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Exploring the Antioxidant and Hepatoprotective Potential of *Prunella Vulgaris* Extract: A Comprehensive Biochemical and Mechanistic Investigation in Thioacetamide Liver Injury Model and FRAP Assay

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Abstract: The current work used the Ferric Reducing Antioxidant Power (FRAP) assay model and a thioacetamide-induced liver damage model to investigate the pharmacological effects of *Prunella vulgaris* stem and leaf extract. The study explored the hepatoprotective and antioxidant potential of plant extract. The hepatoprotective effect of the plant extract was studied in thioacetamide induced liver damage model and the antioxidant effect was evaluated in FRAP assay model. Moreover, various molecular and biochemical markers wer also investigated. These markers included lipid profile analysis viz, total cholesterol (TC). triglycerides (TG), HDL, LDL and liver function test including SGPT (ALT), SGOT (AST), ALP, γ-GT levels. Total Serum Protein level, Serum Albumin level, Serum Bilirubin level, and Conjugated Bilirubin level were also estimated. Various hallmark markers of oxidative stress and antioxidant defense system viz, CAT, SOD, GSH and TBARS were also estimated. The results clearly demonstrated amelioration of the harmful and damaging effect of hepatotoxicity in terms of reversal and normalization of all the biochemical markers. The results also revealed the normalization of the oxidative stress markers and improved the antioxidant defense system as a result of the treatment with the plant extract. The findings contribute to the expanding body of knowledge regarding natural botanicals and extracts that promote liver function and may facilitate the development of therapeutic approaches that take advantage of the therapeutic benefits of *Prunella vulgaris* extract in averting oxidative stress-related hepatic

damage.

Keywords: *Prunella vulgaris*, Antioxidant, Hepatoprotective, Thioacetamide liver injury model, FRAP assay

1. Introduction

The most significant organ that is vital to the preservation of the body's many physiological functions is the liver. It is involved in metabolism, secretion, and storage, among other essential processes. It is essential for the excretion and detoxification of several endogenous and foreign substances. As a result, any harm done to it or impairment of its functionality has detrimental effects on the health of the impacted individual. Viral infection is one of the primary causes of hepatic damage; nonetheless, hepatitis-related liver cirrhosis accounts for around 18,000 deaths annually. It serves as a repository for metals, proteins, glycogen, and other vitamins. By moving blood from the portal to the systemic circulation and its reticulo-endothelial system, it also contributes to the immunological process and aids in the control of blood volume (Bahashwan et al. 2015; Drotman and Lawhan 1978; Kaplowitz and DeLeve 2013; Sonika and Kar 2012; Wolf 1999).

Nearly all medications are recognised by the human body as alien substances (xenobiotics), and in order to prepare them for excretion, they go through a number of chemical reactions including metabolism. Chemical changes must be made in order to: (a) alter biological activity; and (b) decrease fat solubility. The smooth endoplasmic reticulum in the liver serves as the primary "metabolic clearing house" for both endogenous chemicals (such as cholesterol, steroid hormones, fatty acids, and proteins) and exogenous substances, despite the fact that practically every tissue in the body has some capacity for chemical metabolism (e.g., drugs). The liver is vulnerable to damage from drugs due to its essential function in the elimination and modification of substances (Bahashwan et al. 2015; Drotman and Lawhan 1978; Kaplowitz and DeLeve 2013; Lee et al. 2008; Sonika and Kar 2012; Tukov et al. 2007; Wolf 1999).

