

Resveratrol ameliorating the liver functions against hydrogen peroxide exposed rats

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ABSTRACT

This study is designed to investigate the effect of resveratrol supplement on liver injury induced by hydrogen peroxide in adult rats over 45 days. Forty adult male rats were divided into four equal groups as follows: Controls were given distilled water, group G1: Received tap water containing 0.5% hydrogen peroxide, group G2: Received tap water containing 0.5% hydrogen peroxide and gavaged resveratrol 87mg/kg B.W. orally and group G3 gavaged resveratrol 87mg/kg B.W. orally. Blood samples were taken at pretreatment, after 45 days. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase activities, as well as total serum protein (TSP), albumin and globulin concentrations were measured. At day 45 rats were euthanized and liver samples were taken to estimate malondialdehyde (MDA) and superoxide dismutase (SOD) concentrations. The results showed a significant increase in ALT, AST, ALP and LDH activities, while a significant decrease in TSP, albumin, globulin concentrations accompanied with a significant increase in MDA and decrease in SOD in liver tissues in rats treated with 0.5% hydrogen peroxide. Whereas, resveratrol in group G2 caused an alleviation of the studied criteria related to hepatic dysfunctions through lowering the accumulative negative effects of hydrogen peroxide. Our results clarified that resveratrol, by multiple antioxidant mechanisms interplay, ameliorate liver injury induced by hydrogen peroxide in rats.

Key Words : Resveratrol, hydrogen peroxide, liver enzymes, MDA, SOD.

INTRODUCTION

Reactive oxygen species (ROS), is widely used in medicine and biology (1). These molecules are normal by-products of cellular metabolism and play an important role in normal cellular homeostasis, such as cell defense, hormone synthesis and signaling, activation of G protein-coupled receptors and ion channels and regulators of transcription factors and gene expression (2). The major intracellular sources of ROS and reactive nitrogen species (RNS) are mitochondria, endoplasmic reticulum, peroxisomes, lysosomes and some enzymes in the plasma membrane or cytoplasm, as well as, extracellular metals such as Cu, Fe etc...are considered as a source of their molecules (3). A lower concentration of ROS is essential for normal cellular signaling. The overproduction of ROS via oxidative phosphorylation, induces an oxidative stress (OS) which stimulates to promote damage of cell macromolecules such as proteins, lipids and DNA, eventually resulted in degeneration, necrosis and apoptotic cell death (4). Hydrogen peroxide (H₂O₂) is not a free radical by definition because it lacks free electrons. Hydrogen peroxide is produced in vivo by many reactions through a wide variety of enzymes including monooxygenase and oxidase. It induces damage to the cell membrane and decreases cell viability. It also causes oxidative damage to genomic DNA and mitochondrial (mtDNA) (5). Hydrogen peroxide is a powerful oxidizer kills bacteria, virus, and fungi and used to disinfect wounds externally. (6). Chronic liver disease is the most common prevalence worldwide and is strongly associated with oxidative stress (7). Certain drugs, infection, external exposures to pollutants, tissue injury, and so forth cause increase production of ROS (8). Furthermore, ROS play an important role in induced liver diseases, including ethanolic and non-

alcoholic fatty liver disease and cigarette smokers (9-11) and liver cancer (12). Lower oxygen tension reduces oxidative phosphorylation and participates in generation of formation of a range of other ROS and RNS (13).

Resveratrol found in some plants, fruits and derivatives such as red wine, it exhibits a wide range of beneficial properties, that may be due to its structure (14). It act as antioxidant activity via radical scavenging and metal ion chelating abilities (15) leading to minimizing or preventing lipid oxidation (LPO) through modulation of several cellular antioxidant pathways (16). Resveratrol has received growing attention as antioxidant, anti-platelet, anti-inflammatory, cytoprotective, neuroprotective, anti-hyperlipidemia, anti-carcinogenic, cardioprotective, immunomodulatory, vasorelaxant and antidiabetic effects (17-22). In current study we investigate the effect of resveratrol on ameliorating liver dysfunctions- induced by exposure to hydrogen peroxide in rats.

