Physiological and histological protective role of *Astragalus spinosus* root alcoholic extract against oxidative stress induced by H$_2$O$_2$ in rabbits

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**Abstract.** This study aims to evaluate the role of the plant’s root (*Astragalus spinosus*), in biochemical parameters and histological sections effect in reducing the oxidative stress induced by hydrogen peroxide in rabbits *Oryctalagus cuniculus*, 7-9 months aged and (1300-1500 g) of weight. Animals were distributed into three groups, every group included five rabbits; group (1) control received water and diet. Group (2): animals treated with H$_2$O$_2$ (0.05) and drinking water. Group (3): animals treated with H$_2$O$_2$ and alcoholic extract of the plant root (5mg /kg body weight) for period one month. Study showed high significant increasing in Creatinine and ALP, LDH concentrations of animals treated with H$_2$O$_2$ alone (1.74±0.40) (9.900±1.058), (260.40±26.66) respectively, in compared with control (0.838±0.08) (8.420±1.132), (154.0±49.79) respectively while the concentration of Creatinine, ALP and LDH had been decreased with high significant ($P\leq 0.05$) in group treated with H$_2$O$_2$ and extract of the plant roots (0.79±0.09), (9.220±0.801) and (166.2±36.08) respectively in compared with H$_2$O$_2$ group. And the result of histological sections of liver, kidneys and heart, which showed an improvement of cells in group treated with the extract of the plant which had been induced by oxidative stress.

**Keywords.** Oxidative, Antioxidants, *Astragalus*, liver.

1. Introduction

Oxidative stress represents a situation in which the production of active oxygen varieties (ROS) increases in number that exceeds the ability of antioxidants to get rid of them, which results in an imbalance in the delicate balance between the production of free radicals and the body's ability to remove them or repair their destructive effects, resulting in damage to the various tissues in the body due to fat oxidation by the action of free radicals [1] of the active oxygen varieties is the negative superoxide root (O$_2^-$), which is one of the most toxic free radicals, hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH-) [2]. As for antioxidants, they are substances that prevent or slow down the generation of free radicals and oxidative processes in the body. Therefore, they form a line of defense against free radical activity and reactions [3]. Antioxidants are substances that work to prevent or slow down the generation of free radicals and oxidative processes in the body. Therefore, they
constitute a line of defense against the destructive activity of free radicals in chain reactions [4]. The primary role of antioxidants of various types is to work on giving an electron to the free root to prevent its reactions and stop its destructive activity, or it works on materials that scavenge free radicals, such as the superoxide dismutase (SOD) enzyme that captures the negative superoxide root, or it acts as metal chelating agents [5]. Genus Astragalus, consisting of about 3000 species, is a valuable reference of herbal drugs. The review article comprises scientific data concerning morphological, distribution and phytochemical characters of the genus. Polysaccharides, saponins, and flavonoids are considered main active principles. Astragalus have long been used as medicinal plants in folk medicine as cardiovascular, antihypertensive, diuretic, choleretic, as well as antimicrobial and antiviral agents, this plants of the exhibit a broad spectrum of pharmacological effects, among which major include anti-inflammatory diuretic, antibactericidal, and hypotensive [6]. Plants of the genus Astragalus have been used in traditional medicine in many countries for centuries, which the genus Astragalus being the largest in the family Fabaceae. It is known to contain various metabolites such as saponins flavonoids and polysaccharides. Flavonoids, which possess a wide range of pharmacological activities [7, 8]. The present study aimed to test the effectiveness of the alcoholic extract of Astragalus plant’s root through its protective and antioxidant role on experimental animals by measuring creatinine, basal phosphatase, lactic dehydrogenase, and a histological study of the kidneys, liver and heart to clarify the effect of oxidative stress induced by hydrogen peroxide and the protective role of the plant’s root of Astragalus spinosus.

2. Materials and Methods

2.1. Experience Design

The animals were divided randomly into three groups (5 animals in each group), and the experiment was designed as follows; first group G1: the control group was given only water and food. Second group G2: were given water containing hydrogen peroxide (0.05) and food only. Third group G3: were dosed orally with alcoholic extract at a concentration of 5 mg /kg body weight. The amount was given according to the weight of the animal, and hydrogen peroxide was added to the drinking water at a concentration of (0.05) in addition to the food. After the ending of one-month dosing period, the animals were forbidden from food for 24 hours, after which the blood was collected by cutting the jugular vein and placing the blood in test tubes. The blood was separated by using of a centrifuge at a speed of 3000 rpm for 15 minutes to obtain serum, and then prepared blood serum was withdrawn by micropipette and placed in tubes and kept at -20 °C.