Hepatitis is an inflammation of the liver characterized by the presence of inflammatory cells in the liver's tissue. Five main kinds of viruses have been identified so far: A, B, C, D, and E. These five categories are the most alarming due to the prevalence of disease and death. The disease may progress and result in cirrhosis and fibrosis, or it may be self-limiting and go away on its own. Although jaundice, anorexia (lack of appetite), and malaise are usually the outcomes, hepatitis might present with little or no symptoms. If hepatitis does not go away within six months, it is considered chronic; if it does, it is considered acute. Hepatic problems include parasitic and viral infections; autoimmune disorders; and xenobiotic overdose from alcohol, drugs, herbal remedies, chlorinated solvents, alcohol-containing substances, fungal toxins, peroxidized fatty acids, radioactive isotopes, and industrial pollutants, among other substances. In particular, types A and C are responsible for hundreds of millions of cases of chronic sickness and are the primary cause of both liver cirrhosis and cancer. An estimated 1 million fatalities annually are ascribed to viral hepatitis infection, namely the combined effects of the hepatitis B and hepatitis C viruses. In 78% of instances, this virus is the main culprit behind liver cancer and cirrhosis. Nearly one in three people, or about 2 million people globally, had both HBV and HCV infections. World Hepatitis Day was marked on July 28, 2013, by the World Health Organization (WHO) and its partners. They emphasized that while the number of cases of viral hepatitis is increasing, many policymakers, medical professionals, and members of the public still largely disregard or are unaware of this reality (Lee et al. 2008; Tukov et al. 2007). For millennia, medicinal plants have been used to treat human ailments. Around the world, traditional healers investigate and employ a variety of therapeutic herbs. These plants are used to cure a variety of human illnesses, and they can be found in forests or outside of them. The kidney and liver are two vital organs that perform a wide range of tasks. Because of the excessive use of alcohol, narcotics, and other substances, there is currently a growing number of individuals suffering from liver and renal problems (Bhuyan et al. 2018; Jaswal et al. 2013; Kaplowitz and DeLeve 2013; Sonika and Kar 2012).

Since the beginning of time, medicinal plants have been essential to human health and wellbeing. They provide a wide range of therapeutic chemicals that are the cornerstone of traditional medical systems all over the world. Approximately 80% of the world's population is reliant on traditional medicine, which is derived from medicinal plants. Conventional medicine encompasses a broad spectrum of natural health treatments, such as Ayurveda, Unani, Amchi, Siddha, and tribal/folk traditions. It has been estimated that 7,500 plants are used in India's rural and tribal areas. Out of these, the general public is either unaware of the precise medicinal potential of almost 4,000 plants or just partially aware of it. About 1200 plant species were used in traditional medical systems including Ayurveda, Unani, Tibetan, Amchi, and Siddha. A thorough examination of the plants used in regional healing customs and pharmacognostical assessment can result in the development of essential plant medications for several incurable illnesses (Ravichandra et al. 2013) (Bhuyan et al. 2018; Jaswal et al. 2013; Kaplowitz and DeLeve 2013; Sonika and Kar 2012). Furthermore, studies have proven and verified the effectiveness of a number

of forest-based plant species in the management of renal and liver problems. Forests and other locations have a variety of important plants, shrubs, and trees that are utilised to cure liver disease. These include *Andrographis paniculata, Phyllantus niruri, Emblica officinalis, Cichorium intybus, Boerhavia diffusa, Curcuma longa, Tinospora cordifolia* and *Solanum nigrum*. In the same way, renal problems can benefit from the usage of Aegle marmelos, Amaranthus spinosus, Bacopa monnieri, Bryophyllum pinnatum, Eclipta prostrate, Cyperus rotundus, Tribulus terrestris, etc (Drotman and Lawhan 1978; Kaplowitz and DeLeve 2013; Moron et al. 1979; Sonika and Kar 2012; Zimmerman 1999). The seeds, fruit, flowers, leaves, stems, roots, and/or other plant components contained certain physiologically active chemicals. As a result, many herbal and pharmaceutical firms employ them to prepare potent herbal drugs and to treat ailments of the liver, kidney, and other organs. Although it is unknown exactly how these plants work to treat illnesses of the liver, kidneys, and other organs (Bhuyan et al. 2018; Chance and Maehly 1955; Chopra et al. 1956; Drotman and Lawhan 1978; Kirtikar and Basu 1935; Ravichandra et al. 2013; Talluri et al. 2016; Wang et al. 2020). Scientific research has confirmed many of the traditional assertions about the effectiveness of medicinal herbs in liver therapy in the current period.

