Hindawi
Journal of Food Quality
Volume 2022, Article ID 7381378, 9 pages
https://doi.org/10.1155/2022/7381378

Research Article

Effect of a Combination of *Rosa canina* Fruits and Apple Cider Vinegar against Hydrogen Peroxide-Induced Toxicity in Experimental Animal Models

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Received 24 April 2021; Revised 16 December 2021; Accepted 23 December 2021; Published 5 January 2022

Academic Editor: Constantin Apetrei

Oxidative stress is the trigger of several diseases. It is an imbalance between the production of free radicals and antioxidants. This study aims to evaluate the antioxidant capacity and the protective property of *Rosa canina* fruits and apple cider vinegar combined or not against hydrogen peroxide (H_2O_2)-induced toxicity in Wistar rats. The experiment included five groups: group 1 received distilled water (10 mL/kg b.wt), group 2 received H_2O_2 10% (10 mL/kg b.wt), group 3 received H_2O_2 10% (10 mL/kg b.wt) and apple vinegar (2 mL/kg b.wt); group 4 received H_2O_2 10% (10 mL/kg b.wt) and apple vinegar supplemented with *Rosa canina* fruits extract (300 mg/kg b.wt); group 5 received H_2O_2 10% (10 mL/kg b.wt) and extract of *Rosa canina* fruits (300 mg/kg b.wt). The doses were given once daily via a gavage. The antioxidant capacity of apple vinegar and *Rosa canina* extract was analyzed, and AST, ALT, PAL, urea, and creatinine were determined on day 22 of the experiment. In addition, the kidney and the liver tissues were analyzed. The results showed that H_2O_2 caused a significant elevation of blood urea, blood creatinine, and transaminases. The histopathology examination revealed that H_2O_2 caused congestion, hemorrhage, and Bowman's space enlarged. On the other hand, the results clearly showed that apple vinegar and *Rosa canina* fruits counterbalance the biochemical and histological changes induced by H_2O_2. In conclusion, the two natural products studied in this work are effective against the harmful effect of oxidative stress, which explains their use in traditional medicine.

1. Introduction

Oxidative stress induces an increase in the production of free radicals considered as drivers of different pathogenesis and organ damages. It could disturb the antioxidant system and lead to direct cellular injury stress in the liver and kidney [1]. Numerous factors can induce this imbalance such as acidosis, nitric oxide, transition metals, LDL oxidation [2], and toxic agents including hydrogen peroxide (H_2O_2) [3]. Furthermore, H_2O_2 can permeate across the membrane as a destructive agent and generate hydroxyl radicals [3]. Other producers of reactive oxygen species (ROS) can induce necrosis and apoptosis [4]. Numerous reports have well established that oxidative stress is the major cause of various pathologies.

Nowadays, the use of natural products turns out to be a good alternative to chemicals, the side effects of which can be serious [5, 6]. Fruits and their byproducts have tremendous beneficial properties. Among a long list of fruits wealthy on bioactive compounds with enormous utilizations in folk medicine, we found *Rosa canina* fruits. Several studies evoked that *Rosa Canina* fruits were used in alternative medicine to treat different diseases including diabetes mellitus, colds, fever, scurvy, and kidney diseases [7]. The wealth of hips on phytochemicals with high antioxidant ability improves cell longevity and obstructs skin aging as previously proved by Phetcharat et al [8]. It has been documented that hips extract can be useful in the prevention of histological damages, oxidative stress, and functional

disturbances induced by reperfusion injury [9]. Phytochemical analysis revealed that the *Rosa canina* fruits contain large amounts of phenolic and flavonoids compounds and vitamin C which are responsible for their antioxidant and anti-inflammatory properties [10, 11]. The anti-inflammatory effect of rosehip was early investigated and proved its ability to reduce chemotaxis of peripheral blood polymorphonuclear leukocytes and reduced protein C level [12, 13]. On the other hand, apple cider vinegar as a byproduct of apples is considered a good source of bioactive substances such as phenols, flavonoids, and organic acids. Apple cider vinegar is used as a healthy and safe drink for diverse purposes. In traditional folk, medicine apple vinegar is applied for the treatment of several diseases such as diabetes mellitus, hyperlipidemia, Alzheimer's disease, common cold, and cough [14–17]. Fruits vinegar was the most kind recommended functional product to treat laryngitis, fever, swelling, stomachache [17]. In our previous studies, we noticed that apple cider vinegar can ameliorate the metabolic disorder induced by a hypercaloric diet, and it possesses antibacterial and antifungal activities 18–20]. Recent researchers have explored that vinegar exhibited antioxidative properties by decreasing ROS accumulation and upregulating the expression of antioxidative enzymes [21–23]. It has been shown that vinegar administration exhibits a potent hepatoprotective effect against oxidative damages induced by hydrogen peroxide [24]. Phytochemical analysis of apple cider vinegar revealed the presence of several bioactive compounds with high antioxidant potential such as syringic acid, gallic acid, caffeic acid, p-coumaric acid, *p*-hydroxybenzoic acid, and catechin, which could explain its beneficial properties [25, 26]. The physicochemical properties of apple cider vinegar make it an ecologic solvent to extract bioactive compounds [27]. Recently, the combination of numerous natural products proved their efficiency to prevent and treat several pathologies such as diabetes, cardiovascular ailments, and oxidative stress [28–31]. The incorporation of natural products in daily diet constitutes the first line to prevent the installation of different diseases as dietary therapies [28].