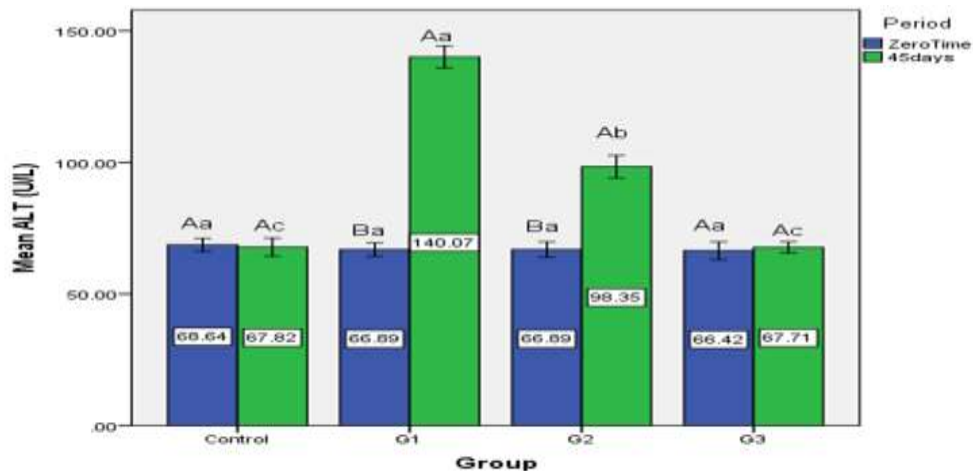
MATERIALS AND METHODS

Mature albino Wistar rats (aged 11-12 weeks and weighted 190 ± 200) were housed in well ventilated room inside plastic cages in the College of Veterinary Medicine-University of Baghdad. Animal were feed on a standard pellet diet and drinking water *ad libitum*, room temperature was $25 \pm 2^\circ\text{C}$ and 12 hrs. ight/dark cycle along during the experimental periods. Forty adult male rats were randomly divided into four equal groups (10 rats /group) and were handled for 45 days as follows: Control group (C): gavaged distilled water, the second group (G1) rats were taken tap water containing 0.5% of hydrogen peroxide (23), group G2 were received tap water containing 0.5% of hydrogen peroxide plus gavaged resveratrol at a dose 87 mg/kg. B.W. Orally. and third group (G3) were gavaged resveratrol at a dose 87mg/kg. B.W. orally. Blood samples were drawn by cardiac puncture technique (24) from anesthetized rats at day zero and 45 days of the experiment. Serum were collected by centrifugation for 15 minutes at 3000 rpm estimation of the following parameters using enzymatic kits (product of Agappe, India), including; Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) (25), alkaline phosphatase activity (ALP) (26), lactate dehydrogenase (LDH) (27) activities. Besides, total serum protein (TSP) (28), albumin and globulin concentrations (29) were calculated. After rats were euthanized, specimens from liver was taken, washed in saline in an ice bath and homogenized for determination of malondialdehyde (MDA) and superoxide dismutase (SOD) by using enzymatic ELISA kits (product of Cohesion bioscience, China). Statistical analysis was performed using SAS version 9.1(30). By using one and two-way of Variance Analysis (ANOVA) followed by the least significant differences (LSD) at $P < 0.05$ to assess significant differences among means (31).

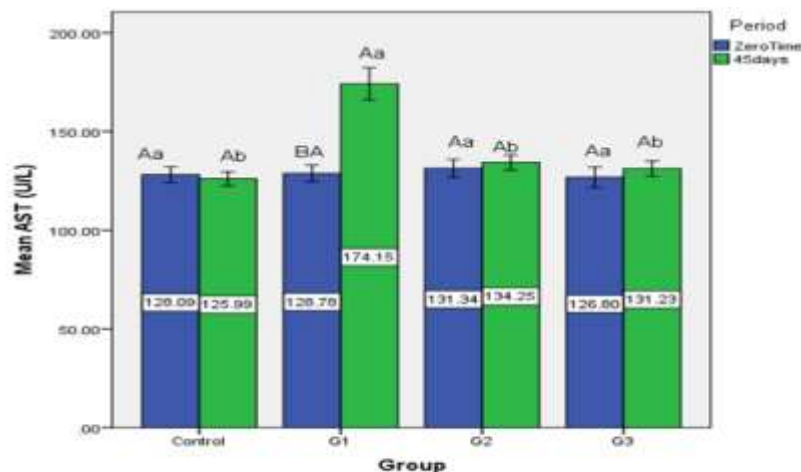
RESULTS

Rats exposed to hydrogen peroxide (group G1) or in combination with resveratrol (group G2) showed a significant ($P < 0.05$) increase in ALT activity after 45 days of the experiment compared to the value in the control and G3 groups, figure1-A. On the contrary, group G2 revealed a significant ($P < 0.05$) decrease in values of this parameter at the end of the experiment comparing to