The stem of *Prunella vulgaris* L. is one to two feet long, and the leaves have a notched edge. The purple blossoms appear near the top of the stalk. Most flowers are at their peak from June to August. Another name for *P. vulgaris* L. (labiatae) is selfheal. It was well-liked in Chinese and European medicine for treating fever, sore throats, and accelerating wound healing. This perennial herb is widely utilized and grows wild in the Kashmir valley. The plant has great medical value, and it is a staple in all traditional Unani composite medicines for headache, sore throat, and common cold. The herb is cooked and breathed as steam, which helps to remove congestion and lessen headaches. In Kashmir, Unani medicine uses the herb as a brain tonic during the cold winter months. In Kashmir, the plant is one of the components of a traditional composite medicine that is used to bathe expectant mothers following childbirth. Herpetic keratitis has recently been treated clinically with an aqueous preparation of this plant. The plant is utilized as an antipyretic, diuretic, hypotensive, antibiotic, antiseptic, antirheumatic, antioxidant, and vermifuge. It also has a high medical value. It is used on wounds, ulcers, and sores. Prunellin, a polysaccharide with anti-HIV properties, is present in the aqueous extract of *P. vulgaris*. After screening several popular herbs, it was discovered that *P. vulgaris* had notable anti-HIV properties. There have also been reports of its antiviral activity against the herpes simplex virus. This herb's aqueous preparation prevents allergic responses and anaphylactic shock. Rat RBC is shielded against hemolysis and lipid peroxidation by it, as are kidney and brain homogenates. *P. vulgaris* was shown to have immune-modulating effects on monocytes. Because of the high concentration of rosamarinic acid in it, the plant is more useful for medicinal purposes (Bhuyan et al. 2018; Chance and Maehly 1955; Chopra et al. 1956; Drotman and Lawhan 1978; Karabay et al. 2005; Kirtikar and Basu 1935; H-L Li et al. 2003; S Li et al. 2016; Mao et al. 2017; Ravichandra et al. 2013; Soga et al. 2012; Talluri et al. 2016; Wang et al. 2020).

Given the foregoing information, the current study's objectives were to extract and assess *Prunella vulgaris* leaves and stems' antioxidant and hepatoprotective potential in the Thioacetamide hepatoxicity model as well as other mechanistic models *in vitro* and *in vivo*, for instance the assessment of ferric reducing ability of plasma.

2. Material and Method

Drugs, reagents and chemicals

The study exclusively used reagents and chemicals that were collected and purchased from reliable, verified suppliers. The source of thioacetamide was Himedia Laboratories located in Mumbai, India. We received gift samples of quercetin and catechin from Rachet Pharma in Baddi, India.

Plant Material and Authentication

Prunella vulgaris, often known as "self-healing," has medicinal value, which has led to research into its potential for therapeutic use by extracting bioactive components from its stems and leaves. The plant material was gathered in the Indian state of Assam's Morigaon area. A botanist had recognized and verified the identity of the plant. An herbarium (MK/2019/198) has been created and maintained in the pharmacy department for reference.

Extraction of the plant and Preliminary phytochemical screening

The plant material was carefully collected, dried in the shade, and then ground into a coarse powder in order to facilitate extraction. The Soxhlet apparatus was utilized in the selected extraction method, which maximized the extraction of a wide range of phytochemicals using a water/methanol solvent mixture. This mixture of solvents, which includes both polar and moderately polar molecules, increases the possibility of extracting a wide range of bioactive components found in *Prunella vulgaris*. The *Prunella vulgaris* extract (PVE) was stored in a vacuum chamber at -4 ◦C until it was needed for the investigation.

Hepatic damage caused by thioacetamide (TAA)

The CYP450 2E1 enzyme bioactivates thioacetamide (TAA) in the liver, resulting in the production of TAA-S-oxide and TAA-S-dioxide. Through the hepatocellular membrane's lipid peroxidation, TAA-S-dioxide causes oxidative stress. TAA binds covalently to liver macromolecules, causing hepatic necrosis throughout the pericentral area with a single dose treatment (50–300 mg/kg). When injured hepatocytes endure TAA therapy (150–300 mg/kg, twice a week for 11–16 weeks), transforming growth factor (TGF)–β/smad3 downstream signaling is initiated, triggering HSCs (hepatic stellate cells) to adopt a myofibroblast-like phenotype. A range of extracellular matrix is produced by the activated HSCs, which causes portal hypertension, cirrhosis, and liver fibrosis. Varied doses, frequencies, animal models, and administration modalities may result in different liver damage from TAA. On the other hand, TAA consistently causes hepatotoxicity, making it a perfect model for assessing the antioxidant, cytoprotective, and antifibrotic substances in test animals. The animal study was previously approved by IAEC as per the guidelines from The Committee for Control and Supervision of Experiments on Animals (CCSEA), India.