In the present work, two natural products (*Rosa canina* fruits and apple cider vinegar) were evaluated on laboratory rats by studying their capacities in renal and hepatic protection against the harmful effects of oxidative stress induced by the oral administration of H_2O_2.

2. Materials and Methods

2.1. Preparation of Herbal Extract. Apple cider vinegar was purchased in the Midelt area, while the *Rosa canina* fruits were collected from Ait Ayach Midelt (32° 41′ 27″ N, 4° 55′ 45″ O) in Morocco. The extraction process was performed with ethanol/water (70 : 30) by maceration. Before extraction, the hips were dried and ground to coarse powder with small particles. The extraction yield was calculated as follows: yield (%) = (W1/W2) × 100, where W1 is the weight of the extract residue obtained after solvent removal (g) and W2 is the weight of *Rosa canina* fruit taken (g). The obtained extract was filtrated and concentrated with a rotary evaporator. Distilled water was added to obtain the concentration of (300 mg/kg b.wt).

2.2. Phytochemical and Antioxidant Analysis. Total phenolic content in apple cider vinegar and *Rosa canina* fruits extract was quantified using the colorimetric method by Folin-Ciocalteu as described by Bakour et al [32]. The values were expressed as mg of gallic acid equivalent per 100 mL for vinegar and mg of gallic acid per Gram of *Rosa canina* extract. Flavonoids were determined using the method described previously by Kong et al [33]. The results were expressed mg of quercetin per 100 mL of apple vinegar and mg of quercetin equivalent per g of *Rosa canina* extract. While the total antioxidant capacity of apple vinegar and *Rosa canina* fruits was evaluated using the phosphomolybdenum method as previously described by Zengin et al [34], the antioxidant capacity of the two products tested was expressed as equivalent ascorbic acid (mg AAE/100 mL vinegar and mg AAE/g extract).

2.3. Experimental Method. Twenty male rats (103 ± 9 g) obtained from animal house breeding center, Faculty of Sciences, Dhar Al-Mahraz Fez, were housed under normal environmental conditions (25 ± 1 °C), 55 ± 5% humidity on a 12-hour light-dark cycle). The experiment was designed in accordance with "Guide for the Care and Use of Laboratory Animals" prepared and published by the National Academy of Sciences and the National Institutes of Health, respectively. The ethical approval of the present work is SNAMOPEQ, USMBA, 2017-03. Animals were randomly allocated into five groups of four rats each: group 1 received deionized water and was used as the control (10 mL/kg b.wt), group 2 received hydrogen peroxide (10 mL/kg b.wt 10% H_2O_2), group 3 received hydrogen peroxide (10 mL/kg b.wt 10% H_2O_2) and apple vinegar (10 mL/kg b.wt), group 4 received hydrogen peroxide (10 mL/kg b.wt 10% H_2O_2) and apple vinegar combined with *Rosa canina* fruits extract (300 mg/kg b.wt), and group 5 received hydrogen peroxide (10 mL/kg b.wt 10% H_2O_2) and *Rosa canina* fruits extract (300 mg/kg b.wt). After 22 days of treatment, the rats were sacrificed by decapitation after light anesthesia with ethyl-urethane, and the liver and kidney were removed and immediately putted in formalin solution (10%). The blood was collected to analyze AST, ALT, PAL, urea, and creatinine.

2.4. Biochemical Analysis. After 22 days of treatment, the blood was collected to analyze liver and kidney markers (AST, ALT, PAL, urea, and creatinine). Aspartate aminotransferase (AST) was determined using kit number 7D81-20, aspartate/NADH method; alanine aminotransferase (ALT) was determined using kit number 7D56-20, alanine/NADH method, and alkaline phosphatase was determined using kit number 7D55-20 and 7D55-20, colorimetric method, while urea was determined using kit number 7D76-20, urease/NADH method and creatinine was determined using kit number 7D-64-20, picric acid/NaOH method (Architect c8000i biochemistry analyzer [31]).

