS software, specifically utilizing One-Way Analysis of Variance (ANOVA) alongside the Tukey Post-Hoc Test to assess mean differences among various groups, and T-test to **compare** between two groups, P-value <0.05 was established to determine the presence of statistically significant differences between groups, and the data were presented as mean ± SEM (Standard Error of the Mean). All graphics were conducted by GraphPad Prism 10 software utilizing SPSS results.

Results and Discussion

Time Impact on Extraction Process and Residue Formation.

The results showed no statistically significant difference ($p > 0.05$) in precipitate weight across different soaking durations, as indicated in Table 2. This suggests that the primary constituents of *H. sabdariffa* are highly water-soluble, leading to rapid diffusion from the calyx into the surrounding solvent.

This experiment demonstrated the presence of phenolic acids and flavonoids, both known for their water solubility. GC-MS analysis further identified multiple water-soluble compounds. Notably, anthocyanins, responsible for the solution's coloration, were initially dissolved in water, explaining the observed results.

A previous experiment examining the components of hibiscus demonstrated that its extract contains various water-soluble substances, including organic acids, carbohydrates, and proteins, which further supports and confirms our findings (45). Moreover, Nguyen et al. (2020) reported that extending the brewing time beyond 30 minutes does not significantly augment the extract weight (46).

It is important to emphasize that this evaluation is solely quantitative, lacking qualitative analysis. Consequently, relying on the extract's weight alone does not adequately assess its efficacy, especially since the active compounds are present in trace amounts.

Table 1: Group design dividing the rats (30 rats) into 5 groups (each group has six rats), and the dosing continues for 28 days.

Group Name	Group Type	Group Treatment	Dose Reference
G1	Negative control	no treatment	
G2	Positive control	CPF 10 mg/kg/day.	(47)
G3	Experiment group	*H. sabdariffa* extract 250 mg/kg/day + CPF 10 mg/kg/day.	(48)
G4	Experiment group	*H. sabdariffa extract* 750 mg/kg/day + CPF 10 mg/kg/day.	(49)
G5	Positive control	Vit C 100 mg/kg/day + CPF 10 mg/kg/day.	(50)(51)

CPF (chlorpyrifos), Vit C (vitamin C).

Table 2: the weight of residue (w), the volume of the solution produced (v), the concentration of solutions produced (w/v), and the yield percentage of crude calyces obtained six times for each soaking period.

soaking time	10 minutes	30 minutes	1 hour	3 hours	12 hours	24 hours
weight of residue (w)	3.94±0.31	4.76±0.27	4.72±0.28	4.46±0.17	4.645±0.29	4.31±0.15
valium of solution (v)	75.83±0.17	75±0.0	73.33 ±2	78.33 ±1.4	68.83 ±0.7	70.83 ±1.6
concentration of solution (w/v)	51.93±4.1	63.48±3.5	64.99 ±5.7	56.98 ±2.3	67.35 ±3.6	60.88 ±1.4
Yield percentage (w/10) *100%	39% ±3.1	48% ±2.7	47% ±2.8	44% ±1.7	46% ±2.9	43% ±1.5

Yield percentage was calculated using the formula = [Weight of residue (g) ÷ 10 (g)] × 100%, where 10 grams represents the fixed weight of crude calyces used in each brewing period. The data are represented by mean ± SEM.

GC-MS analysis of *H. sabdariffa* extract.

Gas chromatography-mass spectrometry (GC-MS) is an advanced analytical technique that combines the separation abilities of gas chromatography with the identification and quantification capabilities of mass spectrometry. In this study, GC-MS analysis of the aqueous extract of *H. sabdariffa* identified eight bioactive compounds, each with documented medical efficacy (Table 3). These findings help explain the positive health outcomes associated with hibiscus tea consumption, including its antioxidant, anti-inflammatory, and anticancer properties. Notably, these results corroborate the findings of Ityo et al. (2023), who also detected numerous active substances in *H. sabdariffa* tea using GC-MS (52).

Qualitative Evaluation of *H. sabdariffa* Extract by DPPH, Total Phenolic Acid, and Flavonoid Analysis.

