Egyptian Journal of Nutrition
Official journal of the Egyptian Nutrition Society
Print ISSN: **1687-1235**
Online ISSN: 2090-2514
Vol. 40 (2): 14 – 35 (2025)
DOI:10.21608/enj.2025.432351
https://ejn.journals.ekb.eg/

Hepatorenal Effect of Truffle on Oxidative Stress in Male Albino Rats

Heba Ibrahim Mahmoud[1], Osama El-Sayed Mostafa[2] Mohammed Hamdy Haggag[1]

1-Nutrition and Food Sciences Department., Faculty of Home Economics, Helwan University, Cairo Egypt.

2- Home economics Dept., Faculty of Specific Education, Ain Shams University, Cairo, Egypt

hebaewais1@gmail.com

ABSTRACT

This research was conducted in order to examine the efficacy of truffle on eliminating oxidative stress of male albino rats. Thirty-six male rats were divided into six groups (6 rats of each) as follow; The 1st was fed on basal diet as a negative control group, the 2nd was as the same of group 1 and was intraperitoneally injected with potassium dichromate at a dose 10mg/kg BW as a positive control group, the 3rd, 4th and 5th as the same of group 2 with 300, 400 and 500 mg hydroethanolic truffle dried extract/Kg BW. Whereas, the 6th group as the same of group 2 with 500 mg truffle powder /Kg BW. At the end of experiment (after 8 weeks), rats were fasted over night before scarifying. Serum kidney and liver functions, oxidative and anti-oxidative markers were analysed. Kidney and liver were removed from each rat to histopathological examination. The results indicated that truffle extract or its powder caused significant decreased ($P<0.05$) in serum CR, urea, AST, ALT, ALP and MDA but significant increase in CAT compared to the +ve control group. Otherwise, histopathological examination showed improvement on kidney and liver of groups treated with truffle extract(as concentration 500mg group 5) or its powder(as concentration 500mg group 6) compared with +ve control group. According to these above results, the usage of truffle extract or its powder as natural agent may be effective in reducing the risk of oxidative stress so may be play a therapeutic role of human beings.

Key words: Truffle, hepatorenal, antioxidants, polyphenols, histopathological examination

Received: 28-5-2025 ***Accepted: 9-6-2025*** ***Published: Issue2-2025***

INTRODUCTION

Oxidative stress is defined as “an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage. The term “antioxidants” in cellular defense against oxidants predominantly includes antioxidant enzymes with their substrates and coenzymes. Exogenous low-molecular-mass compounds also have a role, but this is more limited. Multiple biomarkers of damage due to oxidative stress have been identified for different molecular classes (protein, lipid, carbohydrate, and DNA) **Sies, (2020).**

Aerobic organisms, including humans, can efficiently produce adenosine triphosphate (ATP) using oxygen in the air. However, 1–2% of the oxygen consumed becomes a reactive oxygen

species (ROS) called superoxide ($O_2^{\cdot}$), and mitochondria are constantly exposed to oxidative stress. In addition, a large amount of ROS is produced from inflammatory cells such as neutrophils, and the body is exposed to strong oxidative stress. At the same time, the body is exposed to various stimuli, not only from within the body but also from what is called the exposome (ultraviolet rays, radiation, tobacco, alcohol, food additives, environmental hormones, etc.), and is exposed to oxidative stress from outside the body **Ichikawa, (2025)**

Desert truffle belongs to the Pezizales order (Ascomycetes). It grows naturally after rainfall during the time of Al-Wasm (more than 200 mL from September to October) in arid and semiarid zones. The desert truffle is a macro-fungus that grows under the surface of the earth and is found in the countries of the Arabian Peninsula and Iraq, in addition to the regions of North Africa. Two genera are very famous in these regions of Middle Eastern including *Terfezia sp*. and *Tirmania sp*. The truffle amounts increase according to the average of rainfall, especially in the time of Al-Wasm. The desert truffle also has many health benefits and nutritional value depending on its chemical composition; it enjoys in terms of the presence of proteins, amino acids, carbohydrates, and fibers, in addition to a suitable and low amount of fat and energy **Khan *et al.,(* 2022).**

Truffles provide numerous health benefits due to their bioactive compounds. They have antioxidant, anticancer, antiviral, antimicrobial, liver protective, anti-mutagenic, and anti-inflammatory properties. Truffles are abundant in bioactive compounds, including ascorbic acid, ergosterol, phenolics, flavonoids, terpenoids, phytosterols, and polysaccharides etc. **Elkhateeb *et al*.,(2024).** So, this research was conducted in order to examine the efficacy of truffle on eliminating oxidative stress on experimental rats.

MATERIALS AND METHODS

Materials

Thirty-six male albino rats (150 ± 5 g) were obtained from Helwan Farm, Cairo, Egypt. The basal diet ingredients were procured from El-Gomhoria Pharmaceutical Company, Cairo, Egypt, while soy oil, sucrose, and starch were sourced from the Egyptian local market. Potassium dichromate was also purchased from El-Gomhoria Pharmaceutical Company. Black truffle (Terfezia claveryi) was collected from Sinai, Egypt. All biochemical assay kits used in the study were obtained from Gamma Trade, Giza, Egypt.