2.5. Histological Analysis. The liver and kidney of rats were fixed in the formalin solution (10%) for 24 h and then dehydrated using a series of increasing concentrations of ethanol, and the organs were clarified in toluene and finally embedded in paraffin. The obtained paraffin blocks were cut using a microtome to obtain fine sections (5–6 mm). Hematoxylin and eosin (H&E) were used for staining the slides obtained for observation under an optical microscope [32].

2.6. Statistical Analysis. All data are presented as mean ± SD (standard deviation). Statistical comparisons between the groups were performed with one-way analysis of variance (ANOVA) followed by Tukey test using GraphPad Prism® software (version 5.0; GraphPad Software, Inc, San Diego, USA). Significance was accepted at $p < 0.05$.

3. Results

3.1. Phytochemical Analysis and Antioxidants. Table 1 summarizes the obtained results of phytochemical analysis and antioxidants. The quantification of bioactive molecules showed that the apple cider vinegar and *Rosa canina* fruits extract contain total phenolic content (106.91 ± 1.64 mg GAE/100 mL-30.2 ± 0.2 mg GAE/g dw), total flavonoid content (11.36 ± 0.06 mg QE/100 mL-12.23 ± 0.11 mgQE/g dw), and total antioxidant activity (9.17 ± 1.22 mg AAE/mL-4.04 ± 0.03 mg AAE/g dw), respectively.

3.2. Effect of Hydrogen Peroxide with and without Apple Cider Vinegar and Rosa canina on Hepatic Enzymes. An animal model was used to verify the potential protective activity of apple cider vinegar and ethanolic extract of *Rosa canina* fruits (the extraction yield = 38%) used alone or combined with hydrogen peroxide-induced hepatonephrotoxicity in vivo. Damage of the liver was assessed by the plasma AST, ALT, and PAL levels. Figures 1, 2, and 3 illustrate the obtained results.

Hydrogen peroxide caused a significant change of ALT levels as compared to the control group (Figure 1) whereas the AST and PAL levels were not significantly increased (Figure 2 and 3). On one side, AST and ALT levels were not significantly changed by the apple cider vinegar despite a trend to a decrease of enzyme levels mentioned above (Figures 1 and 2), and it had no impact on PAL levels (Figure 3). On the other side, the chronic treatment with *Rosa canina* fruit extract decreased not significantly the rise in levels of ALT, AST, PAL in H_2O_2-treated rats, and the values did not differ from that registered in the control group. Furthermore, the combination of both products tested in the present work does not significantly modify the ALT and PAL levels despite a trend to a diminution of AST levels.

3.3. Effect of Hydrogen Peroxide with and without Apple Cider Vinegar and Rosa canina on Kidney Function. Concerning kidney function, the results obtained are summarized in Figures 4 and 5. It is clearly shown that the hydrogen peroxide increased insignificantly serum creatinine and urea levels as compared to the normal control (Figures 4 and 5). However, simultaneous administration of apple cider vinegar and *Rosa canina* alone or combined did not affect the level of serum urea. Both of the products studied decreased insignificantly serum creatinine as compared to the H_2O_2 group but decreased significantly when apple cider vinegar and *Rosa canina* extract were simultaneously administrated.

3.4. Histological Study. Histopathological modifications are shown in Figures 6 and 7. Histopathological evaluations of the liver and kidney sections from the control group did not show any abnormal structure. However, liver and kidney of H_2O_2-treated rats showed a significant rise in the severity of organ damages with congestion, Bowman's space enlarged, congestion, and hemorrhage (Figures 6 and 7).

The treatment simultaneously with apple vinegar and *Rosa canina* or combined relieved damages induced by H_2O_2 in the liver and kidney with a good improvement in the group treated by *Rosa canina* alone.

4. Discussion

Hydrogen peroxide is considered a toxic agent that causes redox imbalance which contributes to the pathogenesis of organ injuries [35–37]. It has been confirmed that oxidative stress induced by hydrogen peroxide disrupts the normal function of cells and induces histopathological lesions of the liver [32]. Oxidative stress is a major reason for organ dysfunction and the pathogenesis of numerous ailments [38]. Liver and kidney injuries were manifested by the risen of liver enzymes, creatinine, and urea levels. In this experiment, we have attempted to examine the impact of a toxic agent (hydrogen peroxide) and the possible protective effect of apple cider vinegar and *Rosa canina* combined or not against the toxicity induced by hydrogen peroxide.

