Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
Al-Qadisiyah Journal of Pure Science

Comparative Effects of Conventional and Nano-Formulated Ethanolic Extracts of *Portulaca oleracea* on Oxidative Stress-Induced Physiological Alterations in Male Rats

Muneer Naeem Lafta bio.edu.posta24.36@qu.edu.iq

Hussein A . Selman Hussein.Abbas@qu.edu.iq

Department Biology, College of Education, University of Al-Qadisiyah, Iraq

Received: 04/05/2026; Revised: 12/05/2026; Accepted: 17/05/2026.

Abstract

This study examined the effects of traditional and nano-scale alcoholic extracts of *Portulaca oleracea* on liver and kidney function and oxidative stress in male white rats. Forty rats (aged 6–8 weeks, weighing 120–200 g) were used in the animal laboratory of the Department of Biology, Faculty of Education, Al-Qadisiyah University. The animals were randomly divided into five groups (n=8) for a period of 30 days: the control group (tap water), T1 (0.5% hydrogen peroxide), T2 (H_2O_2 + 10 mg/kg alcoholic extract), T3 (H_2O_2 + 10 mg/kg nano-extract), and T4 (H_2O_2 + 5 mg/kg nano-extract). The results showed significant increases ($P<0.05$) in urea, creatinine, uric acid, ALT, AST, ALP, and markers of oxidative stress, along with a decrease in total antioxidant capacity in the T1 group compared to the control and treatment groups, confirming the presence of hepatic and renal dysfunction resulting from the effect of hydrogen peroxide. The treatment groups (T2–T4) showed significant improvement ($P<0.05$), with decreased biochemical and oxidative stress markers and increased antioxidant capacity compared to group T1. Group T3 demonstrated higher therapeutic efficacy than groups T2 and T4, while groups T2 and T4 showed similar moderate

effects. These results highlight the potential of nanoscale formulations to enhance the bioavailability and therapeutic efficacy of plant extracts in mitigating oxidative damage and restoring organ function under stress-induced conditions in experimental animal models of hydrogen peroxide-induced oxidative stress.

Keyword : Portulaca oleracea, nano-extract, oxidative stress, liver, kidney, antioxidants.

1. Introduction

Evidence suggests that chronic diseases are linked to complex cellular mechanisms, with oxidative stress being one of the most prominent; it arises from an imbalance between reactive oxygen and nitrogen species and the antioxidant system [1, 2]. The accumulation of free radicals, resulting from metabolism and environmental factors, leads to damage to proteins, lipids, and nucleic acids, contributing to the development of heart disease, liver disease, diabetes, and neurological disorders [3, 4]. The liver and kidneys are among the organs most susceptible to such damage, as chronic oxidative stress is associated with inflammation, fibrosis, and functional decline [5].Antioxidants have garnered increasing attention for their ability to neutralize free radicals and reduce cellular damage; they include vitamins (C, E, A), trace elements such as selenium and zinc, as well as bioactive plant compounds such as flavonoids, carotenoids, and polyphenols [6, 7].Concurrently, progress has been made in developing biomarkers to accurately measure oxidative stress, which supports early diagnosis and the evaluation of the effectiveness of therapeutic interventions [8]. Medicinal plants have traditionally been used to treat a wide range of diseases by utilizing their various parts, and have contributed to promoting public health [9] . These plants are a rich source of natural antioxidants, which reduce oxidative stress and protect cells from its damage, thereby reinforcing their role in the prevention of chronic diseases [10]. *Portulaca oleracea* is an annual plant of the Portulacaceae family, widely distributed in tropical and subtropical regions [11, 12]. It is characterized by distinct morphological features, including

fleshy leaves, yellow flowers, and small black seeds [13], and its various parts are used for food and medicinal purposes in traditional medicine across a wide geographic range. It also has high nutritional value due to its content of omega-3 fatty acids, antioxidant vitamins (A, C, E), and B vitamins [14] *P. oleracea* contains bioactive compounds, such as betalains, flavonoids, and alkaloids, which give it a high capacity to neutralize free radicals and reduce oxidative damage [15, 16] . Studies have shown that its extract possesses protective effects on the liver and kidneys by improving their functional parameters, reducing enzymes, creatinine, and urea levels, and minimizing pathological histological changes, reflecting its dual protective role [17, 18]. Despite the therapeutic efficacy of plant extracts, they face limitations related to low bioavailability, instability, and poor tissue penetration [19]. Nanotechnology has emerged as an advanced approach to improve these properties, as nanoscaling (<100 nm) increases surface area and enhances solubility and diffusion across biological membranes [20]. Furthermore, converting *Portulaca oleracea* into a nanoscale formulation improves the release of active compounds, thereby increasing its bioavailability and pharmacokinetic profile compared to conventional extracts [21]. Based on the above, this study aimed to investigate the protective role of the alcoholic extract of *Portulaca oleracea* in its conventional and nano-formulations in reducing oxidative stress. The objective was to evaluate the effect of these extracts on improving liver and kidney function by measuring liver enzymes (ALT) alanine aminotransferase, (AST) aspartate aminotransferase, (ALP) alkaline phosphatase, and renal markers (creatinine, urea, uric acid), as well as assessing oxidative status through total antioxidant capacity (TAC) and malondialdehyde (MDA) levels, thereby helping to elucidate the role of nanotechnology in enhancing the preventive efficacy of medicinal plants.