Experimental design (Talluri et al. 2016)

Thirty-four rats in all were split up into six groups: Groups 2 and 3 each had five rats, and the other four groups had six rats apiece. For one week, Group I, the usual control group, received an oral saline vehicle (pH 7.8) at a rate of 2 milliliters per kilogram of body weight. Group II was administered TAA subcutaneously at a dose of 50 mg/kg body weight on the first day as a 2 percent w/v solution in water. Thereafter, saline therapy was administered continuously. Rats in Group III received a subcutaneous injection of TAA (50 mg/kg body weight) on the first day of treatment. Three weeks later, they were given an oral dosage of silymarin (25 mg/kg body weight). Groups IV, V, and VI received PVE for three weeks at dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight p.o., respectively, and TAA as a 2 percent w/v solution in water on the first day at 50 mg/kg body weight subcutaneously. The purpose of this experiment was to examine how various therapies affected the pathophysiology and function of the liver in the rat model.

Table 1: An unambiguous summary of the experimental groups, the quantity of rats in each group, and the treatments that were given to each group.

Groups	Number of Rats	Treatment
I	6	Saline (2 ml/kg BW) orally for 1 week
II	5	TAA (50 mg/kg BW, subcutaneous) + Saline (continuous)
III	5	TAA (50 mg/kg BW, subcutaneous) + Silymarin (25 mg/kg BW, oral) for 3 weeks
IV	6	TAA (50 mg/kg BW, subcutaneous) + PVE (200 mg/kg BW, oral) for 3 weeks
V	6	TAA (50 mg/kg BW, subcutaneous) + PVE (400 mg/kg BW, oral) for 3 weeks
VI	6	TAA (50 mg/kg BW, subcutaneous) + PVE (600 mg/kg BW, oral) for 3 weeks

Liver Profile and Lipid profile

Total cholesterol (TC), Serum triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol levels have been estimated using standard biochemical kits (Davidson and Rosenson 2009; Finley and Tietz 1996; Latt 1991). The Hitachi 912 clinical chemistry automatic analyzer was utilized to assess the activities of AST (aspartate transaminase), ALT (serum alanine transaminase), γ-GT (gamma-glutamyltransferase), and ALP (alkaline phosphatase) (R & D Systems).

Assessment of oxidative stress

Hepatic tissue that had been homogenised was treated and put through an assay to measure oxidative stress indicators.

Estimation of Catalase (CAT) and Superoxide dismutase (SOD)

The standard procedure previously mentioned was used to measure the amount of catalase (CAT) in liver homogenate (Chance and Maehly 1955; Greenwald and Claiborne 1985). Additionally, SOD was assessed using the conventional procedure earlier mentioned (Kakkar et al. 1984; Misra and Fridovich 1972).

Reduced glutathione activity (GSH)

The standard procedure previously described was followed to assess the GSH activity in liver homogenate using a calorimetric kit (BioVision, USA) at absorbance 412 nm (Tietze 1969).

Estimation of TBARS (thiobarbituric acid reactive substances)

The amount of TBARS has been estimated using a conventional technique that was previously published (Iqbal et al. 1996).

Ferric Reducing Ability of Plasma (FRAP)
Following intraperitoneal injections of PVE (200, 400, and 600 mg/kg), quercetin (50 mg/kg), vitamin C (50 mg/kg), and vitamin E (40 mg/kg) to several animal groups, FRAP was assessed. After two hours, the blood samples were removed, and the plasma was separated for the FRAP examination (Benzie and Strain 1996). At that point, 300 µL of freshly mixed FRAP reagent (10 mM tripyridyltriazine, 20 mM ferric chloride, and 500 mM acetate buffer) was added to 10 µL of plasma, and the absorbance at 593 nm was recorded.
Statistical analysis
Using GraphPad Prism, and Microsoft Office Excel 2019, the measurable data's means, standard deviation (SD), and statistics were determined. One-way analysis of variance (ANOVA) was used to assess the significance of the results, and then the *post hoc* 'Turkey's multiple comparison test' was used. A type 1 error probability of p = or < 0.05 was identified as the cutoff point for statistical significance.