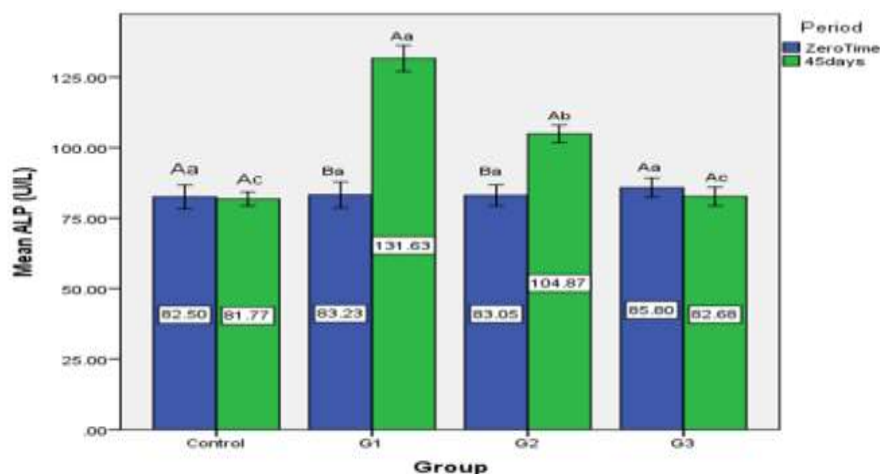
value in G1 treated groups, while there were no significant ($P>0.05$) differences at the end of experiment between G3 and control. The results in figure (4-1-B) showed a significant ($P<0.05$) increase in AST activity after 45 days of the in group G1 comparing to control, G2 and G3 groups. Meanwhile, a non-significant ($p>0.05$) difference in this parameter was observed in control, G2 and G3 groups when compared between each other's. Comparing to group G1, rats received H₂O₂ plus resveratrol orally for 45 days caused a marked significant ($p<0.05$) decrease in serum ALP activity, figure 1-C. The results also revealed that the treatment of normal rats with Resveratrol afforded non-significant ($p>0.05$) differences at the end of the treatment compared to the control group. Depending on the results clarified in figure (1-D) there were a significant ($P<0.05$) increase in serum LDH activity groups G1 and G2 as compared to control and G3 groups. As well as, a significant ($p<0.05$) decrease in LDH activity was observed in the G2 group comparing to G1. Besides, the results showed that the activity of LDH in group G3 near to control group.



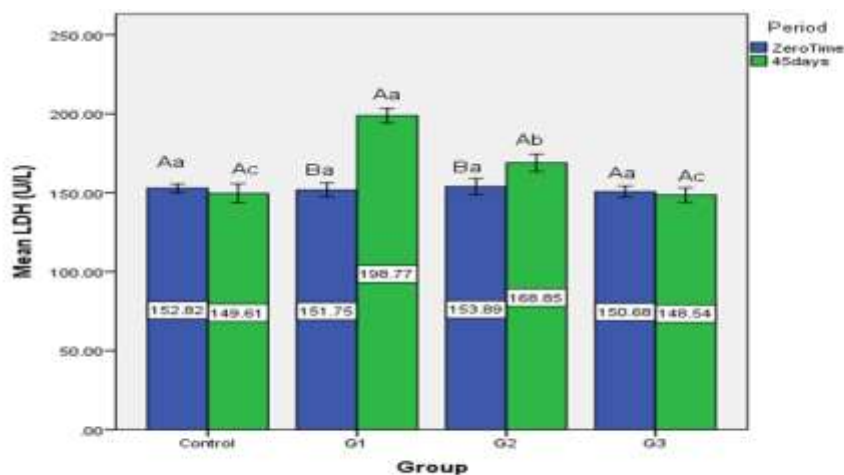
(A)



(B)



(C)

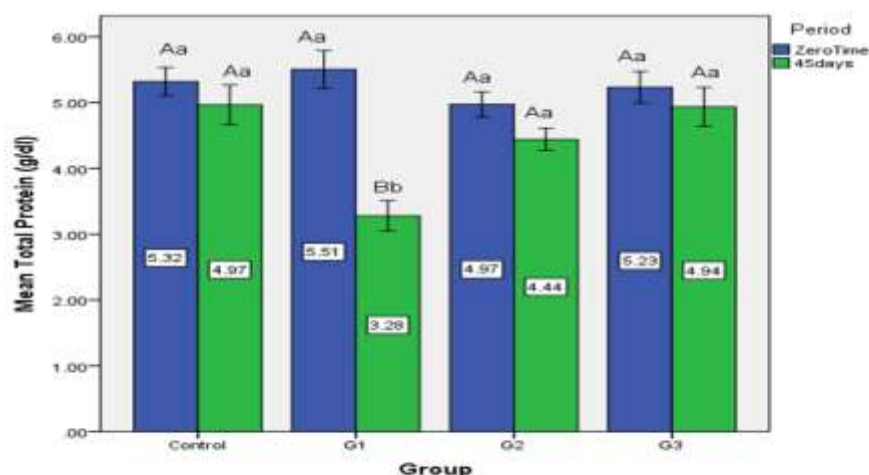


(D)