2. Materials and Methods

Ethanol (alcoholic) extract of *portulaca oleracea* preparation

Portulaca oleracea was collected from local markets in Diwaniya Governorate, then cleaned and washed with sterile water, cut, and dried in the shade at room temperature for ten days. The plant was then ground, and the powder was stored until the extraction process was carried out. The ethanol extraction was performed using a Soxhlet apparatus according to the method described by [22], wherein 50 g of *P.oleracea* leaf powder was extracted using 500 mL of 70% ethanol at a temperature of 70°C. After the extraction was complete, the solvent was removed by evaporating the alcohol in an incubator at 50°C. The extract was then collected and stored in dark, dry glass containers at room temperature until use.

Nano-extract of p. *oleracea* preparation

This procedure was carried out using a British-manufactured homogenizer, following the method described by [23] with certain modifications aimed at producing nanoscale formulations. Briefly, 20 mg of the ethanolic extract of *Portulaca oleracea* was dissolved in 100 mL of ethanol and left under magnetic stirring for 3 hours at room temperature. The resulting solution was then filtered using filter paper, and the filtration process was repeated three times. Subsequently, the filtrate was further filtered once using a syringe filter. The obtained filtrate was then subjected to homogenization at maximum speed for 30 minutes using a homogenizer. After that, the solution was again filtered once through a syringe filter to obtain a nanoscale particle suspension. In the final step, the filtrate was transferred into heat-resistant glass containers and incubated at 50°C until complete drying, after which it was used for subsequent experimental work.

Identification of bioactive compounds of the plant using GC–mass analysis

The chemical profiling of bioactive compounds in both the conventional and nano-ethanolic extracts of *P. oleracea* was performed using Gas Chromatography–Mass Spectrometry (GC–MS) at the Central Laboratory of Kashan University, Iran. Samples were analyzed using an Agilent GC–MS system equipped with a Mass Selective Detector (MSD), with helium employed as the carrier gas. Separation of compounds was achieved based on

differences in their volatility and their affinity to the stationary phase within the chromatographic column. Subsequently, the separated constituents were identified through mass spectral data analysis and comparison with reference spectral libraries.

Characterization of ethanolic and nano-extracts

Atomic force microscope (AFM)

The surface properties of the conventional and nano-sized alcoholic extracts of *Portulaca oleracea* were analyzed using an atomic force microscope (AFM) to determine the diameters, sizes, and aggregation levels of the nanoparticles, as well as to calculate the crystallinity index using the following equation:

Crystallinity Index = Dp / L

The samples were then sent to the central laboratory at Kashan University in Iran for analysis.

Scanning election microscope (SEM)

The conventional and nano-scale alcoholic extracts of *Portulaca oleracea* were examined using a scanning electron microscope (SEM) to study the surface morphology and characterize the structural composition of the extract. The examination was conducted at the Central Laboratory of Kashan University, Iran.

Experimental Animals.

Adult male laboratory rats were obtained from the College of Veterinary Medicine at the University of Kufa; they were 6–8 weeks old and weighed 120–200 g. The animals were acclimatized for two weeks to ensure their health, and the study was conducted at the animal facility of the Department of Life Sciences, College of Education, University of Al-Qadisiyah.

Experimental Design

A total of 40 experimental animals were randomly allocated into five equal groups, with 8 animals in each group, as follows, over a period of 30 days:

Control group (Control): Animals received 1 mL of normal saline daily throughout the experimental period.

Group 1: Animals were administered hydrogen peroxide (H_2O_2) at a concentration of 0.5% to induce oxidative stress.

Group 2: Animals were administered hydrogen peroxide 0.5%, followed by treatment with the conventional ethanolic extract of *P. oleracea* at a dose of 10 mg/kg body weight.

Group 3: Animals were administered hydrogen peroxide 0.5%, followed by treatment with the nano-formulated extract of *P. oleracea* at a dose of 10 mg/kg body weight.

Group 4: Animals were administered hydrogen peroxide 0.5%, followed by treatment with the nano-formulated extract of *P. oleracea* at a dose of 5 mg/kg body weight.

Biochemical Estimation.