Methods

Truffle Extract Preparation

The dry slice samples were ground to powder in order to increase the efficiency of extraction. The sample powder was twice pretreated with 70% ethanol in order to remove small molecular compounds, which can dissolve in ethanol. After being extracted by ethanol, the truffle sample was mixed with deionized water in the ratio of (1:5 v/v). The mixture was but in hot water (40 ˚C) for 2 h and the extract was collected and filtered. Rotary evaporator was used to concentrate hydroethanolic extract **Tongze et al., (2018).**

Induction of Oxidative stress

Oxidative stress was induced by ip injecting potassium dichromate at a dose of 10 mg/kg Body weight., once per 24 h for 5 days **Anita, (2009).**

Proximate chemical composition of Truffle was determined according to AOAC **Chemists and Cunniff, (1990)** methods. Phenolic compounds in truffle powder and its extract were analysed according to the method described by **Hatzidimitriou et al.,(2007).** Free radical scavenging

activity was measured in truffle powder and its extract by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) **Leaves and Leaves, (2014).** Basal diet was formulated according to **Reeves et al., (1993).**

Experimental design

Rats were housed in Post Graduated Animal Lab in Nutrition and Food Science Department Faculty of Home Economic Helwan University. Rats were fed basal diet for one week as an adaptation period. After this week rats were divided into 6 groups as followed, Group1, rats were fed basal diet for 4 weeks and served as -ve control group. Group2, as the same of group 1 but was injected ip with potassium dichromate in order to induce oxidative stress. Groups3, 4 and 5 as the same of group 2 with 300, 400 and 500 mg/Kg bw. of Truffle extract, respectively. Group 6, as the same of group 2 with 500 mg/Kg bw. of powdered truffle.

During the experimental period, water was introduced ad-libitum with hygienic conditions. At the end of experiment (after 8 weeks), rats were fasted over night before scarifying. Blood was collected then centrifuged to obtain serum for biochemical analysis. kidney and liver were removed from each rat to histopathological examination. Feed intake was recorded daily, body weight gain assessed weekly and feed efficiency ratio was calculated at the end of the experiment according to (Chapman et al., 1959). After animals sacrificing, the blood samples were collected into tubes and centrifuged for 15 min at 3,000 rpm then serum was separated into vacuum tubes and stored at -20˚C tell used.

Serum urea and creatinine (CR) were determined according to (Burtis and Ashwood, 1999). Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed according to (IFCC, 1976; IFCC, 1980 and GSCC,1972), respectively. Malondialdehyde (MDA) and catalase (CAT) were determined according to (Ohkawa et al., 1979 and Claiborne, 1985), respectively. For histological examination, auto samples were taken from kidney and liver of each rat in different groups and fixed in 10% formal saline for twenty-four hours (Bancroft and Stevens, 1996). Statistical analysis was carried out using SPSS program Version 16 according to (SPSS, 1986).

RESULTS AND DISCUSSION

The chemical composition of the truffle powder is presented in Table (1). Truffle powder (dry weight basis) contains high amount of carbohydrate followed by fat, protein, Fiber and ash with values 59, 19, 15, 5, 2 g/100g, respectively. Therefore, truffle also contains energy 469 kcal/100g and total solid 96.40%.

Khlaif *et al.*, (2021) and Ljubojević *et al.*, (2023), results agreed with research's who reported that *Terfezia claveryi* rich in carbohydrates, proteins and low in fat. Chemical analysis showed that Tuber aestivum contains about 75.5 % water and about 25.5 % dry matter. The most common group of compounds were carbohydrates, followed by proteins, while the mineral component and fats were much less presented but disagreed with the concentration of lipid that said the truffle content of lipid was high may be due to environmental factors, weather and harvest seasons.

A total of 15 phenolic acids identified in truffle powder as shown in Table (2). The main phenolic compounds presented in truffle powder were catechin 6.95 mg/g, gallic acid 4.9 mg/g, chlorogenic acid 1.33 mg/g and Syringic acid 0.48 mg/g. In addition, truffle powder has a small amount of Caffeic acid 0.28 mg/g and Methyl gallate 0.11 mg/g. truffle powder has a trace of

phenolic acids as (Rutin, Ellagic acid, Coumaric acid, Ferulic acid, Naringenin, Rosmarinic acid, Cinnamic acid ,Kaempferol and Quercetin).

A total of 15 phenolic acids identified in truffle extract as shown in Table (2). The main phenolic compounds presented in truffle powder were gallic acid 162.16 mg/g, chlorogenic acid 18.95 mg/g, Hesperetin 3.04 mg/g, Kaempferol 2.91 mg/g and Syringic acid 1.30 mg/g. In addition, truffle extract has a small amount of caffeic acid, ferulic acid, methyl gallate, rutin, naringenin, ellagic acid, quercetin, rosmarinic acid, Catechin and coumaric acid were the phenolic acid in truffle extract with the levels of 0.99, 0.61, 0.58, 0.46, 0.41, 0.36, 0.28, 0.23 0.15 and 0.13 mg/g, respectively. These results were in harmony with Villares *et al.,* (2012).

The results displayed as the most effective methods DPPH, with IC_{50} values are illustrated in Table (3). The truffle powder and its extract, which have amounts significant of DPPH radical scavenging activity. The strongest DPPH radical scavenging activity with an IC50 of truffle extract was 89.07µg/mL, followed truffle powder with IC_{50} values of 17.4µg/ml.

The highest DPPH radical scavenging activity with an IC_{50} of truffle extract was 74.4, 67.4, 60.4, 53.0, 46.1, 33.3, 25.9, 18.6, 11.3µg/mL, respectively. While the strongest DPPH radical scavenging activity with IC_{50} of truffle powder was 89.4, 83.5, 76.6, 69.5, 62.6, 55.6, 48.5, 41.7, 35.9, 28.5 µg/mL, respectively.

These results were in agreement with **Hamza *et al*., (2016)** that reported that DPPH radical-scavenging activity Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. Extracts of *T. boudieri* truffle were subjected to DPPH radical-scavenging activity, The methanolic extract containing the highest amounts of phenolics and flavonoids. These results agreed with the fact that free radical-scavenging activity is greatly influenced by the phenolic compounds of that show in Table (2).

The results of feed intake (FI) showed that, the +ve control group significantly decreased compared with the -ve control group with mean values 17.22 vs 18.93g/day, respectively. While the FI levels of all treated groups which fed truffle extract or its powder were significantly increased compared to the +ve control group with mean values 18.40, 18.84, 18.76, 18.60 vs. 17.22g/day, respectively. The highest improvement among all treated groups was recorded in group 4 which was fed on 400 mg truffle extract orally.

