Evaluation of Ashwagandha (Withania Somnifera) and its Extract to Protecting the Liver From Damage

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Abstract: This study was achieved to the safety evaluation of the ashwagandha (Withania somnifera) and total phenolic acid and total flavonoids compounds were determined, as well as ABTS and DPPH radical scavenging activities assay were studied. In addition, the biological experimental and histological assay was an examination of hepatitis rats.

The results observed that the ashwagandha root extract was safety due to do not evidence of toxic effect or mortality in mice. This safety from the ashwagandha root extract caused the extract had contained high amounts of phenolic and flavonoids compounds which directly role of in free radical scavenging, thus the extract from ashwagandha root is a source of antioxidant activity.

At the end of biological experimental after eight weeks the results were found that the effects of ashwagandha roots extract on carbon tetrachloride CCl₄-treated induced alterations in serum hepatic enzymes were decreased than control CCl₄ positive and give nearly equal control negative at level 400mg/kg body weight for a rat. Moreover, the results found that the effect ashwagandha roots extract on antioxidant enzymes hepatic as glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GPx), and catalase (CAT) parameters showed that the ashwagandha roots extract was improvement the antioxidant enzymes.

Furthermore, different concentrations extract alleviated histopathological changes in rats' liver treated with CCl₄. Ashwagandha root extracts confirmed that the protection of the rats' liver CCl₄-induced hepatotoxicity. This influence may be due to activated, the antioxidant activities of these extracts.

In recommending that the obviously results supported that the possible anti hepatic damage effect of ashwagandha root extract against CCl₄-induced hepatic damage. This anti hepatic damage influence may be due to activated, the antioxidant activities

Keywords: Ashwagandha, hepatic, liver enzyme, toxicity

INTRODUCTION

Ashwagandha (Withania somnifera, family Solanaceae) is a significant great significance herb of Ayurveda system of medicine in India. It is utilized widely as possessing different health useful (1). Ashwagandha is Know recognized to its biologically active chemical compositions like the alkaloids, steroidal lactones, and saponins (2). The roots of ashwagandha are recognized for their pharmacological influence due to the presence of a group of steroidal lactones. Theutilize of ashwagandha has been apparent in the therapy of leukoderma, asthma, bronchitis, Parkinson’s disease, and chronic liver disease, etc. (3, 4).

Ashwagandha had contained various natural antioxidants that preservation the antioxidant liver enzymes which are responsible as a health promoter. It has been utilized to therapy inflammation, fevers and to protect against illness. It has been utilized to protect the immune system, become better memory and to promote overall wellness (6).

The roots of Withania somnifera had contained for the most of compounds known as withanolides, which are steroidal compounds and bear a resemblance to the active constituents of Asian ginseng (Panax ginseng) known as ginsenosides. Ashwagandha's withanolides have been the influence on many statuses; including the immune system and even cancer (7).

Chemical analysis of Ashwagandha observed that the major components to be alkaloids and steroidal lactones. Moreover, the Ashwagandha leaves had contained steroidal lactones, which are commonly called withanolides (8).
Aim of this study was achieved to estimate the Ashwagandha root powder for natural antioxidant content and activity and also and its extract as protect of the liver from damage.

MATERIALS AND METHODS

Materials

Ashwagandha (*Withania somnifera*) roots were obtained from local market and it was properly washed under running water to remove adhering foreign particles, mud, dust, etc., dried at low temperature (50°-60° C) and ground under hygienic conditions to powder form using traditional stone pestle and mortar as well as an electric grinder. The powder so obtained was sieved twice to remove the coarse particles and stored in airtight containers until further analysis.