The study evaluated a set of biochemical parameters related to liver and kidney function by measuring liver enzymes (ALT, AST, and ALP) and markers of kidney function (creatinine, urea, uric acid), as well as an assessment of oxidative status through the measurement of total antioxidant capacity (TAC) and markers of oxidative stress (MDA**).**

Statistical Analysis

Statistical analysis was performed using SPSS software. Moral status was assessed using a one-way ANOVA, and statistically significant differences were identified using Duncan's multiple range test ($p \leq 0.05$)

3. Results and discussion

Liver Enzymes

Statistical analysis (Table 1) revealed a significant increase ($P<0.05$) in liver enzyme levels (AST, ALT, and ALP) in the first treatment group (T1) compared with the control group and the other treatments, where (T1) recorded the highest values. In comparison with (T1), the therapeutic treatments showed a significant reduction ($P<0.05$) in enzyme levels; however, these values remained significantly higher than those of the control group, which exhibited the lowest levels. Among the treatment groups, (T3) demonstrated a significantly greater reduction ($P<0.05$) in enzyme levels compared with (T2) and (T4), indicating the best therapeutic response, with some parameters approaching control values without significant differences, particularly for ALP. Conversely, no significant differences ($P>0.05$) were observed between (T2) and (T4) for certain parameters, despite a relative decrease in (T4), which overall exhibited the lowest therapeutic efficacy. The study results indicate the occurrence of liver toxicity resulting from oxidative stress, manifested by a statistically significant ($P < 0.05$) increase in liver enzymes (ALT, AST, ALP) in the hydrogen peroxide group compared to the control, reflecting the liver's susceptibility as a primary target organ for toxins. These results are consistent with the study [24], which demonstrated that a 0.5% concentration of hydrogen peroxide causes an imbalance in the redox status and depletion of antioxidant enzymes, along with exacerbated liver damage in rats. The elevation of liver enzymes in treatment group (T1) is attributed to hydrogen peroxide's ability to generate excessive amounts of free radicals, which target liver cell membranes and induce lipid peroxidation. This results in a loss of membrane integrity and increased permeability, leading to the leakage of enzymes into the bloodstream. This mechanism is consistent with the findings of [25] regarding the association of changes in liver enzymes with cellular damage resulting from the failure of antioxidant defense systems. The study by [26] also supported this explanation by linking elevated malondialdehyde levels to increased lipid peroxidation, altered membrane permeability, and leakage of liver enzymes. In contrast, the results of the experiments (T2, T3, T4) involving the alcoholic extract of *Portulaca oleracea* (conventional and nano) showed a significant ($P \leq 0.05$) reduction in liver enzymes compared to the

oxidative stress group (T1). This effect is attributed to the plant's richness in bioactive compounds such as phenols, flavonoids, and (omega-3), which possess antioxidant properties that scavenge free radicals, thereby preserving plasma membrane integrity and reducing lipid peroxidation, and consequently preventing the leakage of enzymes into the serum [27]. These findings are consistent with previous studies [28, 29], that confirmed the extract's protective role in reducing lipid peroxidation, improving the antioxidant system, and restoring liver function, as well as the study by [30] , which demonstrated its ability to reduce liver damage and preserve tissue structure. The current study also demonstrated the superiority of the nano-extract, as treatment (T3) at a dose of 10 mg/kg showed the best therapeutic response, with enzyme levels returning to values close to those of the control group. The results also showed that the low-dose nanodose produced a protective effect similar to that of the high dose of the conventional extract. This is consistent with the findings of [31] when comparing the phenolic extract of the stinging nettle Urtica dioica with its nanoscale formulation, where they demonstrated that low nanoscale doses (5 and 10 mg) were more effective in repairing liver damage compared to high doses of the conventional extract (250 mg). These results suggest that the nanoformulation of *P.oleracea* extract, particularly at a concentration of 10 mg/kg, has higher antioxidant and anti-inflammatory efficacy, attributed to enhanced biological activity through the inhibition of inflammatory cytokines such as TNF-α and IL-6, thereby contributing to the reduction of cellular damage and improvement of tissue response [32, 33] . This helps reduce the activation of hepatic stellate cells (HSCs) and decrease the production of fibrous collagen [34], while limiting cellular necrosis and steatohepatitis [35]. The therapeutic equivalence between the low nanodose (5 mg/kg) and the high alcohol dose (100 mg/kg) is attributed to the properties of nanotechnology, as the small size and increased surface area improve penetration of cell membranes and enhance bioavailability within target cells, thereby protecting phenolic compounds from degradation and increasing their efficacy against oxidative stress [36].

Table (1): Shows the effect of conventional and nano-alcoholic extracts of the *Portulaca oleracea* on liver enzyme levels in white rats exposed to hydrogen peroxide H_2O_2 .

Group / Parameters	ALT (U/L) Mean ± SE	ALP (U/L) Mean ± SE	AST (U/L) Mean ± SE
Control	53.94±2.64 E	127.53±1.23 C	35.99±1.13 D
H_2O_2 (0.5%)	106.50±2.26 A	195.65±1.38 A	95.03±0.25 A
H_2O_2 (0.5%) + PO Ethanolic extract (100 mg/kg)	75.45±1.74 C	167.20±3.75 B	67.10±2.44 B
H_2O_2 (0.5%) + PO nano extract (10 mg/kg)	65.21±1.77 D	132.39±2.68 C	47.89±2.38 C
H_2O_2 (0.5%) + PO nano extract (5 mg/kg)	87.53±1.48 B	172.26±1.54 B	70.90±0.93 B
LSD	6.37	7.32	5.24

Data are expressed as mean± standard error, values with the same column with different letters are significantly different at P <0.05.