The administration of hydrogen peroxide 10% during 22 days induced an increase in the serum AST, ALT, and PAL levels. This elevation of hepatic enzymes levels may be the result of hydrogen peroxide-induced toxicity. Hydrogen peroxide penetrates across membranes and generates hydroxyl radicals which cause toxic effects on the liver [3, 39]. Hydrogen peroxide is considered as an inducer of oxidative stress-inducing inflammation, ROS production, and cells destruction which enhance enzymes leakage to plasma [32]. In this study, our finding provided that the administration of H_2O_2 increased hepatic enzymes which were consistent with the previous report in vivo conducted by Bakour et al. [32].

In contrast, the treatment with apple cider vinegar and *Rosa canina* extract resulted in an improvement in hepatocellular enzymes elevation due to the protective potential of both natural products. Natural substances used in the current work revealed their ability to stabilize the hepatocellular membrane and prevent hepatocytes from the toxicity induced by hydrogen peroxide that can drop down enzyme leakage to blood circulation.

Treatment with apple vinegar prevents animals from the variations of hepatic enzymes (AST and ALT) levels induced

Table 1: Antioxidant content of apple cider vinegar and *Rosa canina* extract.

Sample	Total phenolic content	Total flavonoid content	TAC
Apple cider vinegar	106.91 ± 1.64 mg GAE/100 mL	11.36 ± 0.06 mg QE/100 mL	9.17 ± 1.22 mg AAE/mL
Rosa canina extract	30.2 ± 0.2 mg GAE/g dw	12.23 ± 0.11 mg QE/g dw	4.04 ± 0.03 mg AAE/g dw

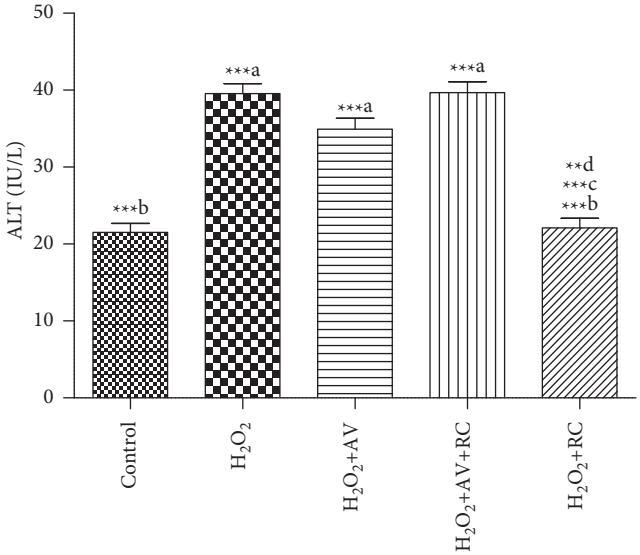


Figure 1: Effects of hydrogen peroxide administration and apple cider vinegar combined with *Rosa canina* extract or not on ALT. (a) Comparison between all groups and the control group, (b) comparison between all groups and the H_2O_2 group, (c) comparison between the H_2O_2 + AV + RC group and the two groups of H_2O_2 + AV and H_2O_2 + RC, (d) comparison of H_2O_2 + AV and H_2O_2 + RC. H_2O_2 = hydrogen peroxide; H_2O_2 + AV = hydrogen peroxide + apple vinegar; H_2O_2 + AV + RC = hydrogen peroxide + apple vinegar + *Rosa canina* extract; H_2O_2 + RC = hydrogen peroxide + *Rosa canina* extract. $^{**}P < 0.01$; $^{***}P < 0.001$.

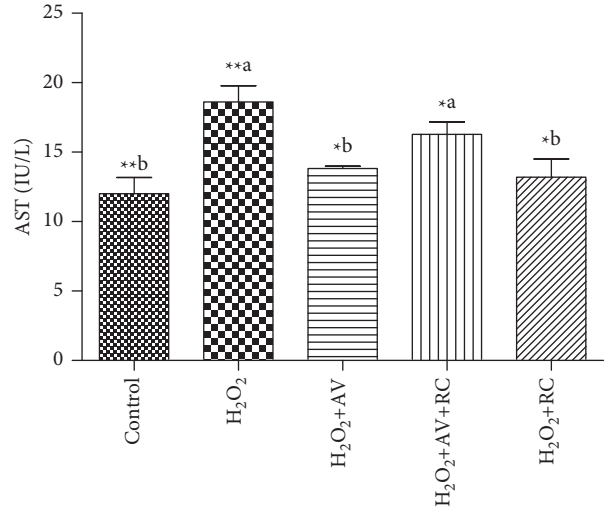


Figure 2: Effects of hydrogen peroxide administration and apple cider vinegar combined with *Rosa canina* extract or not on AST. (a) Comparison between all groups and the control group, (b) comparison between all groups and the H_2O_2 group. H_2O_2 = hydrogen peroxide; H_2O_2 + AV = hydrogen peroxide + apple vinegar; H_2O_2 + AV + RC = hydrogen peroxide + apple vinegar + *Rosa canina* extract; H_2O_2 + RC = hydrogen peroxide + *Rosa canina* extract. $^{*}P < 0.05$, $^{**}P < 0.01$.