METHODS

Acute oral toxicity for animals

Healthy young male albino mice were used for acute oral toxicity study. Eighteen mice (weighing 20–30 g) were purchased from Pharmacy College at King Saud University. The rats were randomly divided into 3 groups of 6 mice per cage to measure the LD50 of the Ashwagandha root. The ambient temperature in the experimental laboratory was maintained at 22 °C (±3 °C). A standard pellet diet was given with water ad libitum. For experiment designed to determine oral LD50 of ashwagandha (*Withania somnifera*) sighting study was done on one rat which was administered 2000 mg/kg ashwagandha roots powder. Signs of toxicity were observed for 14 days. No toxic effects were seen in the sighting study; hence 2000 mg/kg dose was selected for the main study in five rats. Rats were administered the dose of 2000 mg/kg of ashwagandha (*Withania somnifera*) and signs and symptoms of toxicity were observed (9). On day 15, all the animals were euthanized and gross pathological examinations were done.

Subacute toxicity

Healthy male albino mice (24 mice weighing 20–30 g) were purchased from Pharmacy Collage at King Saud University and delivered to the King Fahd Medical Research Center in Jeddah) were divided into 4 groups of 6 mice per cage randomly. Rats were given oral (gavages) ashwagandha of root in the doses of 0 (Group I control), 500 (Group II low-dose), 1000 (Group III mid dose), 2000 (Group IV high-dose) mg/kg/day for 28 days. During the course of sub-acute study, all animals were provided an ad libitum feed, until the day prior to the scheduled euthanasia (10). After 28, animals from Groups I to IV were euthanized, blood was collected from a retro-orbital vein for estimated Red blood cells (RBCs) and white blood cells (WBCs) were measured as recommended by Riley (11). Blood hemoglobin (Hb) and platelets were determined using a whole blood sample and the method described by Dacie and Lewis (12) and PCV was determined according to the equation:

\[
PCV = \frac{The \ volume \ of \ erythrocytes \ in \ a \ given \ volume \ of \ blood}{Total \ blood \ volume}
\]

Estimation of total phenolic acids and total flavonoids compounds

The total phenolic content in the extract was measured using the method of Qawasmeh *et al.* (13) with Folin-Ciocalteu reagent. The UV reading was measured at 760 nm. Gallic acid was used as standard (1 mg/ml) and the results were expressed as gallic acid equivalents (GAE mg/100g of dry weight).

The total flavonoids content was determined by the method of Eghdami and Sadeghi (14). The absorbance was measured against a blank solution at 510 nm and the total flavonoid content was expressed in terms of milligrams of quercetin equivalent per gram dry weight (mg QE/100g DW).

Determination methods of antioxidant activity in ashwagandha root powder

ABTS (2, 2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)

The ABTS assay was performed according to the method used by Miller and Rice-Evans (15) and Ramesh Kumar and Sivasudha (16). For the analysis, 1 mL ABTS reaction solution was added to 100 µL sample extract, and the absorbance was measured at 734 nm immediately after 1 min of initial mixing.

DPPH (1,1-Diphenyl-2-picylhydrazyl)

The DPPH assay was performed as described by Ravichandran *et al.* (17) the absorbance of the mixture was measured at515 nm with the UV-Visible spectrophotometer. The following formula was used to determine the percentage of scavenging activity,
Percentage of inhibition (%) = \[
\frac{(A_{control} - A_{sample})}{A_{control}} \times 100
\]

Where, 
\( A_{control} \) - absorbance of DPPH, 
\( A_{sample} \) - absorbance reaction of mixture (DPPH with Sample)

**Biological experimental**

Male albino rats (n=36 rats), 150-200g per each, were purchased from Pharmacy Collage at King Saud University and delivered to the King Fahd Medical Research Center in Jeddah. Rats were housed in individual cages with screen bottoms and fed ad libitum on a basal powdered diet appropriate for growing the rats which containing casein (20 % ≥ 85% protein), corn oil (8%), corn starch (31%), sucrose (31%), cellulose (4%), salt mixture (4%) and vitamin mixture (1%) according to Pell et al. (18).