The results of body weight gain (BWG) of the +ve control group significantly decreased compared with the -ve control group with mean values 0.99±0.023 vs 1.45±0.018 g/day, respectively. While the BWG values of all treated groups which fed truffle extract or its powder were significantly increased compared to the +ve control group with mean values 1.22±0.017, 1.25±0.017, 1.44±0.083, 1.24±0.020 vs. 0.99±0.023 g/day, respectively. The highest improvement among all treated groups were recorded in group 5which was fed 500mg truffle extract.

The results of feed efficiency ratio (FER) of the +ve control group significantly increased compared with the -ve control group with mean values 0.05±0.001 vs 0.07±0.009, respectively. While the FER ratio of all treated groups which fed truffle extract or its powder were significantly increased compared to the +ve control group with mean values 0.06±0.007, 0.06±0.008, 0.07±0.004, 0.06±0.009 vs. 0.05±0.001, respectively. The highest improvement among all treated groups was recorded in group 5which was fed 500mg truffle extract.

These results agreed with **Dyary, (2020)** who reported that the rats treated with the methanolic extract of *T. claveryi* exhibited normal water intake and feeding, compared to the control; there were no differences between the control and treated groups in the amount of feed and water consumption ($P>0.05$). There was no significant difference ($p>0.05$) in the rate of

weight gain between the control and groups treated with T. *claveryi* methanolic extract. The results of body weight gain (BWG) showed the +ve control group significantly decreased compared with the -ve control group. While the (BWG) levels of all treated groups which fed truffle extract or its powder were significantly increased compared to the +ve control group as may be the chemical composition of truffle which has high content of lipid and protein as shown in **Table 1.**

Regarding to kidney functions (Table 5), data revealed that serum creatinine (CR) of the +ve control group significantly increased compared to the -ve control group with mean values 0.59±0.003 vs. 0.32±0.008mg/dl, respectively. While the level of serum creatinine (CR) of all treated groups which fed truffle extract or its powder were significantly decreased compared to +ve control group with mean values 0.50±0.005, 0.46±0.004, 0.35±0.006, 0.34±0.006 vs. 0.59±0.003 mg/dl, respectively. The highest improvement among all treated groups was recorded in group 6 which was fed 500mg truffle powder.

Concerning the serum urea, the mean value of +ve control group significantly increased compared with -ve control group with mean values17.08 ±00.82 vs. 13.74± 01.84 mg/dl respectively .Whereas, the level serum urea of all treated groups which fed truffle extract or its powder were significantly decreased compared to +ve control group with mean values 16.03±01.54, 15.11±00.96 , 14.42±00.72 , 14.15±00.51 vs. 17.08±00.82 mg/dl, respectively. The highest improvement among all treated groups was recorded in group 6 which was fed 500mg truffle powder.

These above results were in harmony with **Dyary, (2020)** who reported that the creatinine and urea levels in *T. claveryi* treated rats' serum did not differ significantly from the -ve control group rats. *Tuber brumale vittadini* (ST) extract caused a significant decrease of creatinine level in the blood. This may be due to antioxidants of ST extract. Higher content of vitamin E and Zinc in ST extract may be decreased creatinine levels in the blood as reported by **Moussa, (2011)**.**Wu *et al.*, (2022)** and **Althobaiti *et al.*, (2022)** proved the phenolic and flavonoid compounds and their main derivatives (e.g., flavanones, isoflavonoids, flavanols, flavones, flavonols, and anthocyanidins) content of the black truffle extract display their anti-cancer effects through induction of apoptosis, control of the activities of reactive oxygen species (ROS)-scavenging enzymes, suppressing the cell cycle, hang-up of the cell proliferation, etc.; instead, it has been proven that flavonoids are considered as antioxidants during common situations and are effective pro-oxidants in cancer cells activating the pathways of apoptotic and reducing the pro-inflammatory mediators as cleared in Table 2 regulate various metabolic pathways.

Concerning to liver functions (Table 6), the activity of serum aspartate aminotransferase (AST) of the +ve control group significantly increased compared with the -ve control group with mean values 178.00±2.99 vs 121.17±3.20 U/L, respectively. While the AST activity of all treated groups which fed truffle extract or its powder were significantly decreased compared to the +ve control group with mean values 164.20±3.06, 151.60±1.97, 133.00±0.99, 145.33±1.55 vs. 178.00±2.99 U/L, respectively.

Regarding to the results of serum alanine aminotransferase (ALT), there was significant increase in the +ve control group compared to the -ve control group with mean values 92.20±1.800 vs 52.83±1.67 IU/L, respectively. While the ALT activity of all treated groups which fed truffle extract or its powder were significantly decreased compared to +ve control group with mean values 80.80±2.00, 73.40 ±1.49, 59.80±0.89, 63.00 ±1.02 vs. 92.20±1.800 U/L, respectively.

Concerning the serum alkaline phosphatase (ALP) significant increases was observed in the +ve control group compared with the -ve control group with the mean values 142.80±0.95 vs 83.00±0.89 U/L, respectively. Whereas, the ALP activity of all treated groups which fed truffle extract or its powder were significantly decreased compared to +ve control group with mean values 109.20±1.01, 95.00±1.11, 87.60 ±0.79, 91.33 ±1.00 vs 142.80±0.95 U/L, respectively.

The highest improvement among all treated groups of all tested liver functions was recorded in group 5 which was fed 500mg truffle extract.**Mohamed *et al.*, (2022)** results agreed with this obtained research results which reported that black truffle (BT) and its extracts induced a remarkable reduction in the concentrations of liver functions, the effect can be explained by the high antioxidant contents in BT such as vitamin C and ß-carotene. Both materials help to keep the integrity of the plasma membrane, and the destruction of the plasma membrane which in turn prevents the release of cytosolic enzymes such as ALP, ALT, and bilirubin into the blood stream and suppressed the decrease of albumin concentration**. Janakat and Nassar,(2010**) reported that can be attributed to the high antioxidants contents in T. *claveryi*, such as vitamin C and ß-carotene) which stop the mounting of peroxyl radical formation and preventing plasma membrane bleb formation, which conserve the integrity of the plasma membrane from rupturing and cytosolic enzymes such as ALP, ALT and AST from being released into the blood stream as Table 2 and 3.