All concentrations of *H. sabdariffa* showed significant DPPH inhibition, with an IC50 of 0.503 ± 0.13, compared to 1.48 ± 0.03 for Vitamin C. Statistical analysis indicated no significant differences, with a p-value (> 0.05) as shown in Figure 3.

The phenolic content in *H. sabdariffa* extract is 10.4 mg of Gallic Acid/gram of *H. sabdariffa* extract, and the flavonoid content is 1.562 mg of Quercetin/gram of *H. sabdariffa* extract.

Table 3: includes the compounds identified through (GC-MS).

	RT	Calculated RI	Literature RI	M. F	Name	Activity
1	22.853	1924	1927(55)	$C_{17}H_{34}O_2$	n-Hexadecanoic acid (Palmitic acid)	anti-inflammatory (56)
2	21.432	1618	1614 (57)	$C_{14}H_{28}O$	Tetradecanal	Promote sleep (58)
3	14.951	1235	1241 (59)	$C_6H_6O_2$	Hydroquinone	depigmenting agent (60)
4	15.359	1240	1233.2 (61)	$C_6H_6O_3$	5-ydroxymethylfurfural	Anticancer (62)
5	13.309	1170	1178 (63)	$C_7H_6O_2$	Benzoic acid	Antifungal (64)
6	16.106	1365	1373 (65)	$C_{10}H_{12}O_2$	Eugenol	anti-inflammatory, antioxidant, analgesic (66)
7	23.45	2032	2024 (67)	$C_{18}H_{36}O$	Octadecanal	Antimicrobial (68)
8	24.542	2133	2141 (69)	$C_{19}H_{36}O_2$	Oleic Acid	cardio-protective antioxidant and antimicrobial properties (70)

RT (retention time), RI (refractive index), and MF (molecular formula).

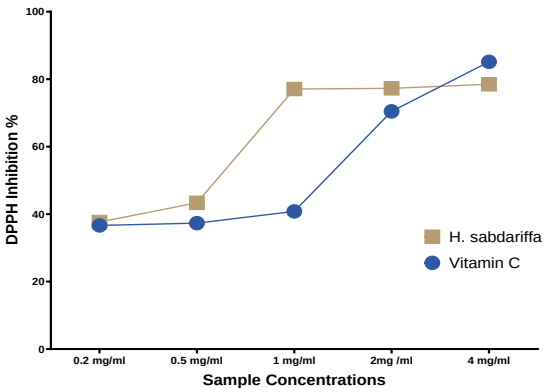

Figure 3: The comparison of Vitamin C and *H. sabdariffa* extract in their ability to scavenge DPPH free radicals.

The DPPH assay is a widely recognized, simple, and cost-effective method for evaluating antioxidant activity. It operates on the principle that antioxidants in a sample donate electrons to the DPPH free radical (1,1-diphenyl-2-picrylhydrazyl), stabilizing it and inducing a visible color shift from deep violet to pale yellow. This change is quantitatively measured using a spectrophotometer, with the degree of discoloration directly correlating to the sample's free radical scavenging ability, making the assay a reliable measure of antioxidant capacity.

This study found that *H. sabdariffa* extract had a lower IC50 value than vitamin C, indicating superior free radical scavenging ability. Accordingly, the raw calyces extract exhibits pronounced antioxidant activity due to its diverse compounds with unique chemical structures that facilitate electron donation to neutralize free radicals. This effect is substantiated by our experimental findings, which revealed the presence of phenolic acids, flavonoids.

The results align with Hamrita et al. (2022), who reported robust antioxidant activity via the DPPH assay (53). Likewise, our findings on total phenolic content correspond with those of Pacheco-Coello F (2021) (37), and Hussein Rassem et al. (2024) similarly detected flavonoids in the aqueous extract (54).

Evaluation of the Antioxidant Capacity of *H. sabdariffa* Extract by Measuring Rat Serum Biomarkers, MDA and GSH Level:

For MDA levels, no significant differences were observed among the control (G1), G3, G4, and G5 groups, whereas G2 exhibited a marked increase compared to all other groups (Figure 4). Similarly, GSH levels remained statistically similar across G1, G3, G4, and G5, but were significantly reduced in G2 relative to these groups (Figure 5). Furthermore, no significant differences were detected between groups G3 and G4 for either

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biomarker.