Kidney Function (Urea , Creatinine, Uric acid).

The results in (Table 2) showed a statistically significant increase ($P < 0.05$) in the first treatment (T1) compared to the control group and the remaining treatments. In contrast, the treatment groups (T2, T3, T4) achieved a significant reduction ($P<0.05$) in the indicators compared to (T1), but they remained significantly higher than the control group. Among the

treatment groups, T3 outperformed the others in reducing creatinine and urea levels; it did not differ significantly from the control group in creatinine levels and significantly outperformed ($P<0.05$) T2 and T4 in most indicators. As for uric acid, (T2) showed a clear improvement with no significant differences from the control group, while (T3) and (T4) showed gradual improvement with significant differences ($P<0.05$) compared to the other groups. The group treated with oxidative stress (T1) showed a significant increase in markers of renal function (creatinine and urea), reflecting a decrease in glomerular filtration rate (GFR) and the occurrence of structural and functional abnormalities in renal tissue. This is attributed to the effect of hydrogen peroxide in increasing the production of reactive oxygen species and inducing oxidative stress, which is a major factor in renal damage through the activation of NF-κB and the promotion of inflammatory cytokine secretion and the inflammatory response within the renal tissue [37]. Oxidative stress contributes to the activation of mitochondrial-mediated apoptosis due to an imbalance in proteins that regulate cell death, thereby damaging renal cells [38]. Free radicals also stimulate lipid peroxidation, damage cellular and glomerular membranes, and impair renal tubular function, while inhibiting urea cycle enzymes and causing elevated creatinine and urea levels [39], in addition to renal cell hypertrophy and urinary tract narrowing [40].This is also associated with a metabolic disorder that increases protein breakdown and elevates its nitrogenous byproducts [41]. Furthermore, oxidative stress leads to a disturbance in purine metabolism through the activation of xanthine oxidase, which raises uric acid levels and increases ROS production, causing a vicious cycle of oxidative damage [42]. In contrast, the treatments (T2, T3, T4) that treatment with the alcoholic extract (conventional and nano) of *Portulaca oleracea* resulted in a significant improvement in renal function parameters, as evidenced by a decrease in creatinine, urea, and uric acid levels compared to (T1), although these levels remained relatively higher than those in the control group, consistent with [43]. This effect is attributed to its antioxidant properties and its ability to scavenge free radicals and enhance cellular defense, in addition to reducing inflammation by inhibiting NF-κB and lowering

inflammatory cytokines such as TNF-α and IL-6 [44]. Improvements in urea and creatinine levels have been reported, along with positive histological changes in renal toxicity models, which support the current findings [45]. A study by [46], confirmed that the methanolic extract of *Portulaca oleracea* improved urea and creatinine levels and increased antioxidant levels in models of renal toxicity, which had a positive effect on kidney function. The results also demonstrated the superiority of the nanoscale formulations, with (T3) showing the best response by returning the indicators to levels close to control, compared to a lesser improvement in (T4). This is attributed to increased bioavailability and a higher ability to inhibit inflammatory pathways [47], in addition to protecting renal cells from apoptosis by maintaining mitochondrial integrity and regulating gene expression [48].

Table (2): Shows the effect of conventional and nano-alcoholic extracts of the *P. oleracea* on kidney functions in white rats exposed to hydrogen peroxide H_2O_2

Group / Parameters	**Creatinine (mg/dl) Mean ± SE**	**Urea (mg/dl) Mean ± SE**	**Uric acid (mg/dl) Mean ± SE**
Control	1.135±0.022 C	2.046±0.104 D	33.150±1.183 D
H_2O_2 (0.5%)	1.963±0.073 A	7.233±0.367 A	52.263±1.566 A
H_2O_2 (0.5%) + PO Ethanolic extract (100 mg/kg)	1.637±0.094 B	4.618±0.321 B	35.973±0.789 D
H_2O_2 (0.5%) + PO nano extract (10 mg/kg)	1.246±0.080 C	3.761±0.207 C	39.610±0.543 C

H_2O_2 (0.5%) + PO nano extract (5 mg/kg)	1.724±0.157 B	5.314±0.216 B	43.913±1.233 B
LSD	0.301	0.819	3.534

Data are expressed as mean± standard error, values with the same column with different letters are significantly different at P <0.05.

Oxidative Stress Parameters (TAC and MDA).

The results of the study, shown in (Table 3), revealed that the first treatment (T1) showed a statistically significant increase in malondialdehyde (MDA) concentration and a statistically significant decrease in total antioxidant capacity (TAC) compared to the control group and the remaining treatments, whereas the treatment groups (T2, T3, T4), which were subjected to stress, showed a significant improvement (P<0.05) characterized by a decrease in MDA and an increase in TAC compared to the T1 group; however, their levels remained dissimilar to those of the control group. When comparing the treatment groups, the third treatment (T3) showed a statistically significant advantage (P<0.05) in both indicators, recording the lowest MDA level and the highest TAC level compared to both T2 and T4, and was closest to the control group. Treatment 2 (T2) also showed statistically significant moderate efficacy (P<0.05) compared to Treatment 4 (T4), which recorded the lowest therapeutic efficacy among the groups. Statistical analysis revealed a significant increase in malondialdehyde and a significant decrease in total antioxidant capacity in treated group (T1) compared to the control and the remaining groups, reflecting the occurrence of oxidative stress. This is attributed to the effect of hydrogen peroxide in increasing the production of reactive oxygen species, leading to an imbalance in the redox state and the occurrence of structural and functional damage to cell membranes and the denaturation of cellular components, in addition to tissue damage and necrosis and the inhibition of antioxidant