Table 8 showed oxidative and antioxidative biochemical parameters of rats fed truffle extract or its powder. Data revealed that serum malondialdehyde (MDA) of +ve control group significantly increased compared with -ve control group with mean values 2.01±0.07vs. 00.88±0.01 nmol/ml, respectively. While the level of MDA of all treated groups which was fed truffle extract or its powder were significantly decreased compared to +ve control group with mean values 1.79±0.04, 0.93±0.02, 0.90±0.02, 0.98± 0.01vs. 2.01±0.07nmol/ml, respectively. The highest improvement among all treated groups was recorded in group 5 which was fed 500mg truffle extract.

Concerning the serum catalase (CAT), the mean value of CAT of +ve control group significantly decreased compared with -ve control group with mean values 12.85±1.07 vs. 15.73±1.01 U/L, respectively. Whereas the level CAT of all treated groups which fed truffle extract or its powder were significantly increased compared to +ve control group with mean values 13.66±0.09, 15.08±1.10,15.34±1.05,14.50 ±0.99vs. 12.85±1.01 U/L, respectively. The highest improvement among all treated groups was recorded in group 5 which was fed 500mg truffle extract.

These results were in harmony with **Ayed *et al.*, (2021)** who reported that *T. claveryi* exhibit a higher oxidative inhibition on lipid peroxidation as well as deoxyribose and able to scavenge nitric oxide radical. attributed that decreasing the level of malondialdehyde during using truffles due to it contains of antioxidants such as vitamin A, C, β -carotene and phenolic compounds in according to **Table 2** and **3**, which can scavenge peroxyl radicals and chelate ferric ions, thus reducing lipid peroxidation**. Mohamed *et al.*, (2022)** results agreed with this research which was proved that rats treated with both aqueous and methanolic BT extracts showed a significant improvement in the antioxidant status of the liver tissues. The results showed that TAC, SOD, CAT enzymes as sort of antioxidants significantly increased in the liver of treated rats accompanied by a reduction in the expressed levels of both MDA and 8-Oxo-dG. Whenever rats treated with aqueous BT extract showed more enhancements in the levels of TAC, SOD, and CAT enzymes with a reduction in the levels of MDA and 8- Oxo-dG.

Histopathological examination of live

Microscopically, liver of rats from group 1 showed hydropic degeneration of hepatocytes (photos. 1a and 1b). On contrary, liver of rats from group 2 demonstrated histopathological alterations characterized by hepatocellular steatosis (photos. 2a – 2d), Kupffer cells activation (photo. 4), fibroplasia in the portal triad and newly formed bile ductulus (photos. 5 and 6). Meanwhile, liver of rats from group 3 exhibited vacuolar degeneration of some hepatocytes

(photos 3a, 3b and 3c) and small focal hepatocellular necrosis (photo. 3c). On the other hand, liver of rats from group 4 showed improved picture than the control positive group, hepatic tissue revealed hydropic degeneration of some hepatocytes (photos. 4a, 4b and 4c) and portal infiltration with few inflammatory cells (photo. 4c). Furthermore, liver of rats from group 5 exhibited vascular degeneration of hepatocytes (photos. 5a, 5b and 5c). Additionally, examined hepatic sections of rats from group 6 showed vascular degeneration of hepatocytes (photos. 6a and 6c), hepatocellular steatosis of some hepatocytes (photo. 6b) and focal hepatocellular necrosis associated with inflammatory cells infiltration (photo. 6c).

Histopathological examination of kidneys:

Light microscopic examination of kidneys sections of rats from group 1 revealed the normal histological structure of renal parenchyma (photos. 7a and 7b). In adverse, kidneys of rats from group 2 exhibited histopathological lesions characterized by vacuolar degeneration of epithelial lining renal tubules (photo. 8a) and eosinophilic proteinaceous material in the lumen of renal tubules (photo. 8b). Likewise, kidneys of rats from group 3 exhibited vacuolar degeneration of epithelial lining some renal tubules (photos. 9a and 9b) and eosinophilic proteinaceous material in the lumen of renal tubules (photo. 9c). On the other hand, some examined sections from group 4 revealed apparent normal renal parenchyma (photo. 10a), whereas other sections demonstrated slight vacuolization of epithelial lining some renal tubules (photos. 10b and 10c). Furthermore, kidneys of rats from group 5 showed vacuolization of epithelial lining some renal tubules (photos. 11a and 11b) and eosinophilic proteinaceous material in the lumen of sparse renal tubules (photo. 11c). Moreover, kidneys of rats from group 6 described vacuolization of epithelial lining some renal tubules (photos. 12a, 12b and 12c) and eosinophilic proteinaceous material in the lumen of sparse renal tubules (photo. 12c).

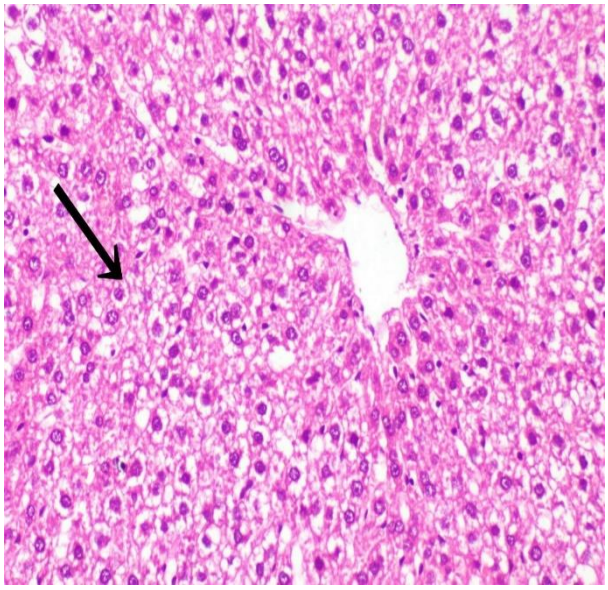

photo. (1a): Photomicrograph of liver of rat from group 1 showing hydropic degeneration of hepatocytes (black arrow) (H and E X 400).