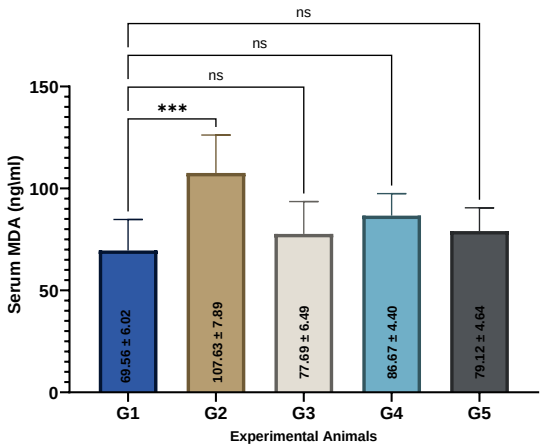

Figure 4: serum MDA level in different experimental groups. Data are presented as mean ± SEM, with (***) indicating a statistically significant difference (p-value < 0.001) compared to other groups.

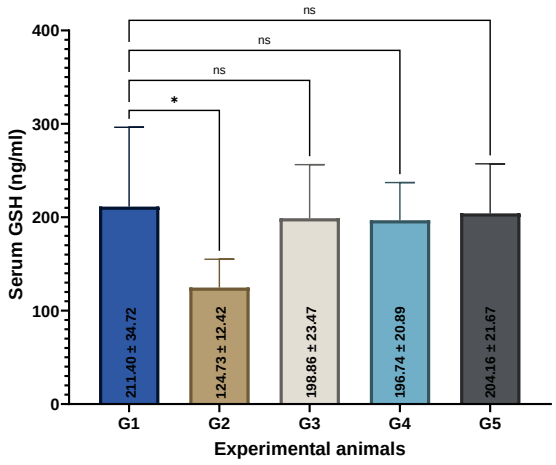

Figure 5: serum GSH level in different experimental groups. Data are presented as mean ± SEM, with (*) indicating a statistically significant difference (p-value < 0.05) compared to other groups.

An elevated level of (MDA) is a critical indicator of oxidative stress, revealing an imbalance where the production of oxidants surpasses the neutralizing capacity of the antioxidant system. In group G2, this is evident through increased ROS, particularly those associated with CPF exposure, and the depletion of (GSH), which is consumed in its attempt to counteract the excess free radicals.

Coadministration of *H. sabdariffa* extract with CPF in Groups G3 and G4 effectively decreased MDA levels and restored GSH levels to near those of the control group (G1). This outcome is attributed to the extract's potent antioxidant compounds, which neutralize reactive oxygen species (ROS) by donating electrons or enhancing the body's antioxidant defenses. The protective mechanism of *H. sabdariffa* against the harmful effects of CPF mirrors that of vitamin C, a well-established antioxidant. This is evident in Group 5, where no significant differences were observed among Groups 3, 4, and 5, confirming their comparable antioxidant efficacy. These findings align with Hamadjida A. and Mbomo R. (2024), who demonstrated that *H. sabdariffa* extract mitigates alloxan-induced oxidative stress in rat serum by reducing MDA and restoring GSH levels (71).

It is worth noting that increasing the dose of *H. sabdariffa* extract does not correlate with improved effectiveness. This conclusion is supported by the lack of statistically significant differences in oxidative stress markers between Group G3, which received a lower dose, and Group G4, which received a higher dose.

Evaluation of the Anti-Inflammatory Capacity *of H. sabdariffa* Extract by Measuring Rat Serum IL10 Biomarker.

Figure 6 demonstrates that IL-10 levels in groups G3, G4, and G5 did not differ significantly from the control (G1). In contrast, G2 exhibited a significant decrease in IL-10 levels compared to all other groups ($p < 0.05$). No significant differences were noted between G3 and G4.