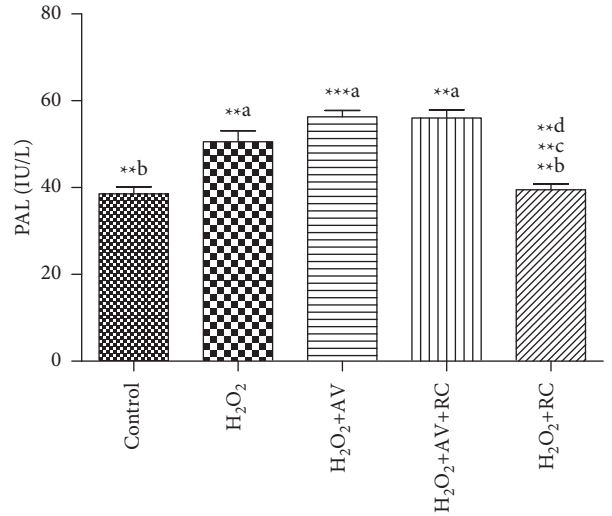


Figure 3: Effects of hydrogen peroxide administration and apple cider vinegar combined with *Rosa canina* extract or not on PAL. (a) Comparison between all groups and the control group, (b) comparison between all groups and the H_2O_2 group, (c) comparison between the H_2O_2 + AV + RC group and the two groups of H_2O_2 + AV and H_2O_2 + RC, (d) comparison of H_2O_2 + AV and H_2O_2 + RC. H_2O_2 = hydrogen peroxide; H_2O_2 + AV = hydrogen peroxide + apple vinegar; H_2O_2 + AV + RC = hydrogen peroxide + apple vinegar + *Rosa canina* extract; H_2O_2 + RC = hydrogen peroxide + *Rosa canina* extract. $^{**}P < 0.01$, $^{***}P < 0.001$.

by hydrogen peroxide, but we noticed that the apple vinegar alone had no significant effect on PAL levels. In addition, the administration of *Rosa canina* extract decreases but not significantly the modifications of hepatic enzymes levels induced by hydrogen peroxide compared to the negative control (group 2). On the other hand, the combination of apple vinegar and *Rosa canina* extract did not affect ALT and PAL levels despite a trend to a diminution of AST levels.

The hepatoprotective effect of apple vinegar and *Rosa canina* has been reported in previous studies [12, 23, 40, 41].

Apple cider vinegar attenuates the degenerative cell changes in the liver induced by several toxic agents including nicotine, mercuric chloride, and a high-fat diet [23, 41, 42]. As a typical fermented food and owing to its rich content in bioactive components, apple cider vinegar had strong detoxifying capacities and high free radical scavenging abilities. It has been proved that apple cider vinegar contains different bioactive compounds with strong antioxidant properties which can impede the oxidative stress induced by hydrogen peroxide [4, 32, 43]. The administration of apple cider vinegar alleviated the leakage of transaminases through the protection of the liver tissue against oxidative damages induced by H_2O_2.

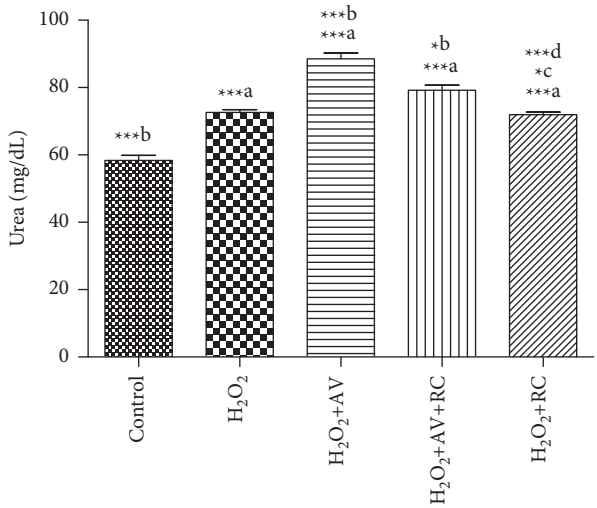


Figure 4: Effects of hydrogen peroxide administration and apple cider vinegar combined with *Rosa canina* extract or not on urea. (a) Comparison between all groups and the control group, (b) comparison between all groups and the H_2O_2 group, (c) comparison between the H_2O_2 + AV + RC group and the two groups of H_2O_2 + AV and H_2O_2 + RC, (d) comparison of H_2O_2 + AV and H_2O_2 + RC. H_2O_2 = hydrogen peroxide; H_2O_2 + AV = hydrogen peroxide + apple vinegar; H_2O_2 + AV + RC = hydrogen peroxide + apple vinegar + *Rosa canina* extract; H_2O_2 + RC = hydrogen peroxide + *Rosa canina* extract. $^{*}p < 0.05$, $^{***}P < 0.001$.