defense systems, which is consistent with [49-51]. Elevated levels of malondialdehyde (MDA) indicate increased lipid peroxidation resulting from an imbalance in favor of free radicals, which leads to the attack of unsaturated fatty acids and the formation of lipid hydroperoxides, Malondialdehyde (MDA) is one of its highly toxic end products, capable of causing damage to DNA, proteins, and lipids [52, 53]. Exposure to hydrogen peroxide also leads to the generation of highly reactive hydroxyl radicals, which accelerate oxidation and elevate MDA levels in serum and tissues as an indicator of the severity of oxidative stress [54]. The decrease in total antioxidant capacity is attributed to the continuous depletion of antioxidant defense systems due to an increase in reactive oxygen species, as TAC represents the total sum of enzymatic and non-enzymatic antioxidants. A study by [55] supported this by demonstrating elevated MDA and reduced TAC in the stress group compared to the control group in rats exposed to hydrogen peroxide. Other studies have also indicated that this exposure leads to the depletion of antioxidants and a decrease in their efficacy [56], in addition to reducing the gene expression of antioxidant enzymes such as GSH, SOD, and CAT [57]. In contrast, the groups treated with the alcoholic extract (conventional and nano) of *Portulaca oleracea* an increase in total antioxidant capacity and a decrease in malondialdehyde, reflecting a preventive and therapeutic role associated with the enhancement of antioxidant enzyme activity, such as catalase and glutathione peroxidase. This is attributed to the plant's content of bioactive compounds such as flavonoids, phenols, alkaloids, and fatty acids (omega-3) [58], which contribute to free radical scavenging and the protection of cell membranes. Flavonoids are also among the most important antioxidants [59], as quercetin (50 mg/kg) improves the antioxidant status in plasma [60]. Studies indicate that purslane extract reduces malondialdehyde (MDA) and enhances antioxidant activity due to its content of active vitamins and minerals, in addition to its ability to neutralize free radicals through hydrogen donation [61] and by regulating Na^+/K^+ ATPase activity [62] . Hepatotoxicity model studies have also shown that P. oleracea significantly reduces oxidative damage when administered with paracetamol [63]. With regard to the nano-extract, (T3)

showed a clear advantage in increasing total antioxidant capacity and reducing malondialdehyde levels compared to the standard alcoholic extract (T2), This is attributed to the reduction in particle size and the accompanying greater release of phenolic compounds, as well as an increase in their bioactivity and ability to scavenge free radicals [64]. Nanoparticles are also characterized by high bioavailability and rapid absorption, along with a greater ability to penetrate cell membranes and reach the blood and tissues [65, 66]. Studies have shown that reducing particle size may enhance antioxidant activity several-fold compared to the parent compound [67], which explains the superiority of the nanodose (10 mg/kg) in the current study. It was also found that the higher dose was more effective than the lower one, which is attributed to the dose-dependent response principle and the achievement of a better balance between TAC and MDA through broader coverage of oxidative stress sites caused by hydrogen peroxide [68].

Table (2): Shows the effect of conventional and nano-alcoholic extracts of the *P. oleracea* on Oxidative Stress Parameters (TAC and MDA) in white rats exposed to hydrogen peroxide H_2O_2

Group / Parameters	**MDA (µMol/ L) Mean ± SE**	**TAC (µMol/ L) Mean ± SE**
Control	2.536±0.261 E	4.966±0.166 A
H_2O_2 (0.5%)	6.211±0.171 A	1.476±0.290 E
H_2O_2 (0.5%) + PO Ethanolic extract (100 mg/kg)	3.897±0.094 C	3.667±0.162 C

H_2O_2 (0.5%) + PO nano extract (10 mg/kg)	3.249±0.036 D	4.307±0.039 B
H_2O_2 (0.5%) + PO nano extract (5 mg/kg)	4.808±0.304 B	3.062±0.117 D
LSD	0.630	0.552

Data are expressed as mean± standard error, values with the same column with different letters are significantly different at P <0.05.

Conclusion

The study results showed that hydrogen peroxide-induced oxidative stress led to significant disturbances in liver and kidney function, manifested by elevated biochemical markers and increased indicators of oxidative stress, along with a decrease in total antioxidant capacity. In contrast, treatments with the alcoholic and nano extracts of Portulaca oleracea significantly improved these changes, indicating that they possess effective protective effects. The nano-extract at a dose of 10 mg/kg demonstrated higher therapeutic efficacy compared to the other treatments, suggesting that the nano-formulation is superior in enhancing biological activity and reducing damage associated with oxidative stress.