Interleukin-10 (IL-10) is an immunosuppressive and anti-inflammatory cytokine that functions mainly through the Jak1/Tyk2/STAT3 signaling cascade. Activated STAT3 inhibits pro-inflammatory mediators, including the MAPK and NF-κB pathways (72), and cytokines like IFN-γ, TNF, IL-1, and IL-6. Disruption of IL-10 levels can compromise this regulation and worsen the inflammatory response (72).

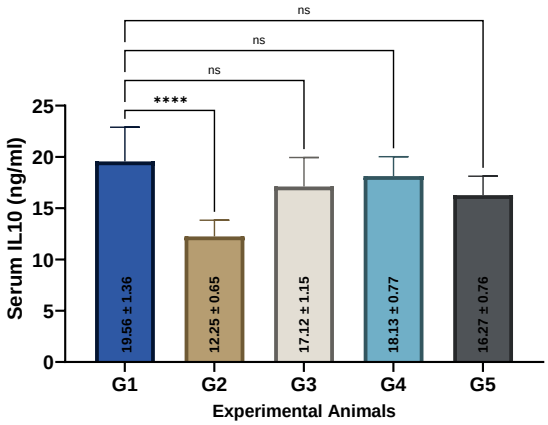

Figure 6: serum IL10 level in different experimental groups. Data are presented as mean ± SEM, with (***) indicating a statistically significant difference (p-value < 0.001) compared to other groups.

CPF-induced oxidative stress, evident in Group G2, led to a significant decrease in IL-10 levels. This aligns with Alruhaimi (2023), who reported similar reductions in IL-10 levels after a 25-day CPF administration at 10 mg/kg (73). Antioxidant treatment effectively restored IL-10 levels to near those of the control group (G1), indicating that oxidative stress caused the observed reduction.

Vitamin C co-administration in Group G5 mitigated oxidative stress and raised IL-10 levels. The lack of significant differences between Group G5 and groups co-treated with *H. sabdariffa* extract (G3 and G4) highlights the comparable antioxidant and anti-inflammatory properties. This finding is further supported by ShamsEldeen et al. (2023), who demonstrated that *H. sabdariffa* extract exhibits antioxidant and anti-inflammatory effects by increasing IL-10 and reducing NF-κB and TNF-α levels under oxidative stress.

Evaluation of the Liver Protection Effect of *H. sabdariffa* Extract is Estimated by: Biomarker Evaluation (AST, ALT, and ALP) and Hepatic Histological Examination.

In the G2 group, levels of AST, ALT, and ALP showed a significant increase compared to all other groups (G1, G3, G4, and G5). Conversely, no statistically significant differences were observed in these enzyme levels among the G3, G4, G5, and control (G1) groups. Moreover, the enzyme levels in G3 and G4 were similar to each other and showed no differences when compared to G5. These findings are illustrated in Figures 7, 8, and 9.

The measurement of (AST, ALT, and ALP) levels in serum is one of the most important ways to assess liver function, as elevated levels above the normal range suggest impairment in the functionality of hepatocytes. The elevation of liver enzymes observed in the G2 group can be directly linked to oxidative stress induced by CPF. This process involves the stimulation of ROS, which triggers lipid peroxidation, leading to cellular damage. Additionally, CPF enhances inflammatory responses while suppressing IL-10, an anti-inflammatory cytokine.

In groups G3 and G4, the data indicate that *H. sabdariffa* extract preserves hepatic enzyme levels at values comparable to those of the control group (G1), illustrating its hepatoprotective properties against CPF-induced oxidative stress. The similar outcomes observed in the vitamin C-treated group (G5) further suggest that the antioxidant capacity of *H. sabdariffa* extract is equivalent to that of vitamin C. These findings are consistent with the observations of Hamza A. and Heeba G. (2023), who demonstrated that hibiscus extract effectively attenuates oxidative stress and mitigates liver damage induced by cisplatin (74).