Rats were housed individually in wire cages in a room maintained at 25 ± 2°C and kept under normal healthy conditions. All rats were fed on the basal diet for one-week before starting the experiment for acclimatization. After feeding on a basal diet for eight days, rats were divided into six groups. The first group considered normal control fed on basal diet and receiving paraffin oil 3ml/kg-1 body weight, subcutaneous (Sc) two times per week for eight weeks and normal saline 5 ml/ kg-1 body weight, per orally (Po) four times per week for eight weeks. The second groups considerable CCl4 as positive control fed on basal diet and receiving carbon tetrachloride 40% in paraffin oil (3ml/kg-1 body weight, Sc) two times per week for eight weeks and normal saline (5 ml/ kg-1 body weight, Po) four times per week for eight weeks. The third, fourth, fifth and sixth groups were fed on basal diet and receiving carbon tetrachloride 40% in paraffin oil (3ml/kg-1 body weight, Sc) two times per week for eight weeks and 100, 200, 300 and 400 mg / per orally /kg-1 body weight two times per week for eight weeks from Ashwagandha root extract separately as well as normal saline 5 ml/ kg-1 Po body weight, four times per week for eight weeks. Each rat was weighed every two days and the food consumption was calculated.

At the end of the experiment, twenty-four hours after dosing of vehicle, CCl4 or plant extracts, blood samples were collected from the orbital sinus. Serum was separated by centrifugation at 3500 rpm and kept under -7°C for the determination of liver enzymes. Animals were anesthetized with diethyl ether and sacrificed for separation of the liver. Livers were dissected out, divided into two parts. One part was kept in liquid nitrogen for determination of antioxidant status and the other part was immediately fixed in formaldehyde solution 10% and was used for histopathological examination.

Serum Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically following Schumann and Klauke (19), while Serum alkaline phosphatase (ALP) detected according to Belifield and Goldberg (20).

The activity of the antioxidant enzymes, Catalase (CAT) was measured according to the method of Aebi (21). Superoxide dismutase (SOD) was measured by Janknegt et al. (22). Glutathione Peroxidase (GPX) was measured by Paglia and Valentine (23). Reduced glutathione (GSH) level was measured according to the chemical method described by Moron et al. (24), while lipid peroxidation products were determined by measuring malondialdehyde (MDA) content in tissue homogenates, according to the method of Uchiyama and Mihara (25).

**Histopathological examination**

Liver tissues were preserved in 10% formaldehyde solution was used for histopathological study. The kidney tissues were placed in plastic cassettes and immersed in neutral buffered formalin for 24 h. The fixed tissues were processed routinely, embedded in paraffin, cut into 4-5 µm-thick sections and stained with hematoxylin and eosin (H&E) according to Wang et al. (26). The extent of CCl4-induced liver damage was evaluated by assessing the morphological changes in the liver sections.

**Statistical analysis:**

All chemical analyses were performed in three replicates and the results were statistically analyzed. Statistical analysis was performed using the GLM procedure with SAS (27) software. Duncan’s multiple comparison procedure was used to compare the means. A probability to p ≤ 0.05 was used to establish the statistical significance.

**RESULTS AND DISCUSSION**

**Acute and Sub-acute toxicity for ashwagandha (Withania somnifera) roots**

In acute toxicity study, oral LD50 of ashwagandha (Withania somnifera) roots extract in mice was taken orally at level 2000 mg/kg body weight. During fourteen days it could not be observed in the acute oral toxicity in rats and no abnormal behavior during the experimental period. Moreover, it could be noticed that the ashwagandha (Withania somnifera) roots extract did not produce any organ atrophy and hypertrophy.
Sub-acute toxicity was evaluated if the extract from ashwagandha roots has any effect on blood parameters and packed cell volume (PCV) and the results are reported in Table (1). The results observed that no statistical variations (P>0.05) when PCV were compared among the groups at the post-treatment groups (after 28 day). Moreover, the PCV was slightly decreased in the treated group at levels 500, 1000 and 2000mg/kg/day were 50.51, 50.29 and 50.16%, respectively, than control group was 50.78% after 28 days. These results confirm that the ashwagandha root extract was taken up to 2000mg/kg/day to rats for 28 days has no significant influence on the parameters were found. These results confirmed by Dewick (28) and Jones and Kinghorn (29) observed that the ashwagandha (Withania somnifera) roots extract is relatively safe for mice when given orally. A slight lowering in PCV showed in behave toward groups in sub-acute toxicity exam could be a reason may be these plant had contained saponins in the extract which is known to due to hemolysis by growing the permeability of the plasma membrane.