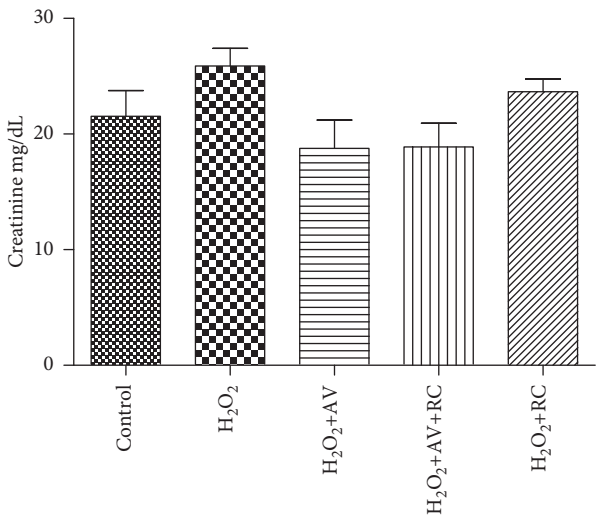


Figure 5: Effects of hydrogen peroxide administration and apple cider vinegar combined with *Rosa canina* extract or not on creatinine. H_2O_2 = hydrogen peroxide; H_2O_2 + AV = hydrogen peroxide + apple vinegar; H_2O_2 + AV + RC = hydrogen peroxide + apple vinegar + *Rosa canina* extract; H_2O_2 + RC = hydrogen peroxide + *Rosa canina* extract.

Similarly, the administration of hips extract showed a protective effect in renal disturbances against oxidative stress and histopathological damage induced by reperfusion injury [9]. Several studies reported that oxidative stress is the main contributor to the occurrence of organ problems, and antioxidants can protect the extent of lipid peroxidation by their ability to scavenge free radicals [23, 28, 32, 41]. The ability of *Rosa canina* to fight free radicals in vivo was confirmed by Fetni et al. [40]. Researchers evoked that the extract of RC exhibits a high protective effect against cardiac and hepatorenal toxicities [40]. In addition, *Rosa canina* stabilizes and maintains membrane cell integrity by correcting the sorting of membrane-associated proteins [44].

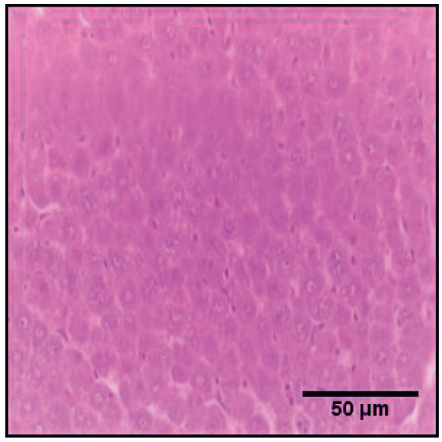
Chronic administration of peroxide hydrogen increases urea and creatinine levels as markers of kidney injury. The outcomes are in accordance with the study conducted by Bakour et al. [32]. Elevation of renal biomarkers is highly related to renal damages supports the role of oxidative stress in H_2O_2-induced nephrotoxicity.

The treatment with apple vinegar did not affect urea levels. The same results were observed following the administration of the apple cider vinegar and *Rosa canina* extract, alone or in combination. While apple vinegar and *Rosa canina* each reduced nonsignificantly creatinine levels, the combination of both products decreased significantly creatinine levels. On one side, results are consistent with those reported by Mahmoodi et al. [45]. On the other side, previous reports proved the ability of vinegar to reduce uric acid and creatinine levels [46]. In the same context, *Rosa canina* extracts proved their ability to reduce urea and creatinine levels [9]. The renoprotective effect of *Rosa canina* was proved against toxicity induced by vancomycin through reducing MDA levels in serum and kidney, thereby preventing the lipid peroxidation in organs. References [47, 48].

The analysis of the histological sections of the kidney and liver reveals that the toxic effect of hydrogen peroxide (H_2O_2) was manifested by tissue injuries. Bakour et al. proved the toxic effect of hydrogen peroxide through oxidative stress which increases lipid peroxidation and organs damage [32].