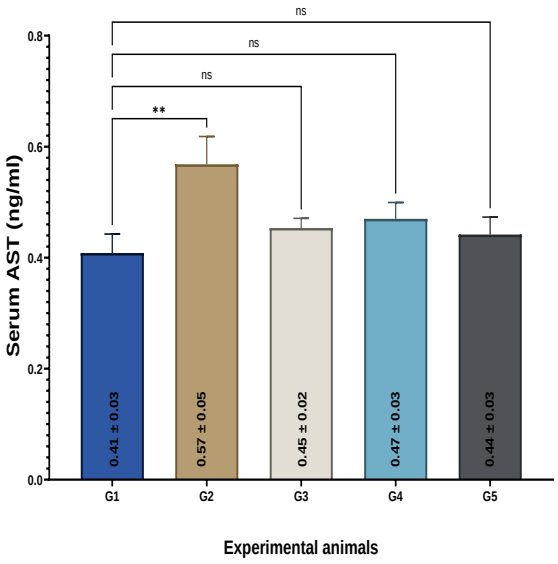

Figure 7: serum AST level in different experimental groups. Data are presented as mean ± SEM, with (**) indicating a statistically significant difference (p-value < 0.01) compared to other groups.

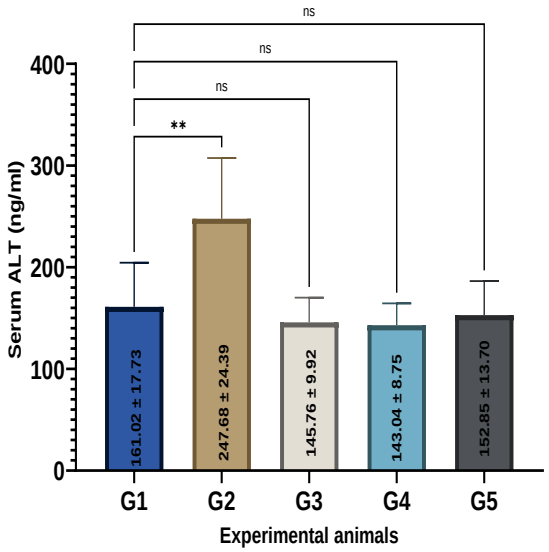

Figure 8: serum ALT level in different experimental groups. Data are presented as mean ± SEM, with (**) indicating a statistically significant difference (p-value < 0.01) compared to other groups.

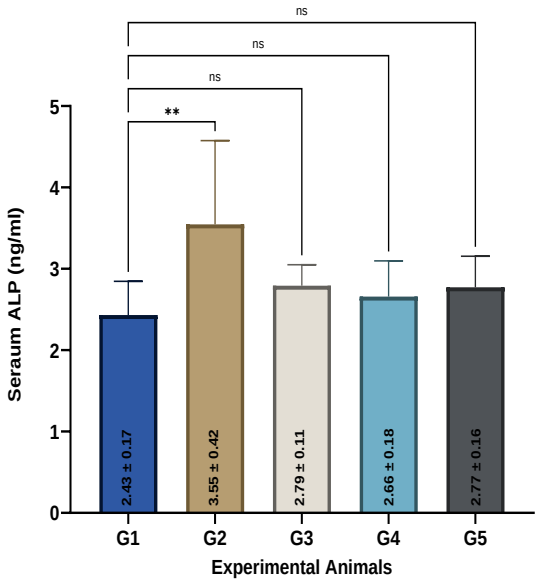

Figure 9: serum ALP level in different experimental groups. Data are presented as mean ± SEM, with (**) indicating a statistically significant difference (p-value < 0.01) compared to other groups.

The results of Hepatic Histopathological Examination are represented in Figure 10 (A, B, C, D, and C)

The biomarker results align with the changes observed in the liver tissue structure. Specifically, in group G2, histological examination revealed noticeable degeneration of the liver parenchyma, which adversely affects its function. This damage is likely due to the lipophilic nature of CPF, which enables cellular infiltration and leads to increased levels of reactive oxygen species (ROS) within the cells. This impact on hepatocyte structure was also observed in a study by Milošević et al. (2022), where chlorpyrifos was administered for 28 days, successfully inducing oxidative stress (43).