From the results it could be noticed that the increase in WBC, RBC, Hemoglobin and Platelets were observed however the values of all the experimental groups were in the normal range. Therefore, the previous variations were as incidental and are not therapy concerning. Hemoglobin is observed to be elevated in normal limits when the animals’ are therapy with ashwagandha (Withania somnifera) roots extract (30). A sub-acute toxicity study with hydroalcoholic extract of ashwagandha (Withania somnifera) roots showed no evidence of toxic effect or mortality in mice (31).

Table (1): Sub-acute toxicity tests of the Ashwagandha (Withania somnifera) roots extract in mice.

| Parameters     | Control     | 500 mg/kg   | 1000 mg/kg  | 2000 mg/kg  |
|----------------|-------------|-------------|-------------|-------------|
| RBC 10^7 /cm   | 8.41 ± 0.39 | 8.75 ± 0.35 | 8.99 ± 1.07 | 9.95 ± 1.12 |
| WBC 10^9 /cm   | 9.15 ± 0.33 | 10.82 ± 1.02| 11.52 ± 1.08| 12.24 ± 1.14*|
| PCV %          | 50.78 ± 2.58| 50.51 ± 1.74| 50.29 ± 1.21| 50.16 ± 1.76 |
| Platelets 10^4 /cm | 922.1 ±30.27| 941 ± 42.54 | 968.5 ± 30.28 | 988.6 ± 33.37 |
| Hemoglobin g/dl| 11.34 ± 0.4  | 12.99 ± 1.05| 13.19 ±1.13*| 15.00 ± 1.21*|

PCV: Packed cell volume, RBC: Red blood cell, WBC: White blood cell. Values are represented as mean ± SD of 3 replicates. Means marked with the same superscript letters are not-significant (p>0.05), whereas others with different superscript letters are significant (p<0.05).

Antioxidant content and activity from ashwagandha roots extract

Antioxidant content as phenolic content and flavonoids compounds and antioxidant activity as DPPH and ABTS radical scavenging ability were determined in ashwagandha (Withania somnifera) roots extract and the results are recorded in Table (2). The results showed that the total phenolic content and flavonoids compounds were 45.32 mg gallic acid /100 g and 32.27 mg quercetin equivalent (QE)/ 100g, respectively. These results confirmed with Harikrishnan et al. (32) reported that the extract from Ashwagandha roots had contained several alkaloids, a few flavonoids and reducing sugars besides rich in iron. Moreover, Udayakumar et al. (33) reported that the total phenolic acids and flavonoids compounds were lower may be caused by the various sources of W. somnifera and polyphenols compounds of the different plant parts which may be concerning to the color, maturity, and environment. The scavenging capacity of DPPH free radicals is utilized to screen the antioxidant potential of foods and plants. The antioxidant capacity of the extract of Withania somnifera roots had contained a good antioxidant capacity as shown in Table (2). A significant lowering was found in the DPPH radical activity may be caused by the scavenging capacity of the extracts.

The ABTS radical scavenging capacity was reported to be increased in Withania somnifera root extract. ABTS• radical is usually utilized for the examination of antioxidants in the beverages and plant extracts for their antioxidant activities for the reason that of its power in both the organic and aqueous media (34, 35).