2.2. Collection and preparation of the plant root

The roots of the Astragalus plant were obtained from the desert areas in the west of the Tigris River between Samarra and Tikrit.

2.3. Preparation of extract

The alcoholic extract was prepared from the plant roots according to the method mentioned by [9]. The amount of extract obtained from every 100 g of the plant’s root was 2 g.

2.4. The active dose of the plant

Twenty one animals were used to know The effective dose of plant, animals were randomly divided into 7 groups ( 3 animals for each group ), and in the table below was determined the effective dose of the alcoholic extract .The experiment was carried out during three days, and the animals were dosed orally at a single dose every 24 hours, and after 3 days, blood samples were collected through the
ear’s vein, and the concentrations of sugar and cholesterol were measured, and the effective alcohol dose was concentration (5 mg / kg body weight).

2.5. Determination of Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (ALP) Enzymes

The activities of LDH were determined spectrophotometrically by measuring the oxidation rate at 340 nm [10]. The activity of ALP enzyme was determined according to the colour method, as the amount of liberated phenol is measured after its reaction with potassium free cyanide and 4-aminoantipyrine to produce a coloured complex with the highest absorption at the wavelength 510 nm.

2.6. Determination of Serum Creatinine

The concentration of creatinine was estimated by using of an analysis kit (BIOLABO SA, France) kit [11]. The microscopical and histological slides were prepared according to [12] for histological study.

2.7. Statistical analysis

For a statistical data, Statistical Package Social Science (spss) version 22 was used, and Student's t-test, the level of significance was P≤ 0.05.

3. Results

The results finding (Table 1) show significant increasing (P≤0.05) in the level of Lactate dehydrogenase (LDH), ALT, and Creatinine in group G2 which were treated with H2O2 when compared with control group G1, while group G3 which treated with plant’s root alcoholic extract showing significantly decreasing in the level of LDH, ALT and Creatinine when compared with group G2 and showed no significant differences when compared with control group G1.

| Groups    | Parameters | LDH     | ALP     | Creatinine |
|-----------|------------|---------|---------|------------|
| G1 control |            | 154.0±49.79 | 8.420±1.132 | 0.838±0.08 |
| G2        |            | 260.40±26.66 | 9.900±1.058 | 1.74±0.40 |
| G3        |            | 166.2±36.08  | 9.220±0.801 | 0.79±0.09 |

N+5, P P≤0.05, ± = Standard error

Results in the present study showed increasing in the level of LDH significantly (P≤0.05) in group G2 giving H2O2, while group G3 showed no significant differences when compared with control group G1 (Figure 1).
Results preserved increasing in the level of ALT significantly (p≤0.05) in group (G2) were treated with H$_2$O$_2$, while group G3 showed no significant differences when compared with group control (Figure 2).

(Figure 3) Results showed increasing in the level of Creatinine level significantly (p≤0.05) in group (G2) were treated with H$_2$O$_2$, while group G3 showed no significant differences when compared with group control G1.

Histological study results in (Figures 4, 5, 6) showed the changes in the heart tissues of study animals in the group G1,G2 and the group G3.Figures (7, 8, 9) that show the changes in liver tissues. Figures (10, 11, 12) show the changes in kidney tissues.
(CMF), B- nuclei (N), C- Endomysium.

**Figure 5.** Cross section of heart in animal treated with hydrogen peroxide (0.05) G2 show:
A- heart, atrophy of cardiac muscle, B- Degeneration of sarcoplasm with disappearance of its nuclei (H&E stain 400x)

**Figure 6.** Longitudinal section of heart in animal treated with hydrogen peroxide (0.05) using dose of (5mg/k body weight) of the plant’s root extract, group G3 show:
A- cardiac muscle fibres with its normal archete chare (sarcoplasm and nuclei).