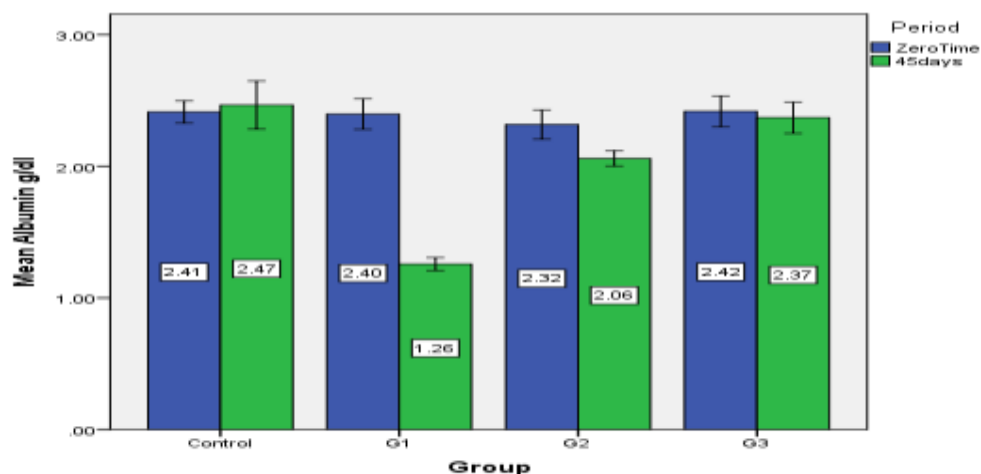
Figure -1, Shown the effect of resveratrol, hydrogen peroxide and their combination in serum ALT (A), AST (B), ALP (C) and LDH (D) activities of control and treated adult male rats.

Values are expressed as mean $\pm$ SE. n=10/each group. G1: Rats received 0.5% of H₂O₂ in drinking water. G2: received a combination of hydrogen peroxide and resveratrol 87 mg/Kg. B. W. G3: received 87 mg/Kg. B. W of resveratrol. Means with a different small letters are significantly different (P<0.05) between groups. Means with a different capital letters are significantly different (P<0.05) within group.

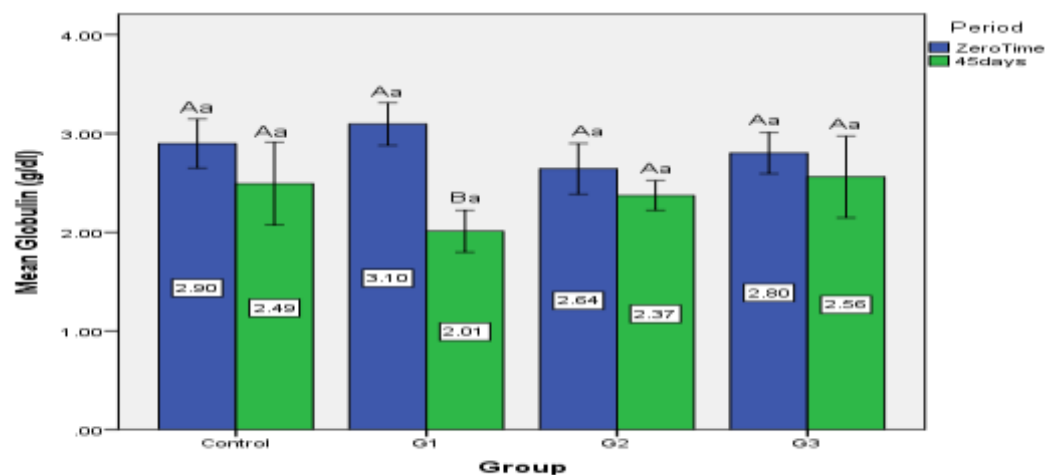
Statistical analysis in figure 2-A showed significant ($P<0.05$) differences in TSP compared to control, G2 and G3 groups. Whereas, group G2 (that received H₂O₂ plus resveratrol) restore the TSP concentration near to control group with non-significant ($P>0.05$) differences between each other's, due to overwhelm the effect of H₂O₂ by resveratrol. Data pertaining in figure (2-B) showed a significant ($P<0.05$) decrease in serum albumin concentration in G1 and G2 treated groups as compared to control group. Besides, group G2 revealed significant ($P<0.05$) increase in this parameter at the same period compared to G1. Meanwhile, no significant ($P>0.05$) differences were observed in serum globulin concentration after 45 days as pointed between four groups. Within the time, the results showed a significant ($P<0.05$) decrease in serum globulin concentration in group G1 at the end of the experiment as compared to the pretreated period (2-C).