3. Results and Discussion

Extraction and Preliminary phytochemical screening
The plant material, leaves and stems of *Prunella vulgaris* was carefully gathered, dried in the shade, and ground into a coarse powder for Soxhlet extraction. A number of phytochemical substances from the classes alkaloids, flavanoids, glycosides, saponins, phenols, phytosterols, fatty acids, carbohydrates, and proteins were found in the preliminary phytochemical screening results.
Evaluation of hepatoprotective activity in Thioacetamide (TAA) induced liver injury
Body weight gain and Liver weight
In comparison to the control group, thioacetamide (TAA) significantly reduced body weight ($p<0.0001$) while increasing absolute liver weight ($p<0.001$). PVE therapy was observed to provocatively ($p<0.001$) diminish and recover the increased absolute liver weight caused by TAA intoxication (Figure 1). PVE demonstrated a body weight restoration that was dose dependent (*$p<0.001$*).

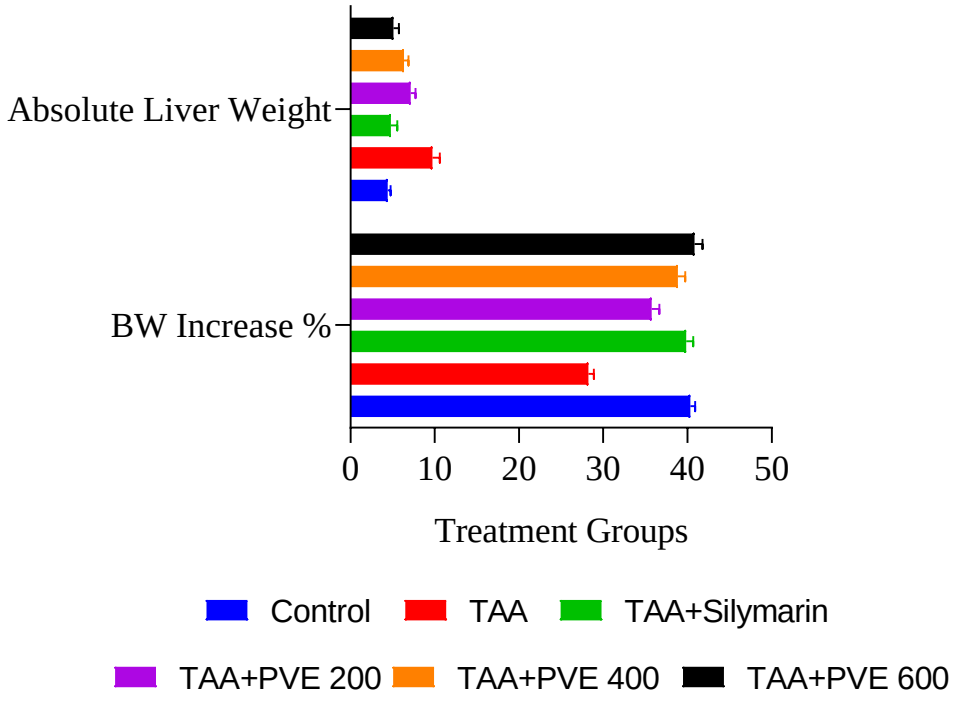

Figure 1: Gains in body weight and the proportion of absolute liver weight.

Lipid profile and Liver Functions
When compared to animals in the control group, thioacetamide was shown to considerably reduce ($p<0.001$) the levels of blood triglycerides (TG), total cholesterol (TC), and low-density lipoprotein and considerably ($p<0.001$) raise the levels of high-density lipoprotein (HDL) (LDL). When compared to the animals in the TAA group, the PVE treatment at dosages of 400 and 600 mg/kg dramatically reduced and restored the blood levels of TC, LDL,

and triglycerides (**p<0.01 and ***p<0.001). Additionally, the PVA group's HDL levels considerably rose in comparison to the TAA group. However, PVE at a dose of 200 mg/kg did not cause HDL levels to rise noticeably (Not significant). Table 2 presents the findings. The hepatoprotective advantages of PVE are shown in Table 3 with reference to liver function markers. In comparison to the TAA group animals, PVE treatment at all tested dosage levels (200, 400, and 600 mg/kg) showed a substantial drop and restoration (p<0.001) of the ALT, γ-GT, AST, and ALP levels to basal value. However, the administration of TAA was seen to increase the levels of liver profile tests, such as ALT, AST, γ-GT, and ALP, in comparison to normal control animals. The blood levels of albumin, total protein, bilirubin, and conjugated bilirubin were also seen to rebound to normal levels when treated with PVE at all dosage levels (Table 2 and 3).