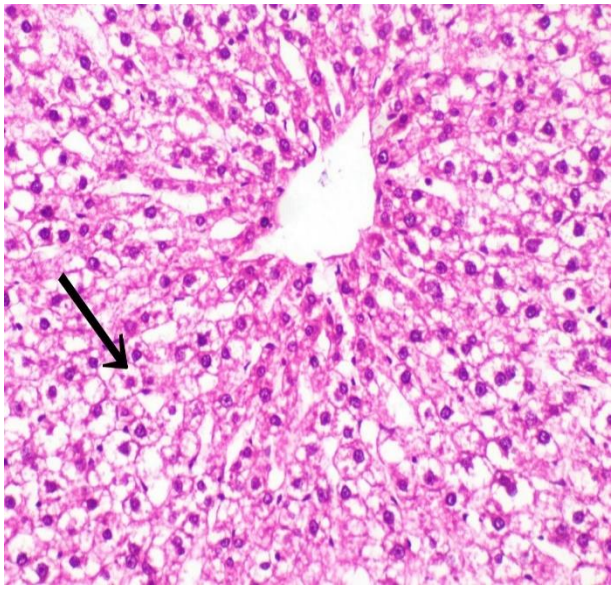

photo. (1b): Photomicrograph of liver of rat from group 1 showing hydropic degeneration of hepatocytes (black arrow) (H and E X 400).

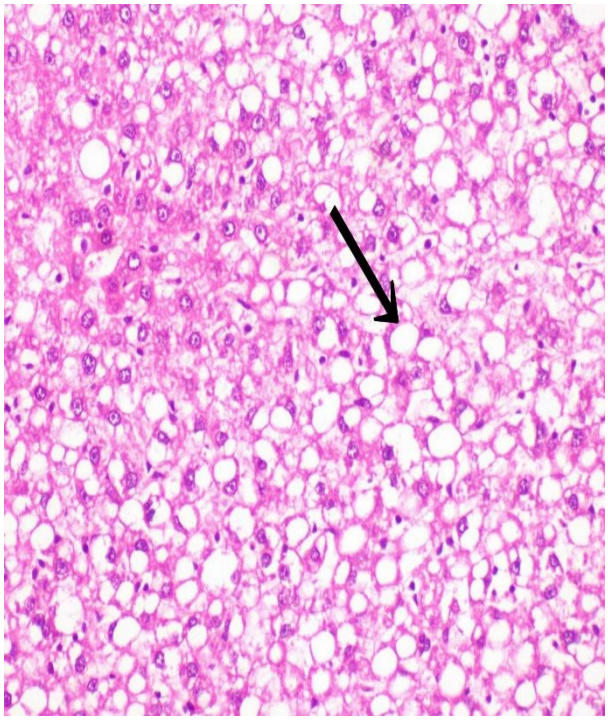

photo. (2a): Photomicrograph of liver of rat from group 2 showing hepatocellular steatosis (black arrow) (H and E X 400).

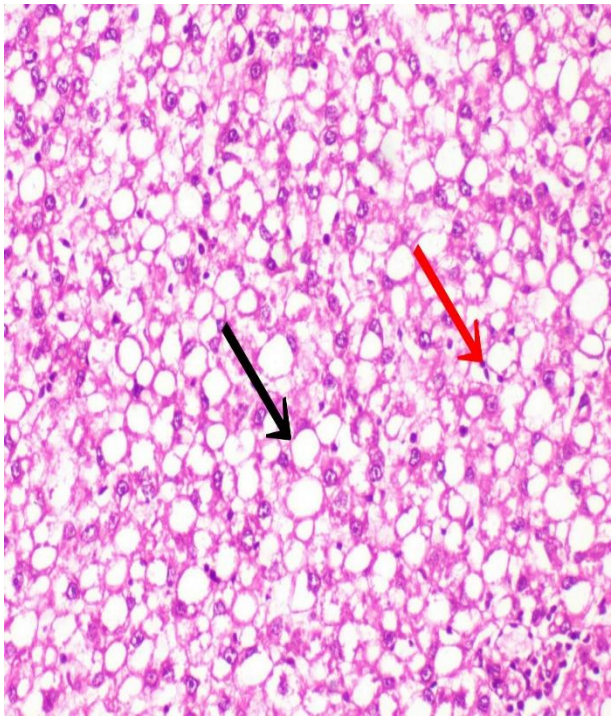

photo. (2b): Photomicrograph of liver of rat from group 2 showing hepatocellular steatosis (black arrow) and Kupffer cells activation (red arrow) (H and E X 400).

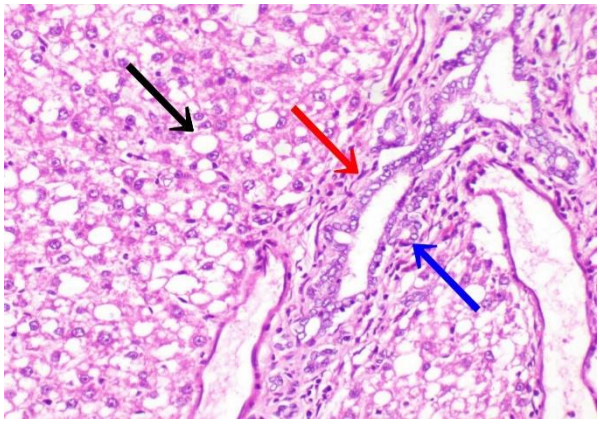

photo. (2c): Photomicrograph of liver of rat from group 2 showing hepatocellular steatosis (black arrow), fibroplasia in the portal triad (red arrow) and newly formed bile ductuoles (blue arrow) (H and E X 400).