Histological examinations of groups G3, G4, and G5 show less tissue damage caused by CPF compared to group G2. Although the tissue isn't as well-preserved as in group G1, and some damage is still visible, it's much less severe than in group G2. This matches the findings from liver enzyme measurements.

These results are in line with the research by Abaker A and Ahmed A (2023), which showed that *H. sabdariffa* effectively protects the liver structure from N-diethylnitrosamine-induced damage in rats (75).

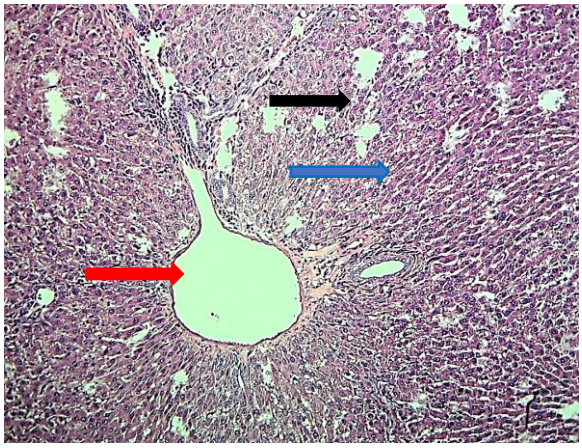

Figure 10-A: Histological sections of rat livers from the control group (G1) exhibited normal hepatocyte architecture (black arrow), a normal central vein (red arrow), and normal sinusoids (blue arrow), all within normal limits (WNL).

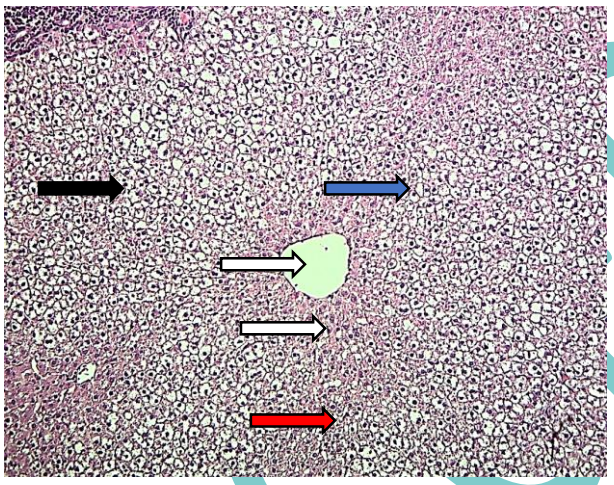

Figure 10-B: The liver of a rat treated with chlorpyrifos at a dosage of 10 mg/kg exhibited enlarged hepatocytes (black arrow) alongside shrunken and necrotic nuclei in some hepatic cells (blue arrow). There was a noticeable narrowing or possible collapse of the sinusoids, accompanied by a constricted hepatic vein (white arrow). Additionally, a focal area of hepatic necrosis was observed, characterized by infiltration of leukocytic cells (red arrow).

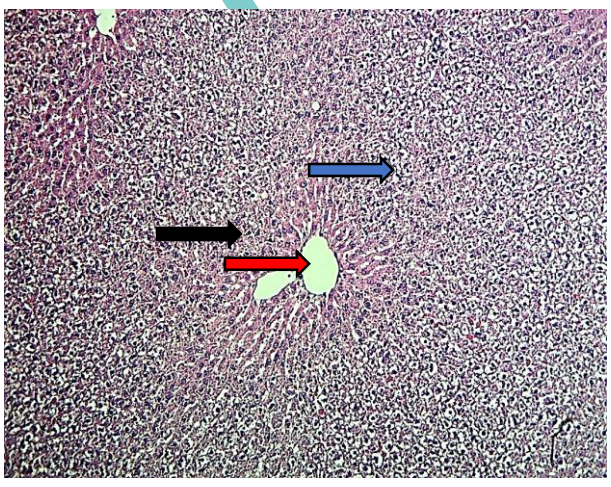

Figure 10-C: The liver of a rat treated with 250mg/kg of *H. sabdariffa* and 10mg/kg of chlorpyrifos noticed a normal hepatic vein (red arrow) with hepatocytes close to the vein appearing normal (black arrow), while hepatic cells farther away appear shrunken with pyknotic nuclei (blue arrow) with leucocyte cells infiltration was present (white arrow).