Table 2: PVE's impact on the lipid profile

	TC (mg/dl)	**TG (mg/dl)**	**LDL (mg/dl)**	**HDL (mg/dl)**
Normal Control	3.23±0.84	4.83±0.84	1.87±0.43	3.01±0.62
Thioacetamide (TAA)	[a] 8.33±0.25***	[a] 8.14±0.65***	[a] 7.82±0.23***	[a] 1.51±0.35***
Silymarin + TAA	[b] 3.52±0.26***	[b] 5.22±0.34***	[b] 2.24±0.28***	[b] 2.91±0.58**
PVA 200 + TAA	[b] 6.19±0.69***	[b] 7.10±0.75*	[b] 6.20±0.46***	[b] 2.37±0.45[ns]
PVE 400 + TAA	[b] 4.24±0.48***	[b] 5.13±0.84***	[b] 4.25±0.17***	[b] 2.72±0.47**
PVE 600 + TAA	[b] 3.46±0.74***	[b] 4.85±0.28***	[b] 2.30±0.19***	[b] 2.94±0.54**

*Results are presented as Mean ± SD (for n=6 values). [a] vs normal control group at ***p<0.001. [b] vs TAA treated group at *p<0.05, **p<0.01, ***p<0.001.*

Table 3: Effect of PVE on biomarkers of hepatic injury

	SGPT (ALT) (IU/L)	**SGOT (AST) (IU/L)**	**ALP (IU/L)**	**γ-GT (nM/min /mg protein)**
Control	60.16±2.63	79.50±1.65	182.24±2.87	106.45±2.74
Thioacetamide (TAA)	[a] 119.24±3.25***	[a]237.74±3.59***	[a]454.65±5.75***	[a] 155.34±2.65***
Silymarin + TAA	[b] 64.48±1.67***	[b] 88.99±1.87***	[b]190.65±2.91***	[b]110.87±3.74***
PVA 200 + TAA	[b] 93.80±1.87***	[b]150.94±2.92***	[b]305.67±2.76***	[b]128.87±3.91***
PVE 400 + TAA	[b] 71.78±1.85***	[b]93.74±2.26***	[b]209.36±2.65***	[b]112.65±3.69***
PVE 600 + TAA	[b] 63.87±1.74***	[b] 85.83±1.71***	[b]193.94±2.79***	[b]109.93±3.97***

*Mean ±SD for the six numbers. At the ***p<0.001 probability level, the control group's significance is shown. The TAA group's significance is indicated by b at the ***p<0.001 probability level.*