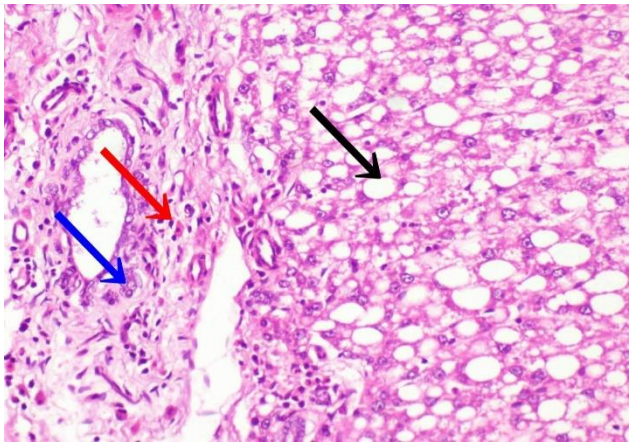

photo. (2d): Photomicrograph of liver of rat from group 2 showing hepatocellular steatosis (black arrow), fibroplasia in the portal triad (red arrow) and newly formed bile ductuoles (blue arrow) (H and E X 400).

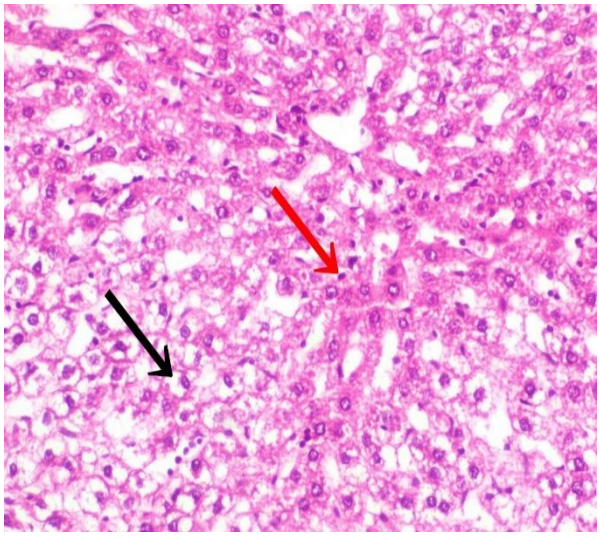

photo. (3a): Photomicrograph of liver of rat from group 3 showing vacuolar degeneration of some hepatocytes (black arrow) and slight Kupffer cells activation (red arrow) (H and E X 400).

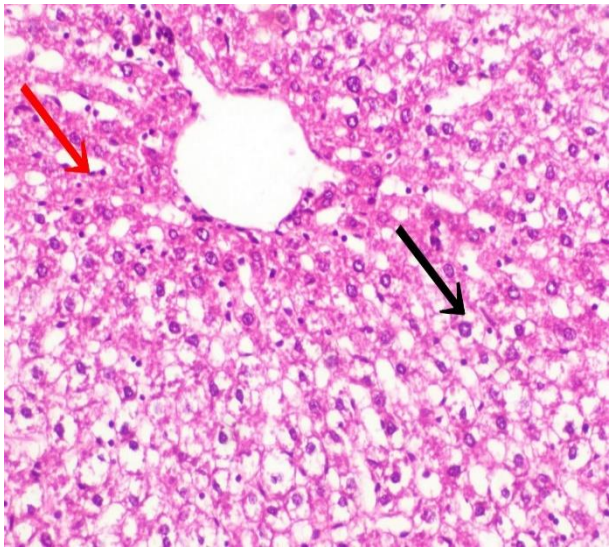

photo. (3b): Photomicrograph of liver of rat from group 3 showing vacuolar degeneration of some hepatocytes (black arrow) and slight Kupffer cells activation (red arrow) (H and E X 400).

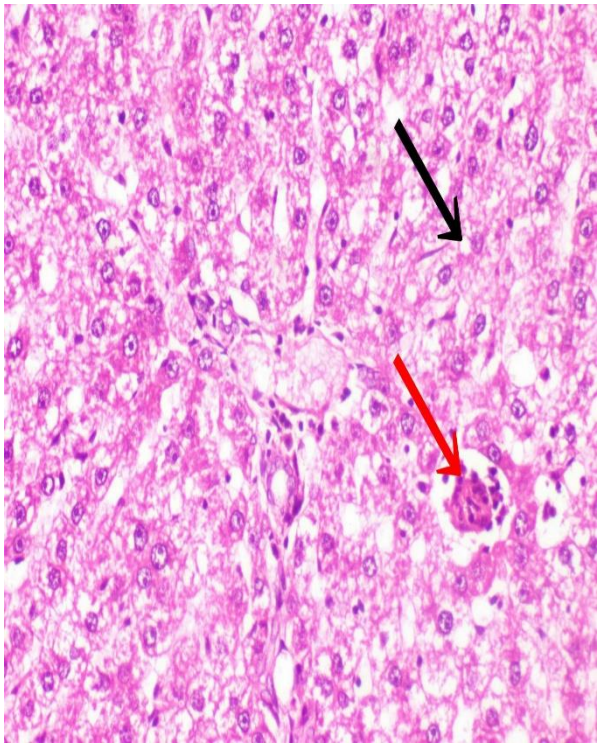

photo. (3c): Photomicrograph of liver of rat from group 3 showing vacuolar degeneration of some hepatocytes (black arrow) and small focal hepatocellular necrosis (red arrow) (H and E X 400).