**Figure 7.** Histological section of liver in control group G1 show:
A- central vein (CV) B- Columns of hepatic cells with its spherical nuclei (HC), C- Blood sinusoids with Kuepfer cells (BS) (H&E stain)
Figure 8. Cross section of liver in animal treated with hydrogen peroxide (0.05) G2 show: A- blood clot in the central vein, B- Lymphocytic nodular aggregation, C- Hypertrophy of Kuepfer cells in sinusoid, D- Disappearance of nuclei Frome certain number of hepatic cells (H&E stain 400X).

Figure 9. Histological section of liver in animal treated with hydrogen peroxide (0.05) and dose of (5mg/kg body weight) of the alcoholic extract of the plant’s root group G3 show: A- normal Architecture of hepatic cells with its spherical nuclei, B- Kuepfer cell in the blood sinusoid (H&E stain 400X).

Figure 10. Figure (10)  Histological section of kidney in control group G1 show: A- renal cortex;
glomerulus G, B- proximal convoluted tubule PCT, C- Distal convoluted tubule DCT
D- Bowman’s capsule BC (H&E stain 400X).

Figure 11. Histological section of kidney in animal treated with hydrogen peroxide (0.05) group G2 showing: Renal medulla, epithelia, desquamation inside the lumen of collecting ducts (A), degeneration of epithelial cells of collecting ducts (B) RBC present in between Henle loop (C) (H&E stain 400X).

Figure 12. Histological section of kidney in animal treated with hydrogen peroxide (0.05) and dose of ( 5 mg/k body weight) of alcoholic extract of the plant root G3 show: A- renal cortex, hyperplasia of glomerular epithelia cell, B- Proximal convoluted tubule, C- distal convoluted tubule, D- Capsule space.