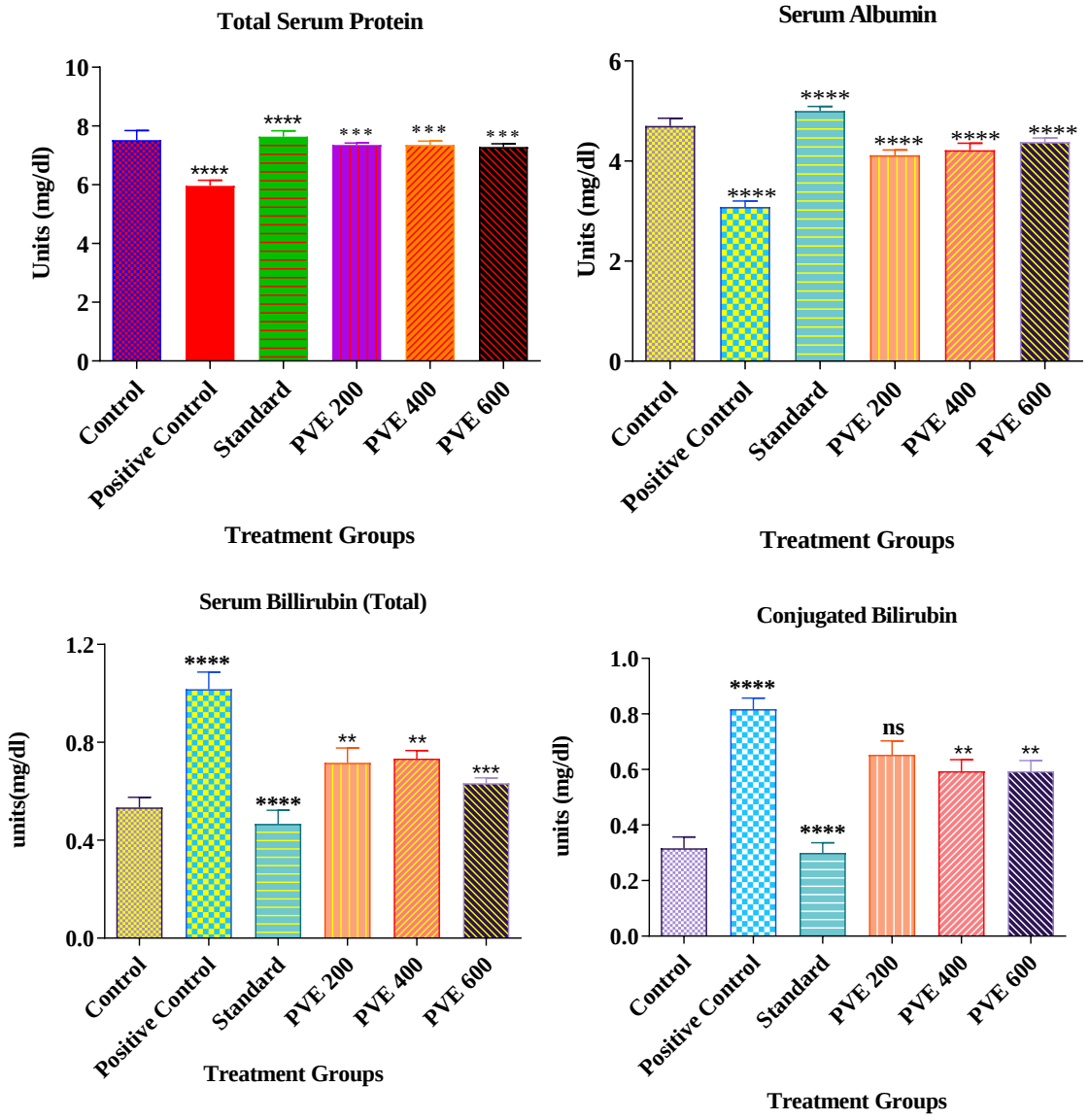

Figure 2: Effect of PVE on Total Serum Protein level, Serum Albumin level, Serum Bilirubin level, and Conjugated Bilirubin level. Data are expressed as mean ± SD (n = 6). One-way ANOVA Tukey *post hoc:* ** *p < 0.001*, **** *p < 0.0001*

Assessment of oxidative stress markers

Rats treated with TAA showed significantly lower levels of CAT, SOD, and GSH activity (**p<0.01 and ***p<0.001) in their liver tissues compared to normal control rats, but their TBARS levels were elevated (***p<0.001). Higher TBARS levels are indicative of increased lipid peroxidation caused by free radical formation and oxidative stress. This, in turn, lowers the levels of SOD, CAT, and GSH, which weakens the antioxidant enzyme defense system. The PVE therapy greatly enhanced and repaired the natural antioxidant defense enzyme system by restoring normal levels of SOD, CAT, and GSH. At every dosage level (200, 400, and 600 mg/kg b.w.), the PVE treatment dramatically raised the levels of CAT, SOD, and GSH in comparison to the animals in the TAA group (** p<0.01, ***p<0.001). Table 4 presents the findings.