(A)



(B)

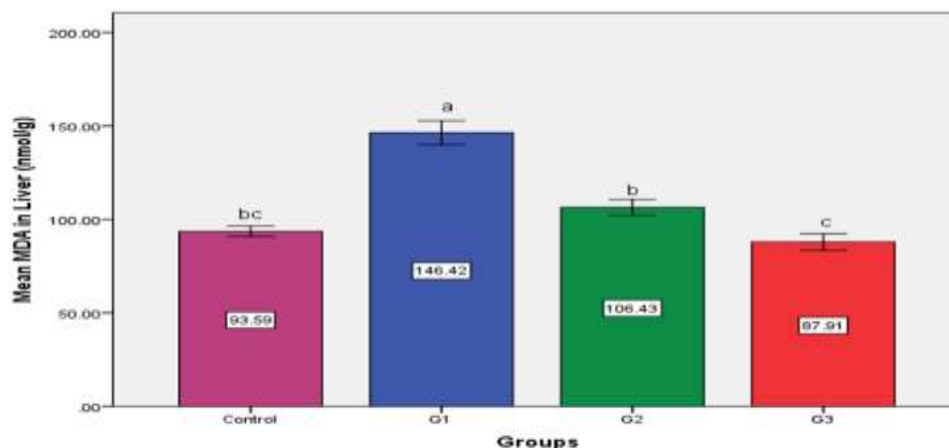


(C)

Figure -2, Shown the effect of resveratrol, hydrogen peroxide and their combination in TSP (2-A), albumin (2-B) and globulin (2-C) concentrations of control and treated adult male rats.

Values are expressed as mean $\pm$ SE. n=10/each group. G1: Rats received 0.5% of H₂O₂ in drinking water. G2: received a combination of hydrogen peroxide and resveratrol 87 mg/Kg. B. W. G3: received 87 mg/Kg. B. W of resveratrol. Means with a different small letters are significantly different (P<0.05) between groups. Means with a different capital letters are significantly different (P<0.05) within group.

Figures (3-A) showed a significant ($P<0.05$) increase in MDA concentration in liver tissue homogenate in group G1 compared to other experimental groups. Whereas a non-significant ($P>0.05$) differences in MDA concentration in liver was observed between G2 and control group. In contrast, statistical analysis of the results in figure 3-B showed a significant ($P<0.05$) decrease in the hepatic tissue of SOD concentration in G1 and G2 treated when compared to other groups. As well as, non-significant ($P>0.05$) difference was found in this enzyme in liver tissues was reported between control and group G3



(A)

(B)

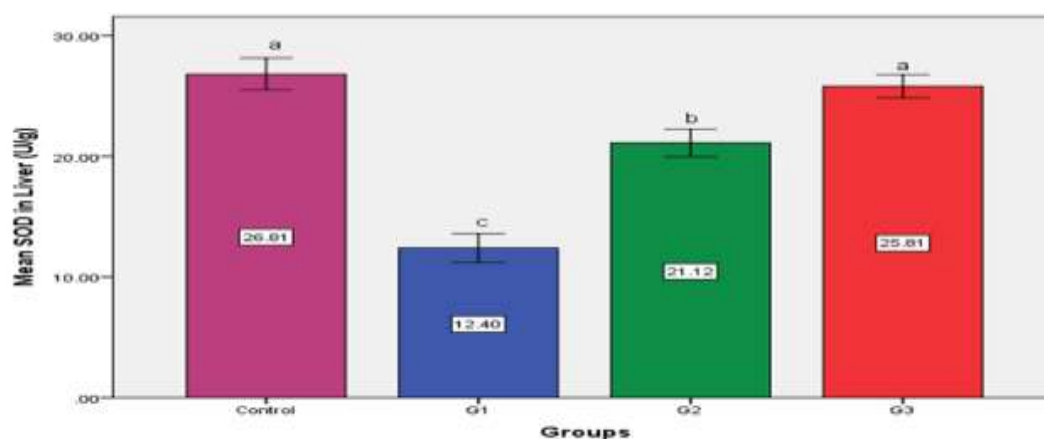


Figure 3, Shown the effect of resveratrol, hydrogen peroxide and their combination in MDA (A) and SOD (B) concentrations in liver tissue homogenate of control and treated adult male rats at the end of the experiment.