References

1. Pham-Huy, L.A., H. He, and C. Pham-Huy, *Free radicals, antioxidants in disease and health.* Int J Biomed Sci, 2008. **4**(2): p. 89-96.
2. Reddy, V.P., *Oxidative Stress in Health and Disease.* Biomedicines, 2023. **11**(11).
3. Muscolo, A., et al., *Oxidative Stress: The Role of Antioxidant Phytochemicals in the Prevention and Treatment of Diseases.* International Journal of Molecular Sciences, 2024. **25**(6): p. 3264.

4. Sikder, M.M., et al., *Reactive Oxygen Species: Role in Pathophysiology, and Mechanism of Endogenous and Dietary Antioxidants during Oxidative Stress.* Chonnam Med J, 2025. **61**(1): p. 32-45.
5. Cichoż-Lach, H. and A. Michalak, *Oxidative stress as a crucial factor in liver diseases.* World J Gastroenterol, 2014. **20**(25): p. 8082-91.
6. Balali, A., et al., *The effect of selenium supplementation on oxidative stress, clinical symptoms and mental health status in patients with migraine: a study protocol for a double-blinded randomized clinical trial.* Trials, 2024. **25**(1): p. 209.
7. Wong, C.P., L.M. Beaver, and E. Ho, *Protective role of dietary zinc on DNA damage, oxidative stress, and metal toxicity.* Front Mol Biosci, 2025. **12**: p. 1618318.
8. Li, J., et al., *Recent progress of oxidative stress associated biomarker detection.* Chemical Communications, 2023. **59**.
9. Zafar, F., et al., *A comprehensive review on medicinal plants possessing antioxidant potential.* Clinical and Experimental Pharmacology and Physiology, 2023. **50**(3): p. 205-217.
10. Lee, S.E., et al., *Screening of medicinal plant extracts for antioxidant activity.* Life sciences, 2003. **73**(2): p. 167-179.
11. Kumar, A., et al., *A review on bioactive phytochemicals and ethnopharmacological potential of purslane (Portulaca oleracea L.).* Heliyon, 2022. **8**(1).
12. Rahimi, V.B., et al., *A pharmacological review on Portulaca oleracea L.: focusing on anti-inflammatory, anti-oxidant, immuno-modulatory and antitumor activities.* Journal of pharmacopuncture, 2019. **22**(1): p. 7.
13. Iranshahy, M., et al., *A review of traditional uses, phytochemistry and pharmacology of Portulaca oleracea L.* Journal of ethnopharmacology, 2017. **205**: p. 158-172.
14. Venkateshwari, V., et al., *Leaf lipidome and transcriptome profiling of Portulaca oleracea: characterization of lysophosphatidylcholine acyltransferase.* Planta, 2018. **248**(2): p. 347-367.

15. Ghorani, V., et al., *Phytochemical Characteristics and Anti-Inflammatory, Immunoregulatory, and Antioxidant Effects of Portulaca oleracea L.: A Comprehensive Review.* Evid Based Complement Alternat Med, 2023. **2023**: p. 2075444.
16. Yadav, P. and A. Patil, *Review on Portulacaceae (purslane).* International Journal of Creative Research Thoughts, 2023. **11**: p. 1-5.
17. khaled Mohamed, W. and R.K. Shaban, *Role of Portulaca Oleracea Extract to Mitigate Histological Alterations in the Liver and Kidneys Induced by the Depakine Drug in Male Rats.* Migration Letters, 2023. **20**(S10): p. 66-76.
18. Yousef, S.M., *The Protective Effects of Portulaca oleracea L. Leaves Extract Against Lead Toxicity in Experimental Rats.* 2024. **10**(4.2): p. 553-569.
19. Budiman, A., et al., *Advancing the Physicochemical Properties and Therapeutic Potential of Plant Extracts Through Amorphous Solid Dispersion Systems.* Polymers (Basel), 2024. **16**(24).
20. Scholz, P. and C. M Keck, *Nanocrystals: from raw material to the final formulated oral dosage form-a review.* Current pharmaceutical design, 2015. **21**(29): p. 4217-4228.
21. Ebokaiwe, A.P., et al., *Loranthus micranthus nanoparticles abates streptozotocin-instigated testicular dysfunction in Wistar rats: Involvement of glucose metabolism enzymes, oxido-inflammatory stress, steroidogenic enzymes/protein and Nrf2 pathway.* Andrologia, 2020. **52**(10): p. e13749.
22. Hajzadeh, m.-a.-r., et al., *The effect of Salvia officinalis leaf extract on blood glucose in streptozotocin-diabetic rats.* Pharmacologyonline, 2010. **1**.
23. Li, Y., et al., *Preparation and characterization of paclitaxel nanosuspension using novel emulsification method by combining high speed homogenizer and high pressure homogenization.* Int J Pharm, 2015. **490**(1-2): p. 324-33.