Table (2): Antioxidant content and activity from ashwagandha roots extract

| Antioxidant content and activity | Ashwagandha extract |
|---------------------------------|----------------------|
| Total phenolic (TP)             | 45.32±7.45a          |
| Total flavonoids (TF)           | 32.27±2.05 a         |
| DPPH                           | 65±8.29              |
| ABTS                           | 85±10.19              |

Data represent average values, standard deviation of three independent extractions from each sample on a dry basis. Different letters in the columns represent statistically significant differences (p < 0.05). TP results were given as mg gallic acid equivalent (GAE)/100 g sample; TF Contents of extracts were given as mg quercetin equivalent (QE)/100 g sample; DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS [(2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)] were given inhibitions percent.
BIOLOGICAL EXPERIMENTAL:

Effect of ashwagandha (Withania somnifera) roots extract on initial, final body weight and feed efficiency ratio in rats:

The results from Table (3) indicated that the effect of ashwagandha (Withania somnifera) roots extract on initial, final body weight and feed efficiency ratio in rats. From the result it could be observed that the normal negative control group was fed on the basal diet had the highest in final body weight (194.5 g, increased in gain bodyweight 27.0 g) and feed efficiency ratio (5.84%) at the end experimental period (eight weeks). While the positive control group was fed on basal diet slightly significantly increased in final body weight (177.0 g increased 6.7g about initial body weight) and feed efficiency ratio was 1.92%. Meanwhile, the rat groups fed on ashwagandha (Withania somnifera) roots extract orally two times per week at levels 400 mg/kg was observed that no significant changes in final body weight and total food intake between them. It means that the rats fed on different parts from ashwagandha (Withania somnifera) roots extract at ratio 400 mg/kg did not affect food intake and body weight may be caused the ashwagandha (Withania somnifera) roots extract had contained rich amounts from the natural antioxidant.

Table (3): Initial, final body weight and feed efficiency ratio in rats fed orally of ashwagandha (Withania somnifera) roots extract

| Groups          | Initial body weight | Final body weight | Gain body weight | Total food intake | Feed efficiency ratio |
|-----------------|---------------------|-------------------|------------------|-------------------|----------------------|
| Control negative| 167.5±7.6          | 194.5±6.6        | 27.0             | 462.7±26.0        | 5.84±0.2             |
| Control CCl4    | 170.3±8.0          | 177.0±7.0        | 6.7              | 348.6±28.3        | 1.92±0.6             |
| 100mg           | 181.7±8.6          | 191.8±7.9        | 10.1             | 450.4±31.3        | 2.24±0.2             |
| 200mg           | 182.3±9.1          | 197.8±8.2        | 15.5             | 445.6±31.1        | 3.48±0.7             |
| 300mg           | 175.8±7.8          | 195.8±8.1        | 20.0             | 435.0±31.2        | 4.60±0.2             |
| 400mg           | 173.2±7.6          | 198.3±9.5        | 25.1             | 430.4±15.4        | 5.83±0.4             |

Data are presented as mean (n=6 rats) ± standard deviation, values with different superscripts within the column are significantly different at P<0.05, while those with have similar or partially are not significant.

Effect of ashwagandha root extract on liver functions

Table (5) showed that the influence of ashwagandha roots on liver function indices of rats. Feeding with the ashwagandha roots at different concentrations extract had significantly increased activity of alanine transaminase (ALT) (23.50, 25.00, 27.98 and 29.33 IU/L, respectively) gradually when the concentration ashwagandha roots at different concentrations extract had significantly increased activity of alanine transaminase (ALT) (23.50, 25.00, 27.98 and 29.33 IU/L, respectively) gradually when the concentration ashwagandha roots (48.21 IU/L) and increased significantly to the group fed orally at concentrate 400mg ashwagandha roots (48.21 IU/L) and nearly to control negative (51.04 IU/L). Rats fed orally on different concentrations extract from ashwagandha roots were considerable lowering in sALP (198.51, 185.70, 180.32 and 175.46 IU/L, respectively) than control CCl4 was 225.00 IU/L. Variation in the activities of these enzymes showed that the damage to organelles like mitochondria most important to the emission of soluble enzymes such as AST (36).