4. Discussion

The results showed a significant increase p (P ≤ 0.05) in the LDH enzyme concentration in the blood serum of animals exposed to oxidative stress mediated by hydrogen peroxide, the results were (260.40±26.66) in compared with the control group (154.0±49.79) and the reason is due to the increase in oxidative stress resulting from the free radicals generated by H₂O₂, which damage the cells and tissues of the body, so the increase in the activity of this enzyme in the blood serum is an indication of the breakdown of the cells of the body organs, Histological section in the heart of treated with hydrogen peroxide (0.05) showing atrophy of cardiac muscle and degeneration of sarcoplasm with
disappearance of its nuclei compared with control group which show histological section in the heart of myocardium; degeneration cardiac muscle fibers. The oval nuclei in the sarcoplasm, endomysium. The cause of the high LDH is attributed to the harmful physiological effects of free radicals and stress on the heart muscle cells, which leads to its destruction and damage, and consequently damage to the cell membranes and the release of enzymes into the circulation [13]. And when the plant extract was treated, it led to a significant decrease in the total LDH level in the blood serum (166.2±36.08) compared to the H$_2$O$_2$ group (260.40±26.66). Also, a clear improvement was observed showing cardiac muscle fibers with its normal architected (sarcoplasm and nuclei) RBC outside the blood vessel. The results are in agreement with [14] where isoflavonoids were isolated from the Astragalus roots where the protective roles and antioxidant effects and increased the antioxidant enzymes, prevented the release of LDH from cells and stabilized the cell membrane structure. The results of ALP were (9.900±1. IU/l) in the group treated with hydrogen peroxide compared with control group (8.420±1.132 IU/l). It is evident from the above results that the level of ALP increased in the serum of the group treated with hydrogen peroxide in compared with control, and this is an indication of the occurrence of liver damage as a result of the effect of free radicals, while the groups treated with plant extract show a significant decrease on probability (P<0.05) of the level of the enzyme ALP (9.220 ± 0.801 IU / l) in compared to the group treated with hydrogen peroxide (9.900±1 IU / l). This indicates an improvement in liver cells and as shown in the Figure (7) Histological section in the liver of animal treated with hydrogen peroxide (0.5%) showing: blood clot with WBC in the Blood clot in CV with WBC Lymphocytic nodular aggregation, Hypertrophy of Kupffer cells in sinusoid. Disappearance of nuclei from certain number of hepatic cell compared with control Figure (8) showing: hepatic lobule central vein, Columns of hepatic cells with its spherical nuclei, Blood sinusoids with Kupfer cells, Draining of blood from blood sinusoid to CV this result is agreed with [14, 15] indicated oxidative tissue damage and acute infections caused by the drug by increasing lipid peroxidation, and this leads to an increase in the permeability of the cell membrane and thus increases the movement of enzymes and their infiltration into the blood, which leads to an increase and decrease in the level of enzymes in the blood serum. In the liver, the study [16] indicated that the presence of such toxic substances leads to self-degradation of hepatocytes as a result of the increased activity of the lysosomes, which leads to the death of hepatocytes that cause an increase in the level of the three enzymes in the blood serum, and here the protective role of Astragalus appears spinous. Because it contains antioxidants, flavonoids can neutralize different types of oxidizing species including superoxide anion(O$^{-}$2), hydroxyl radical (OH), or peroxyl radicals (ROO.) [17] Figure (9) showing section of liver of animal treated with hydrogen peroxide (0.5%) and treated with daily dose of (5mg/k body weight) of the extract of the plant showed normal Architecture of hepatic cell with its spherical nuclei, Kupffer cell in the blood sinusoid with WBC, total flavonoids, obtained from Radix Astragali showed significant antioxidant activity and inhibited the lipid peroxidation caused by O•$^{-}$. H$_2$O$_2$[18, 19] and total flavonoids in Astragalus showed Protective effect from against DNA strand breaks, caused by (OH.) [20]. Flavanonols from Astragalus sinicus showed potent antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [21]. Flavonoid fraction obtained from the seeds of Astragalus complanatus showed significant radioprotective effect against injury induced by γ-irradiation in mice, flavonoids increased survival rate on survival rate of the experimental animals and made the damaged organs recover normal appearance because of enhancing immunity [8]. Observed effect of flavonoid of Astragalus m against reperfusion hepatic injury in hemorrhagic shock [22]. Figure 3 shows a high increase (P<0.05) of creatinine (SCR) of animals exposed to oxidative stress with hydrogen peroxide, where the results were (1.74±0.4 mg / dL) compared to the control group (0.838±0.08mg / dL) and the cause may be due to increased oxidative stress, which leads to impaired renal function, leading to an increase in creatinine in the blood and less excretion in the urine levels of serum creatinine (SCR) manifest the ability of renal tissue to remove the creatinine from blood and gather it in the urine. However, kidney dysfunction could lead to the levels of SCR increased through weaken the ability to filter creatinine. In addition, the injured kidneys could cause the levels of serum urea nitrogen (SUN) elevated on account of the kidney tissue have no ability to remove the urea from
the blood [23, 24], demonstrated that astragalosides, especially astragalo- side V isolated from the root of Astragalus radix, inhibited the formation of advanced glycation end products in vitro. In rats induced diabetes, and treatment with Astragalus membranaceus improved renal function [25]. And when treating animals exposed to oxidative stress mediated by H₂O₂ with Astragalus extract, there was a significantly decrease in the SCR, as the results in figure 1 were (0.784 ± 0.050 mg / dL) compared to the control exposed to oxidative stress with H₂O₂ only (1.526 ± 0.260 mg / dL). While the groups treated with plant extract decreased significant on p (P≤0.05) of the activity enzyme ALP (0.79±0.09IU/l) compared to the group treated with hydrogen peroxide (1.74±0.40IU/l). Figure (10) Histological section in the kidney of animal treated with hydrogen peroxide (0.05) showing: renal medulla; epithelia; desquamation inside the lumen of collecting ducts, degeneration of epithelial cells of collecting ducts, RBC present in between Henle loop, compred with control Figure (11) showing: renal cortex; glomerulus, proximal tubule convoluted, distal convoluted This indicates an improvement in liver cells and as shown in Figure (12), animal treated with hydrogen peroxide (0.05) and treated with daily oral dose (5 mg/kg body weight) of the extract of the plant show: renal cortex; hyperplasia of glomerular epithelia cell, proximal convoluted tubule, distal c.t, Capsule space. The reason is due to Astragalus contains the high capacity of antioxidants that inhibits oxidative processes in the biological system, Astragalus membranaceus has a complex chemicals. Its major active constituents include Astragalus flavonoids, saponins, and polysaccharides [26]. Result is in accordance with [27] with cisplatin induced in mice kidney. The flavonoids made the damaged organ recover normal appearance [20].

5. Conclusion

From our results we can conclude that alcoholic extract of Astragalus spinosus root have a protective role against the oxidative stress effects on biochemical parameters include LDH, ALT, and Creatinine, and have a histological protective from harm effect of hydrogen peroxide and free radicals.

6. References

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