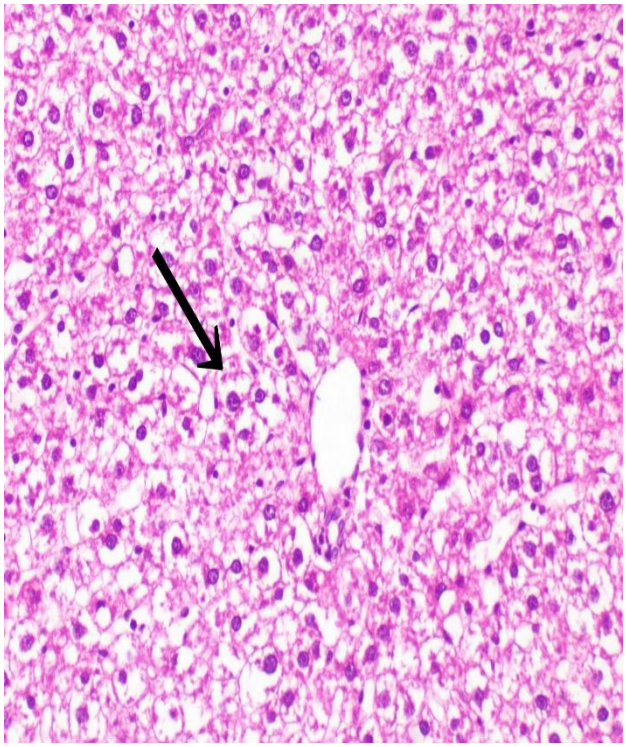

photo. (4a): Photomicrograph of liver of rat from group 4 showing hydropic degeneration of some hepatocytes (black arrow) (H and E X 400).

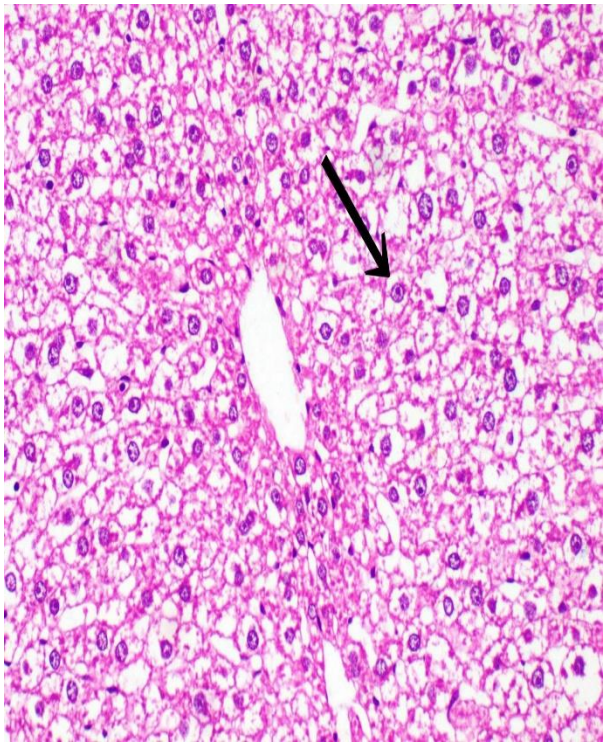

photo. (4b): Photomicrograph of liver of rat from group 4 showing hydropic degeneration of some hepatocytes (black arrow) (H and E X 400).

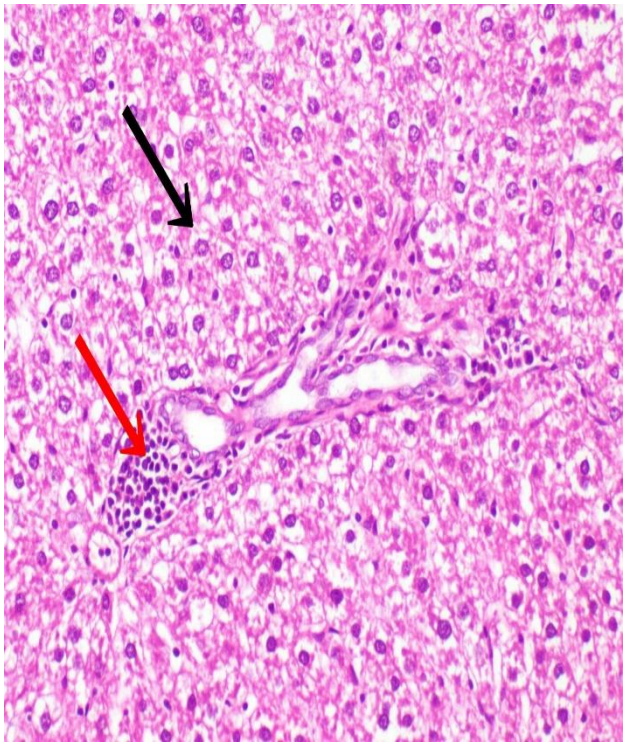

photo. (4c): Photomicrograph of liver of rat from group 4 showing hydropic degeneration of some hepatocytes (black arrow) and portal infiltration with few inflammatory cells (H and E X 400).

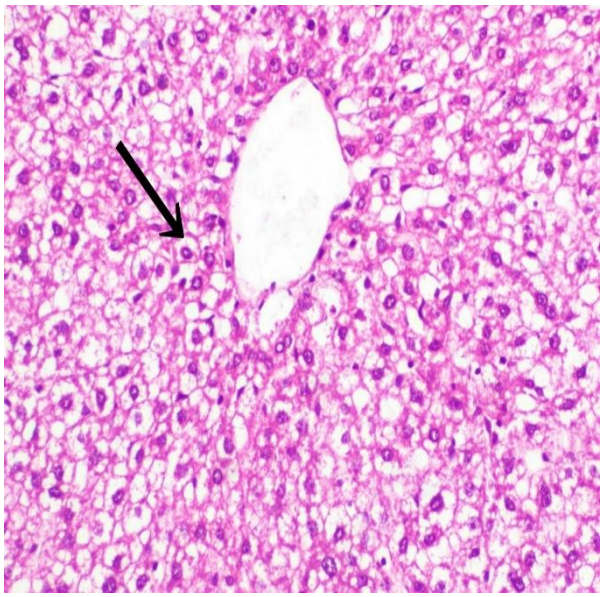

photo. (5a): Photomicrograph of liver of rat from group 5 showing vacuolar degeneration of hepatocytes (black arrow) (H and E X 400).