Table 4: PVE's impact on indicators of oxidative stress

	SOD (U/mg protein)	**CAT (U/min)**	**TBARS**	**GSH (µM /g tissue)**

			(nM /min/mg protein)	
Control	20.73±1.06	3.06±0.74	23.65±1.19	2.78±0.19
Thioacetamide (TAA)	[a] 9.93±0.94***	[a] 1.47±0.29***	[a] 47.39±1.94***	[a]0.41±0.14***
Silymarin + TAA	[b] 18.90±0.98**	[b] 3.46±1.54**	[b]21.89±1.59**	[b]2.68±0.79***
PVA 200 + TAA	[b] 14.50±0.92**	[b] 3.19±0.86**	[b]34.89±1.64**	[b]2.09±0.72***
PVE 400 + TAA	[b] 17.59±0.93**	[b] 3.39±0.69**	[b]26.57±1.79**	[b]2.59±0.79***
PVE 600 + TAA	[b] 19.84±0.89**	[b] 3.48±0.99**	[b]22.94±1.89***	[b]2.86±0.89***

*Results are presented as Mean ± SD (for n=6 values). [a] denote significance from the control group at **p<0.01, ****p<0.0001 probability level. [b] denote significance from the Thioacetamide (TAA) group at **p<0.01, ***p<0.001, probability level*

Ferric Reducing Ability of Plasma (FRAP)
The ferric-reducing capabilities of quercetin (50 mg/kg), vitamin C (50 mg/kg), vitamin E (40 mg/kg), and PVE plasma (200, 400, and 600 mg/kg) were displayed in Figure 3. PVE (200, 400, and 600 mg/kg) was found to be equal to quercetin (50 mg/kg), vitamin C (50 mg/kg), and vitamin E (40 mg/kg) in terms of its considerable enhancement of plasma's ferric reducing activity.

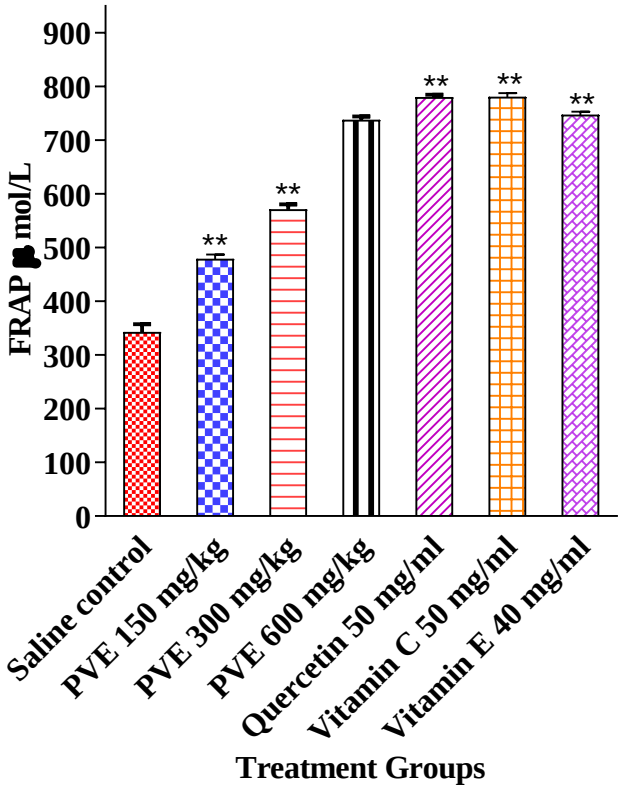

Figure 3: Ferric reducing ability of plasma by PVE. Data are expressed as mean ± SD (n = 6). One-way ANOVA Tukey *post hoc*: ** $p < 0.01$

4. Conclusions

The plant has long been used to treat liver and related problems; the findings of hepatoprotective tests and blood biochemical markers in the extract-treated group confirm the hepatoprotective and antioxidant activity. The leaf

and stem extract had a noteworthy hepatoprotective effect at higher dosages that was similar to silymarin. The extract's natural antioxidant content might be the cause of the hepatoprotective and in vitro antioxidant effects that have been noted as evident from the normalization of the biochemical markers. These imply that mitigation of thioacetamide-induced liver damage is due to a synergy formed between the antioxidant activity and inherent protective properties of the plant extract. The extensive array of tests, which encompass assessments at both the molecular and functional levels, contributes to a robust understanding of the extract's potential applications in liver health. These results pave the way for further research and development of *Prunella vulgaris* extract as a potential medication and therapeutic therapy for liver-related ailments.

Conflict of Interests

The authors declare that there is no known conflict of interest in the manuscript.

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