Values are expressed as mean $\pm$ SE. n=5/each group. G1: Rats received 0.5% of H₂O₂ in drinking water. G2: Rats received a combination of hydrogen peroxide and 87mg/Kg. B. W of resveratrol . G3: Rats received 87mg/Kg. B. W of resveratrol. Means with a different small letters are significantly different (P< 0.05) between groups.

DISCUSSION

Current results showed an increase in the serum liver enzymes in the H₂O₂ treated group accompanied by an increase of MDA content in liver tissue may be due to hepatocytes injury/and or disruption of plasma membrane induced by hydrogen peroxide leading to hepatic dysfunction. These results are supported by many researchers (32-34).

The liver is a primary organ involved in the biotransformation of food and drugs. An increase in gene expression of liver enzymes after long-term of detoxification can be attributed to some pathological changes associated with alterations of hepatocellular architecture as a result of LPO due to oxidative stress (35). Besides, LPO of hepatocytes membrane in alcoholic and non-alcoholic liver diseases lead to loss membrane fluidity, changes in membrane potential and an increase in membrane permeability, consequently lead to leakage and significant increase of the enzymes into blood were reported (36-37).

Resveratrol supplementation (group G2) showed restored the activities of these enzymes in serum close to the control group. These results are in agreement with finding of Seif el-Din et al., (38) and Cheng et al., (39) whom suggested that resveratrol exert a beneficial effects on hepatic damage via scavenging of ROS, enhancing the antioxidant capacity of hepatic cells . and alleviating nuclear and mitochondrial DNA damage (40), then reduced liver injury by a powerful hepatoprotective activity as evidenced by the enhanced decrease in CYP2E1 positive cells (41).

A significant decrease in TSP and albumin concentrations in group the G1 may be due to hepatocytes injury and/or oxidative stress in hepatic tissue, therefore the liver becomes unable to perform its functions (42). These results are in agreement with the results of other researchers (43-44). Such decreases might be due to disturbance of proteins- synthesizing systems by H₂O₂ through suppression of Na-K- ATPase activity, with inhibited incorporation of amino acids into protein , or could be due to either decrease in protein synthesis or increased proteolysis which caused by LPO (45). Previous studies showed that H₂O₂ - induced morphological changes and apoptosis HepG2 cells, increased activities of caspase-3 and caspase-9, release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria and loss of membrane potential leading to a decrease of protein content in the liver of the G1 group (46-47). Besides, a decrease in protein fraction (albumin) in group G1 may be due to increased production of ROS, which can alter many fundamental cellular functions resulted in the progression of several liver diseases (48). These results are in agreement with (Khudiar,2010). Whereas, a significant increase in serum protein and

its fractions concentrations in group G2, may be due to its action of resveratrol as a free radical scavenging activity. These results are agreed with Chen *et al.*, (49); Saleh *et al.*, (50) and Castro *et al.*, (51).

Negative effect of H₂O₂ on oxidant/antioxidant status of liver tissue in group G1, could be due to the ability of H₂O₂ to induced oxidative stress in animal models (rats), in a dose and period dependent. These results were in agreement with (52-54). Most of the deleterious effects of formations of LPO depend on the conversion of H₂O₂ into OH• which is catalyzed by iron and copper, or by cytotoxicity of H₂O₂ and degeneration of DNA leading to various pathological conditions (55) by LPO due to formation lipid hydroperoxides compounds, in particular, malondialdehyde causing several chronic diseases (56). Together the results showed the effective role resveratrol in attenuating the oxidative stress in liver tissues in group G2 may be due its antioxidant properties that counterbalance ROS generated in the cell (Gulcin , 2010). SOD enzymes, including SOD1, SOD2 and SOD3 , which catalyzes the dismutation of the superoxide anion into H₂O, and molecular oxygen (57-58). Also, the antioxidant effect of resveratrol inhibits TNF and H₂O₂-induced NF-κB activation in a dose- and time-dependent manner (59).

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