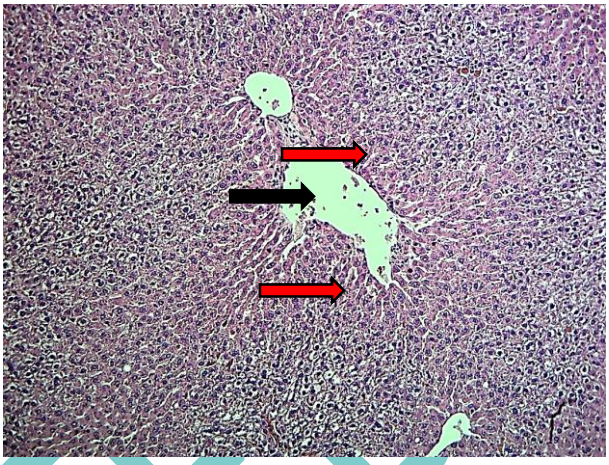

Figure 10-D: liver of a rat treated with 750 mg/kg of *H. sabdariffa* and 10mg/kg of chlorpyrifos showing normal hepatic vein (black arrow) with improvement in liver cells (red arrow) within normal limit.

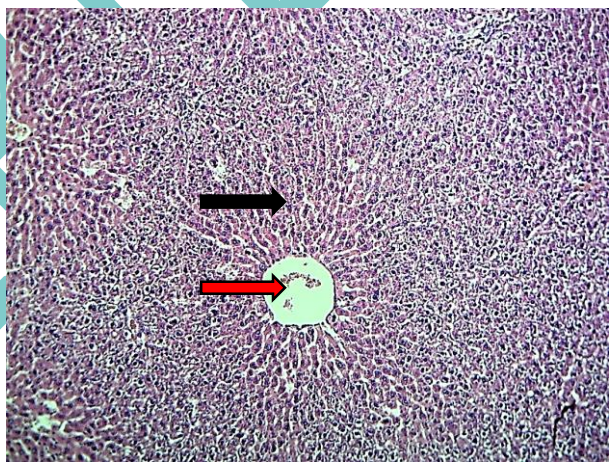

Figure 10-E: The liver of the rat receives vit. C showed a narrow central vein (red arrow) and was normally formed of cords of hepatocytes radiating from the central vein to the periphery of the lobule (black arrow).

Conclusion

The active compounds present in the calyces of *H. sabdariffa* exhibit high solubility in water, enabling the rapid transfer of specific components into the surrounding solvent within minutes. However, this observation is contingent upon the quantitative aspect, which pertains exclusively to the weight of the extract. This metric does not accurately reflect the effectiveness of the extraction process. Therefore, further research is necessary to elucidate the impact of varying soaking periods on the extraction outcomes.

This study underscores the significant therapeutic potential of *H. sabdariffa* as a natural antioxidant and anti-inflammatory agent. When prepared as a tea, its bioactive compounds, including flavonoids, phenolic acids, and other compounds identified through GC-MS analysis, effectively combat oxidative stress caused by CPF exposure. These compounds work by restoring the body's natural antioxidant defenses and reducing inflammatory responses.

Moreover, the extract's safety as a commonly consumed herbal tea makes it a practical and risk-free dietary inclusion. In an era of growing exposure to oxidative stressors, regular consumption of *H. sabdariffa* tea may provide considerable health benefits, protecting vital organs like the liver and enhancing overall well-being.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The raw data required to reproduce these findings are available in the body and illustrations of this manuscript.

Author's contribution

The authors confirm their contributions to the paper as follows: study conception, design, theoretical calculations, and modeling by Mortada E. Sadiq[1]; data analysis and validation by Muhsin S. G. Al-Mozie'l[2]; draft manuscript preparation by Asia S. Abdullah[3]. All authors have reviewed the results and approved the final version of the manuscript.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article

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