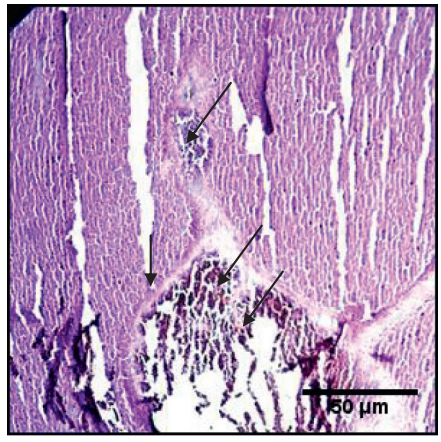
The administration of apple vinegar alone or combined with *Rosa canina* extract resulted in different actions that lead to normalizing organs histology. Beneficial properties of vinegar and *Rosa canina* in the liver and kidney are documented in the literature by controlling organs damage and decreasing hepatic enzymes levels [18, 48–53].

Moreover, the beneficial properties of both natural products could be due to their richness in bioactive components particularly syringic acid, gallic acid, caffeic acid, p-coumaric acid, p-hydroxybenzoic acid, and catechin for apple vinegar [25], and daidzein 7-O-glucoside, dalspinin-7-O-β-D-galactopyranoside, naringenin 5,7-di-O-galactoside, kaempferol 3-O-rutinoside, syringetin-3-glucoside, eriodictyol-7-O-glucoside, isorhamnetin-3-O-rutinoside, and so on, for *Rosa canina* [40]. The synergetic effect of natural products was previously investigated in our previous reports and proved its ability to prevent numerous ailments associated with oxidative stress [29–31].



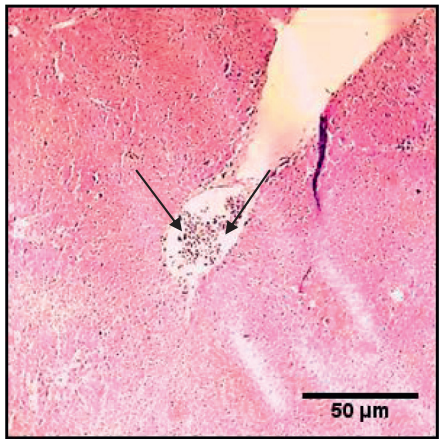
Liver tissue of control group

(a)



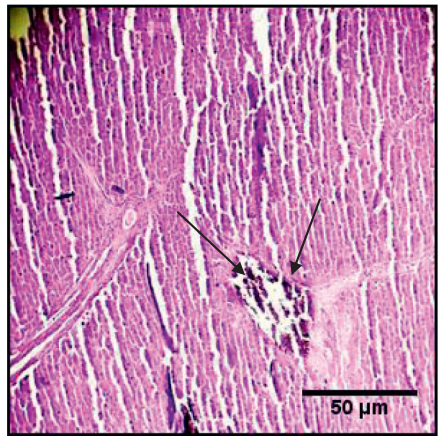
Liver tissue of H_2O_2 group

(b)



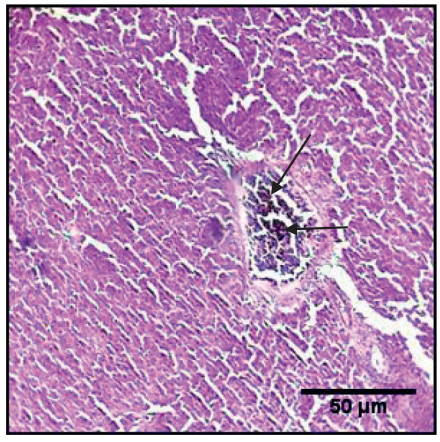
Liver tissue of H_2O_2+ *Rosa canica* group

(c)