Assessment of the antihepatotoxic and antioxidant characteristics of the extract against CCl4-induced liver damage in rats observed that considerable lowering liver function (37). Another research showed that an aqueous extract of the whole plant was estimated for antihepatotoxic activity against CCl4-induced liver damage in male rats. The extract was taken orally at 400 mg/kg body weight dose for 7day. The aqueous extract had important antihepatotoxic activity and lowering the increase levels of serum enzymes like serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, and ALP and increasing total protein (38).

Table (4), The effects of ashwagandha roots extract on carbon tetrachloride CCl4-treated induced alterations in serum hepatic enzymes including aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP).

| Groups          | AST (U/L) | ALT (U/L) | ALP (U/L) |
|-----------------|-----------|-----------|-----------|
| Control negative| 51.04±5.54| 30.00±3.73| 172.05±2.54|
| Control CCl4    | 72.33±3.88| 40.04±3.44| 172.05±2.54|
| 100mg           | 34.72±4.22| 23.50±4.52| 198.51±4.60|
| 200mg           | 35.54±4.10| 25.00±5.55| 185.70±4.44|
| 300mg           | 41.88±6.36| 27.98±8.43| 180.32±4.62|
| 400mg           | 48.21±6.35| 29.33±7.54| 175.46±6.31|
Data are presented as mean (n=6 rats) ± standard deviation, values with different superscripts within the column are significantly different at P<0.05, while those with similar or partially are not significant.

**Effect of ashwagandha roots extract on the activity of the antioxidant enzymes liver parameters:**

Table (5) observed that the rat groups injection of CCl4-induced considerably increased in the antioxidant enzyme level of glutathione reductase (GSH) activities when increasing the concentration of ashwagandha roots extract is taken orally for rat groups was improved to 275.67, 282.52, 295.28 and 310.55 µ/g, respectively, compared with control CCl4 content (188.73 µ/g) and the glutathione peroxidase (GPx) confirmed the results from GSH. In addition, it increased the MDA level in liver tissues (8.54, 8.99, 9.32 and 10.54 nmol/g, respectively) compared to normal control values (8.02nmol/g) and all treatment was decreased than control positive was 14.20 µ/g. Whereas, the results from the SOD in liver tissues was increased from 658.44 µ/g at 100mg ashwagandha root extract to 7.11.0 µ/g at 400mg ashwagandha roots than 470.63 µ/g control positive. As well as the catalase (CAT) was parallel all results from different treatments. These results are occurred by Zarei and Shivanandappa (39) who found that the rats’ pre-therapy with ashwagandha roots the trouble was considerably weakened, which was pointed out by a lowering in lipid peroxidation level and increasing of antioxidant enzymes (SOD, GPx and CAT) than control normal rats.

Superoxide dismutase (SOD), catalase (CAT) and the enzyme of the glutathione redo cycle i.e. glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rd) are the essential intracellular antioxidants and are estimated to be preventive or primary, antioxidant as they inhibit free radical chain reaction by lowering the available concentration of free radical to initiate the process (40).

**Table (5): Effect ashwagandha roots extract on antioxidant enzyme liver as glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GPx), and catalase (CAT) parameters.**

| Groups  | GSH (µ/g) ± SD | GPx (U.g⁻¹ Hb) ± SD | SOD (µ/g) ± SD | MDA (nmol/g) ± SD | CAT (U.g⁻¹ Hb) ± SD |
|---------|----------------|-------------------|---------------|-----------------|-------------------|
| Control | 330.54 ± 18.54| 17.95 ± 1.09      | 600.52 ± 32.43| 8.02 ± 1.30     | 179.12 ± 10.78    |
| CCl4    | 188.73 ± 12.74| 12.04 ± 2.23      | 470.63 ± 27.42| 14.20 ± 1.98    | 132.51 ± 4.84     |
| 100mg   | 275.67 ± 18.47| 14.79 ± 0.89      | 658.44 ± 33.65| 8.54 ± 1.21     | 149.57 ± 5.06     |
| 200mg   | 282.52 ± 16.40| 16.04 ± 1.08      | 673.40 ± 35.55| 8.99 ± 1.54     | 165.35 ± 8.76     |
| 300mg   | 295.28 ± 15.34| 16.95 ± 1.04      | 698.27 ± 31.29| 9.32 ± 1.89     | 170.25 ± 6.75     |
| 400mg   | 310.55 ± 20.22| 17.35 ± 1.27      | 711.00 ± 38.90| 10.54 ± 1.62    | 176.68 ± 9.27     |