24. Ahmed-Farid, O.A., H.A. Rizk, and A.M. Shehata, *Hydrogen peroxide modulates redox status, energy metabolism, and gene expression in a dose-and time-dependent manner in rat liver.* Journal of Biochemical and Molecular Toxicology, 2018. **32**(10): p. e22199.
25. Babarinde, G.O., A.A. Ebun, and P.I. Adegbola, *Hepatotoxicity and biochemical evaluation of a novel breakfast food produced from the blend of fonio (Digitaria iburua Stapf) and pigeon pea (Cajanus cajan (L.) Millspaugh) in albino rats.* Bulletin of the National Research Centre, 2020. **44**(1): p. 157.
26. Mahdi, S.S. and E.R. Al-Kennany, *Programmed Cell Death Induced by Hydrogen Peroxide.* Iraq Medical Journal, 2024. **8**(3).
27. Dkhil, M.A., et al., *Antioxidant effect of purslane (Portulaca oleracea) and its mechanism of action.* Journal of Medicinal Plants Research, 2011. **5**(9): p. 1589-1593.
28. Eidi, A., et al., *Hepatoprotective effects of Portulaca oleracea extract against CCl4-induced damage in rats.* Pharmaceutical biology, 2015. **53**(7): p. 1042-1051.
29. Taha, H. and A. Osman, *Assessment of antioxidant capacity of ethanolic extract of Portulaca oleracea leaves in vitro and in vivo.* Journal of Medicinal Plants Research, 2015. **9**(10): p. 335-342.
30. H Jr, M.A. and S.M. AG, *In vivo hepato-protective properties of purslane extracts on paracetamol-induced liver damage.* Malaysian Journal of Nutrition, 2010. **16**(1): p. 161-170.
31. Jawad, M.M., M.H. Homady, and A.N. Aldujaili, *Protective effect of phenolic extract of Urtica dioica leaves against carbon tetra-chloride induced hepatotoxicity in male rats.* Research Journal of Pharmacy and Technology, 2017. **10**(8): p. 2619-2627.
32. Kakino, S., et al., *Pivotal role of TNF-α in the development and progression of nonalcoholic fatty liver disease in a murine model.* Hormone and metabolic research, 2018. **50**(01): p. 80-87.

33. Li, S., et al., *Insights into the role and interdependence of oxidative stress and inflammation in liver diseases.* Oxidative medicine and cellular longevity, 2016. **2016**(1): p. 4234061.
34. Elsharkawy, A., F. Oakley, and D. Mann, *The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis.* Apoptosis, 2005. **10**(5): p. 927-939.
35. Tarantino, G., et al., *Could inflammatory markers help diagnose nonalcoholic steatohepatitis?* European journal of gastroenterology & hepatology, 2009. **21**(5): p. 504-511.
36. Abdel-Rahman, M.A., et al., *Exploring the antimicrobial, antioxidant, and antiviral potential of eco-friendly synthesized silver nanoparticles using leaf aqueous extract of Portulaca oleracea L.* Pharmaceuticals, 2024. **17**(3): p. 317.
37. Qing, J., et al., *GPX4 expression changes in proximal tubule cells highlight the role of ferroptosis in IgAN.* Scientific Reports, 2025. **15**(1): p. 3886.
38. Khames, A., et al., *Sodium thiosulphate shows promising anti-inflammatory role against doxorubicin-induced renal injury depending on tlr4 pathway inhibition.* Plant Arch, 2020. **20**(2): p. 2948-58.
39. Ibrahem, M., *Effect of aqueous extract Lawsonia inermis leaves on Urea, Creatinine and Histological of kidneys in white male Rats exposed to oxidative stress of H2O2.* Tikrit Journal of Pure Science, 2017. **22**(1): p. 24-30.
40. Sara, A., et al., *Leaves of lavender protect adult mice from hydrogen peroxide-induced injury: evidence fromin vitro and in vivo tests.* Journal of Oleo Science, 2020. **69**(9): p. 1107-1115.
41. Akl, M.G., et al., *Perturbed adipose tissue hydrogen peroxide metabolism in centrally obese men: association with insulin resistance.* PLoS One, 2017. **12**(5): p. e0177268.
42. van der Pol, K.H., et al., *Effects of allopurinol and febuxostat on uric acid transport and transporter expression in human umbilical vein endothelial cells.* Plos one, 2024. **19**(6): p. e0305906.