Liver tissue of H_2O_2+vinegar group

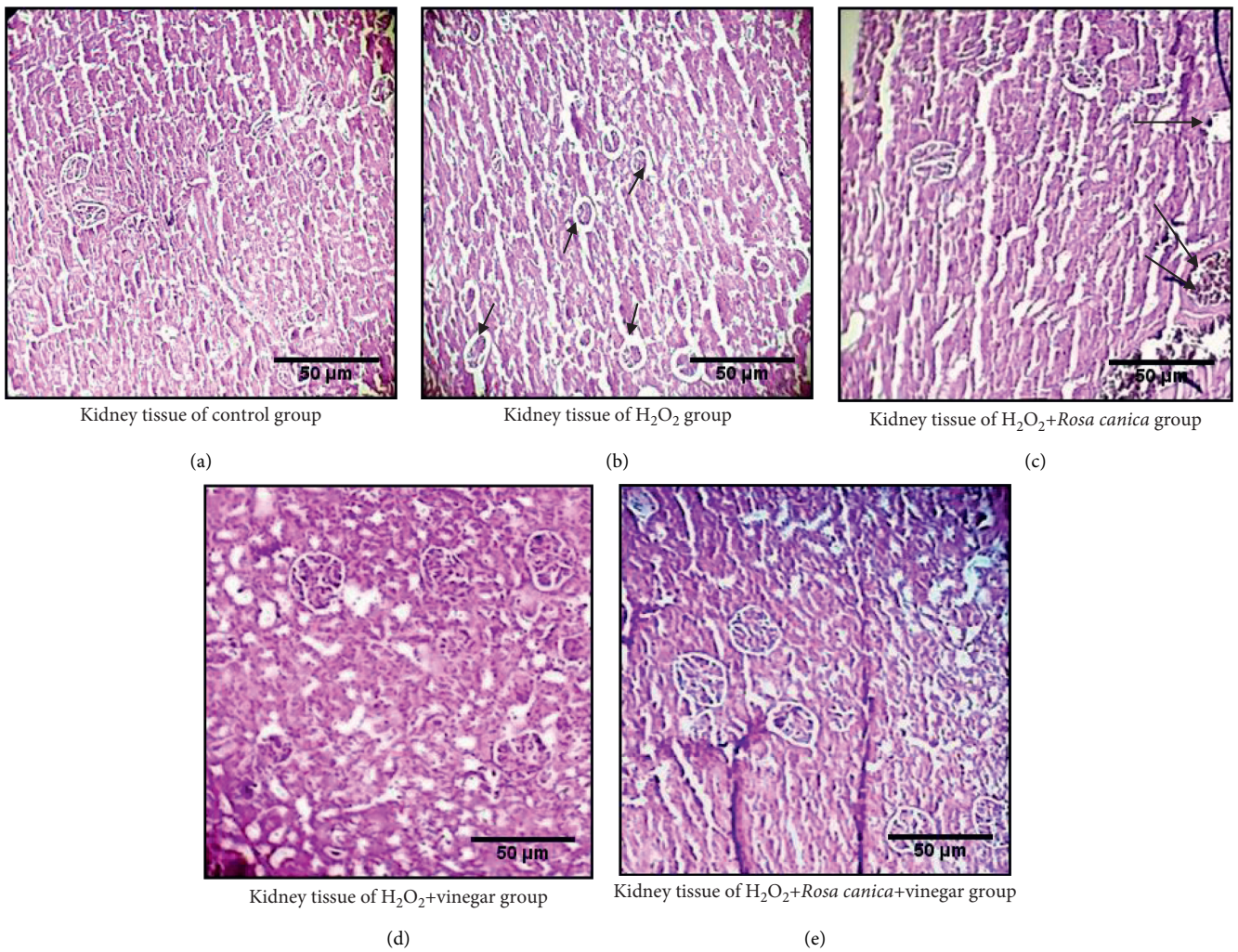
(d)



Liver tissue of H_2O_2+ *Rosa canica*+vinegar group

(e)

Figure 6: (a) Distilled water: normal tissue; (b) H_2O_2 only: arrows show congestion and hemorrhage; (c) H_2O_2 + *Rosa canina*: arrows show congestion; (d) H_2O_2 + vinegar: arrows show congestion; (e) H_2O_2 + *Rosa canina* + vinegar: arrows show congestion.



Kidney tissue of control group (a)

Kidney tissue of H_2O_2 group (b)

Kidney tissue of H_2O_2+*Rosa canica* group (c)

Kidney tissue of H_2O_2+vinegar group (d)

Kidney tissue of H_2O_2+*Rosa canica*+vinegar group (e)

Figure 7: (a) Distilled water: normal tissue; (b) H_2O_2 only: arrows show Bowman's space enlarged; (c) H_2O_2 + *Rosa canina*: arrows show congestion and hemorrhage; (d) H_2O_2 + vinegar: normal tissue; (e) H_2O_2 + *Rosa canina* + vinegar: normal tissue.

5. Conclusion

The outcomes of this study show that hydrogen peroxide leads to oxidative stress which causes histopathological changes in the liver and kidney and alters hepatic enzymes, creatinine, and urea levels. Apple cider vinegar and *Rosa canina* fruits are natural products that can be used to prevent and/or treat the complications of oxidative stress due to their richness in bioactive molecules which exhibit high antioxidant potential.

Abbreviations

AAE: Ascorbic acid equivalent
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
AV: Apple vinegar
DW: Distilled water
GAE: Gallic acid equivalent
H_2O_2: Hydrogen peroxide
LDL: Low-density lipoprotein
MDA: Malondialdehyde
PAL: Alkaline Phosphatase
QE: Quercetin equivalent
RC: *Rosa canina*
ROS: Reactive oxygen species
TAC: Total antioxidant capacity.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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