Data are presented as mean (n=6 rats) ± standard deviation, values with different superscripts within the column are significantly different at P<0.05, while those with have similar or partially are not significant.

**HISTOPATHOLOGICAL EXPERIMENTAL:**

**Effect of ashwagandha roots extract on histopathological disorders:**

One of the four primary categories that have proven beneficial for estimation of hepatic damage in rats in the histological analysis of liver injury, thus biochemical finding in this study, were emphasized by the pathological change of the liver.

The light microscopic examination of normal control group liver sections (Fig. 1) shows the normal structure of the liver. The unit of liver tissue is the classic hepatic lobule (H. L.), which is surrounded by connective tissue. In the center of the hepatic lobule, there is a central vein (C. V.). The plates are radiating from the central vein through directions the periphery of the hepatic. It is clear that from figure (2) which represents the light microscopic examination of the injured group which received CCl4 two times per week for eight weeks there were extensive severe vascular degenerative changes in the hepatocytes. Few hepatocytes show granular degenerative changes. Another section of the hepatocytes of the same group figure (3) shows the congestion of portal blood vessels accompanied by severe degenerative changes hepatocytes are vacuolated and enlarged, cytoplasm appears faintly stained and the nuclei are shrunken. These results may be caused by the CCl4 which increased the production of free radicals which can directly bind to the hepatocellular membrane protein and lipids leading to alkylation reactions and possible enzyme inactivation (41).

Figures (4 and 5) showed that the light microscopic examination of liver sections which received carbon tetrachloride 40% in paraffin oil (3ml/kg-1 body weight, Sc) two times per week for eight week and 100 and 200 mg /kg-1, Po body weight
from ashwagandha roots extract separately in normal saline 5 ml/kg-1 Po body weight, four times per week for eight week that some hepatocytes show necrosis, others were suffering from granular derivative changes.

Figures (6 and 7) represented that the light microscopic examination of liver sections which received carbon tetrachloride 40% in paraffin oil (3ml/kg-1 body weight, Sc) two times per week for eight weeks and 300 and 400 mg/kg-1, Po body weight from ashwagandha roots extract separately in normal saline 5 ml/kg-1 Po body weight, four times per week for eight weeks that hepatocytes show slight granular derivative changes, others were necrotic in figure (6). Whereas figure (7) represents that hepatocytes showed that the slight diffuse vascular degenerative changes, others were necrotic.
CONCLUSION

The results from this study found that the ashwagandha roots extract had contained rich amounts from the natural antioxidant activity which can keep safe the liver from the harm caused by free radicals which came from as the result of CCl₄ metabolism. The results also appear that the ashwagandha roots extract which improved the antioxidant enzyme liver.