43. Askaripour, M., et al., *Effect of purslane (Portulaca oleracea) on renal ischemia/reperfusion injury in rat.* Int J Pharm Pharm Sci, 2015. **7**(1): p. 467-471.
44. Nkhumeleni, Z., W.N. Phoswa, and K. Mokgalaboni, *Purslane ameliorates inflammation and oxidative stress in diabetes mellitus: A systematic review.* International journal of molecular sciences, 2024. **25**(22): p. 12276.
45. Karimi, G., et al., *Protective effect of aqueous and ethanolic extracts of Portulaca oleracea against cisplatin induced nephrotoxicity.* Iranian Journal of Basic Medical Sciences, 2010. **13**(2): p. 31.
46. Seif, M.M., et al., *Hepato-renal protective effects of Egyptian purslane extract against experimental cadmium toxicity in rats with special emphasis on the functional and histopathological changes.* Toxicology reports, 2019. **6**: p. 625-631.
47. Alfarisi, H., et al., *Nanoextract of Acalypha hispida leaves increases antioxidant defense and suppresses microstructure damage in liver and kidney of diabetic rats.* Journal of Applied Pharmaceutical Science, 2022. **12**(10): p. 099-108.
48. Ji, Y., et al., *Mechanisms underlying the impact of interleukin family on acute kidney injury: pathogenesis, progression, and therapy.* Research, 2025. **8**: p. 0738.
49. Khudhur, A.F., R.H. Hamad, and A.A.N. Abdullah, *Effects of bee glue on oxidant-antioxidant balance and renal funication in the male rat exposed to oxidative stress induced by hydrogen peroxide.* Central Asian Journal of Medical and Natural Science, 2024. **5**(4): p. 150-159.
50. Kilicarslan You, D., et al., *Evaluation of the protective role of vitamin E against ROS-driven lipid oxidation in model cell membranes.* Antioxidants, 2024. **13**(9): p. 1135.
51. Montserrat-Mesquida, M., et al., *Effects of chronic hydrogen peroxide exposure on mitochondrial oxidative stress genes, ROS production and lipid peroxidation in HL60 cells.* Mitochondrion, 2024. **76**: p. 101869.

52. Allowitz, K., et al., *Oxidative stress-mediated lipid peroxidation-derived lipid aldehydes in the pathophysiology of neurodegenerative diseases.* Current Neuropharmacology, 2024.
53. Gęgotek, A. and E. Skrzydlewska, *Biological effect of protein modifications by lipid peroxidation products.* Chemistry and physics of lipids, 2019. **221**: p. 46-52.
54. Ayed, H.S., A.S. Khalaf, and T.M. Taki Mohammed. *Effect of Aqueous Extract of Truffle on Some Antioxidants in Rats Exposed to Oxidative Stress induced by Hydrogen peroxide*. in *IOP Conference Series: Earth and Environmental Science*. 2021. IOP Publishing.
55. Al-Rubaei, Z., T.U. Mohammad, and L.K. Ali, *Effects of local curcumin on oxidative stress and total antioxidant capacity in vivo study.* Pak J Biol Sci, 2014. **17**(12): p. 1237-1241.
56. Adwas, A.A., et al., *Oxidative stress and antioxidant mechanisms in human body.* J. Appl. Biotechnol. Bioeng, 2019. **6**(1): p. 43-47.
57. Al-Okialy, B.N. and M.S. Alwan, *Role of Alpha Lipoic Acid in Oxidant/Antioxidant Status and Gene Expression of Glutathione Reductase in Hydrogen Peroxide Exposed Rats:(Part-2).* The Iraqi Journal of Veterinary Medicine, 2018. **42**(2): p. 50-57.
58. Liu, L., et al., *Fatty acids and β-carotene in Australian purslane (Portulaca oleracea) varieties.* Journal of chromatography A, 2000. **893**(1): p. 207-213.
59. Sakai, N., et al., *Portuloside A, a monoterpene glucoside, from Portulaca oleracea.* Phytochemistry, 1996. **42**(6): p. 1625-1628.
60. Justino, G.C., et al., *Plasma quercetin metabolites: structure–antioxidant activity relationships.* Archives of Biochemistry and Biophysics, 2004. **432**(1): p. 109-121.
61. Uddin, M.K., et al., *Evaluation of antioxidant properties and mineral composition of purslane (Portulaca oleracea L.) at different growth stages.* International journal of molecular sciences, 2012. **13**(8): p. 10257-10267.

62. Changizi-Ashtiyani, S., et al., *The effects of Portulaca oleracea alcoholic extract on induced hypercholesteroleomia in rats.* Zahedan J Res Med Sci, 2013. **15**(6): p. 34-39.

63. Ahmida, M., *Evaluation of in vivo antioxidant and hepatoprotective activity of Portulaca oleracea L. against paracetamol-induced liver toxicity in male rats. Am J Pharmacol Toxicol.* American journal of pharmacology and toxicology, 2010. **5**: p. 167.

64. Vaso, M., et al., *Nanosizing Nigella: A cool alternative to liberate biological activity.* Current Nutraceuticals, 2021. **2**(1): p. 37-46.

65. Lau, Y.T., et al., *Product design: a nanomized nutraceutical with enhanced bioactivity and bioavailability.* Industrial & engineering chemistry research, 2012. **51**(21): p. 7320-7326.

66. Saraswaty, V., et al., *Transformation of Melinjo seed micropowders into nanopowders enhances extractability of phenolic compounds and tyrosinase inhibitory activity.* Sains Malaysiana, 2019. **48**(5): p. 983-990.

67. Abraham, A.M., et al., *Improved antioxidant capacity of black tea waste utilizing PlantCrystals.* Molecules, 2021. **26**(3): p. 592.

68. Di Gioia, S., M.N. Hossain, and M. Conese, *Biological properties and therapeutic effects of plant-derived nanovesicles.* Open Medicine, 2020. **15**(1): p. 1096-1122.