REFERENCES

[1] Singh N, Bhalla M, Jager P, Gilca M (2011). An overview on ashwagandha: A rasayana (rejuvenator) of ayurveda. Afr J Tradit Complement Altern Med 8: 208-213.
[2] Mishra LC, Singh BB, Dagenais S (2000) Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): A review. Altern Med Rev, 5: 334-346.
[3] Choudhary M, Kumar V, Malhotra H, Singh S (2015) Medicinal plants with potential anti-arthritis activity. J Intercult Ethnopharmacol, 4: 147-179.
[4] Paliyaguru DL, Singh SV, Kensing TW (2016) Withania somnifera: From prevention to treatment of cancer. Mol Nutr Food Res, 60: 1342-1353.
[5] Mehta J (2013). Development of low cost nutritive biscuits with Ayurvedic formulation. International Journal of Ayurvedic and Herbal Medicine. 2013; 3(3):1183-1190.
[6] Singh, G., Sharma, P. K., Dudhe, R. and Singh S. (2010). Biological activities of Withania somnifera, Annals of Biological Research, 2010, 1 (3) : 56-63
[7] Grandhi, A., Mujundar, A. M. and Patwardhan, B (1994). A comparative pharmacological investigation of ashwagandha and ginseng, Journal of Ethnopharmacology (Ireland), 1994: vol. 3, 131-135
[8] Jayaprakasam, B., Zhang, Y., Seeram, N.P and Nair, M.G. (2003), Growth Inhibition of Human Tumor Cell Lines by Withanolides from Withania somnifera Leaves, Life Sciences, 74,125-132.
[9] Kumar D, Bhat ZA, Shah MY. (2012). Effect of successive extracts of Stachys tibetica Vatke (Lamiaceae) in anxiety. Orient Pharm Exp Med, 12: 247-253.
[10] Patel, S B., Rao, N J. and Hingorani, L L. (2016). Safety assessment of Withania somnifera extract standardized for Withaferin A: Acute and sub-acute toxicity study, Journal of Ayurveda and Integrative Medicine 7 (2016) 30e37
[11] Riley, V. (1960). Adaptation of orbital bleeding technique to rapid serial blood studies. Proc. Soc. Exp. Biol. Med., 109 : 751-754.
[12] Dacie, J. V. and Lewis, S. M. (1984). Practical hematology. Churchill Living Stone. London and New York.
[13] Qawasmeh, A., Obied, H. K., Raman, A., & Wheatley, W. (2012). Influence of fungal endophyte infection on phenolic content and antioxidant activity in grasses: Interaction between Lolium perenne and different strains of Neotyphodium lolii. Journal of Agricultural and Food Chemistry, 60(13), 3381-3388.
[14] Eghdami, A., & Sadeghi, F. (2010). Determination of total phenolic and flavonoid contents in methanolic and aqueous extract of Achillea millefolium. Journal of Organic Chemistry, 2, 81-84.
[15] Miller, N.J.; Rice-Evans, C.A. (1997). Factors influencing the antioxidant activity determined by the ABTS.+ radical cation assay. Free Radic. Res. 1997, 26, 195–199. [CrossRef] [PubMed]
[16] Ramesh Kumar A., and Sivasudha T. (2012). In vitro antioxidant and antibacterial activity of aqueous and methanolic extract of Mollugo nudicaulis Lam Leaves, Asian Pac J Trop Biomed. 2(2), 895-900 (2012).
[17] Pell, J.D., Gee, J.M., Wortley, G.M. and Johnson, I.T. (1992). Both dietary corn oil and guar gum stimulate intestinal crypt cell proliferation in rats, by independent but potentially synergistic mechanisms. J. Nutr., 122, 2447–2456.
[18] Schumann G, Klaue R. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. Clin. Chem. Acta, 2003, 327: 69-79.
[19] Belfield A and Goldberg DM. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. Enzymemol., 1971, 12(5): 561-586.
[20] Aebi, M.E. (1995): Catalase. In: Bergmeyer J, Grabl BM (eds) Methods of Enzymatic Analysis vol. III. Enzymes oxidoreductases, 3rd ed. Weinheim: Verlag-Chemie. Pp 273-286.
[21] Janknegt, P.J.; Rijstenbil, J.W.; van de Poll, W.H.; Gechev, T.S. and Buma, A.G. (2007): A comparison of quantitative and qualitative superoxide dismutase assays for application to low temperature microalgae. J. Photoch em. Photobiol. B Biol., 87, 218-226.