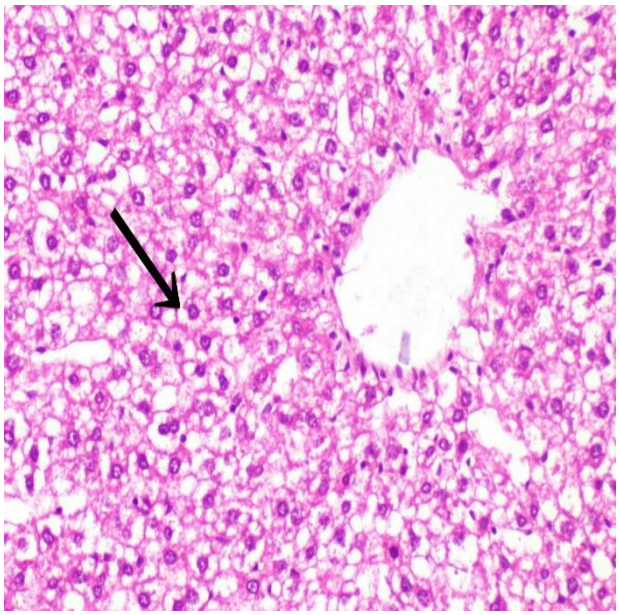

photo. (5b): Photomicrograph of liver of rat from group 5 showing vacuolar degeneration of hepatocytes (black arrow) (H and E X 400).

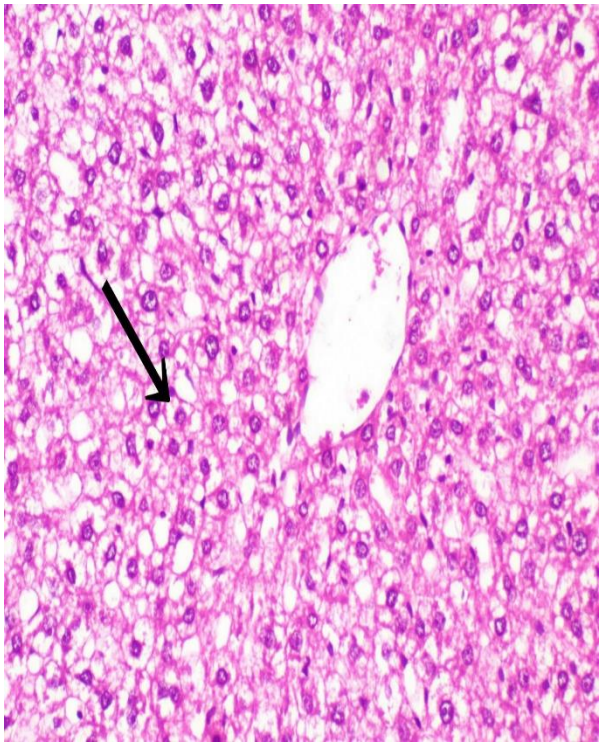

photo. (5c): Photomicrograph of liver of rat from group 5 showing vacuolar degeneration of hepatocytes (black arrow) (H and E X 400).

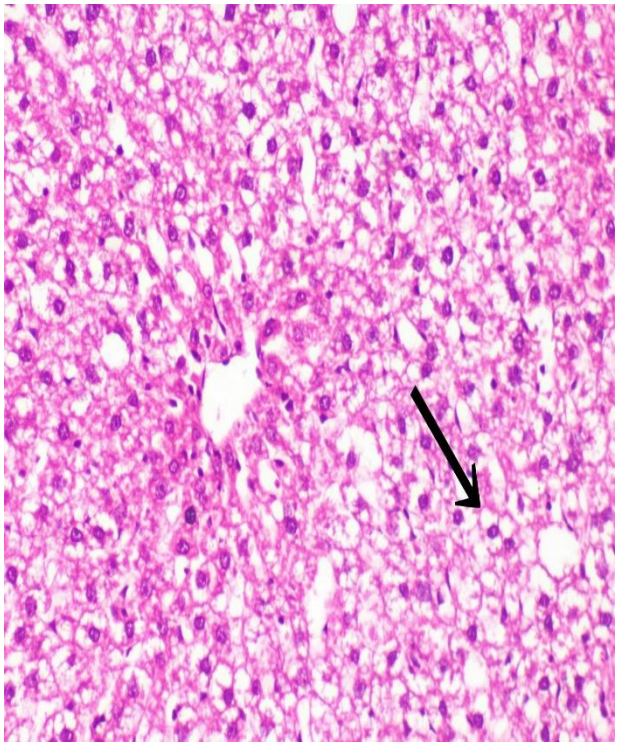

photo. (6a): Photomicrograph of liver of rat from group 6 showing vacuolar degeneration of hepatocytes (black arrow) (H and E X 400).

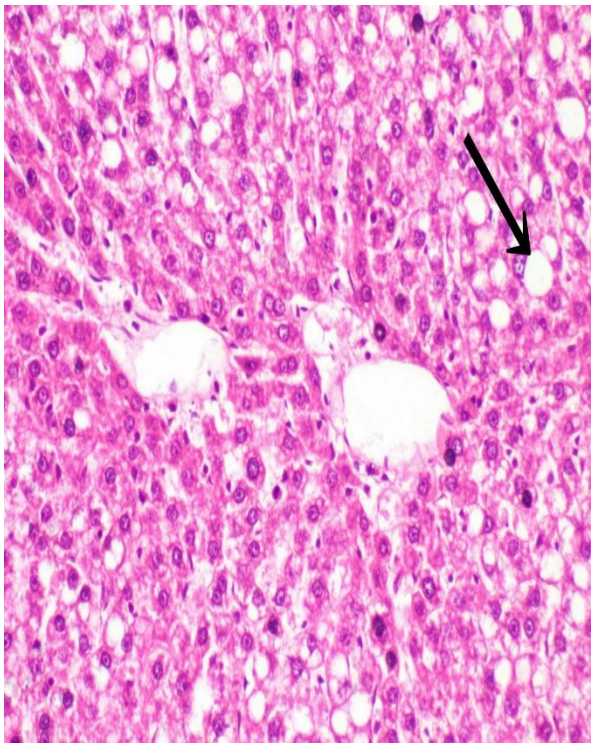

photo. (6b): Photomicrograph of liver of rat from group 6 showing hepatocellular steatosis of some hepatocytes (black arrow) (H and E X 400).

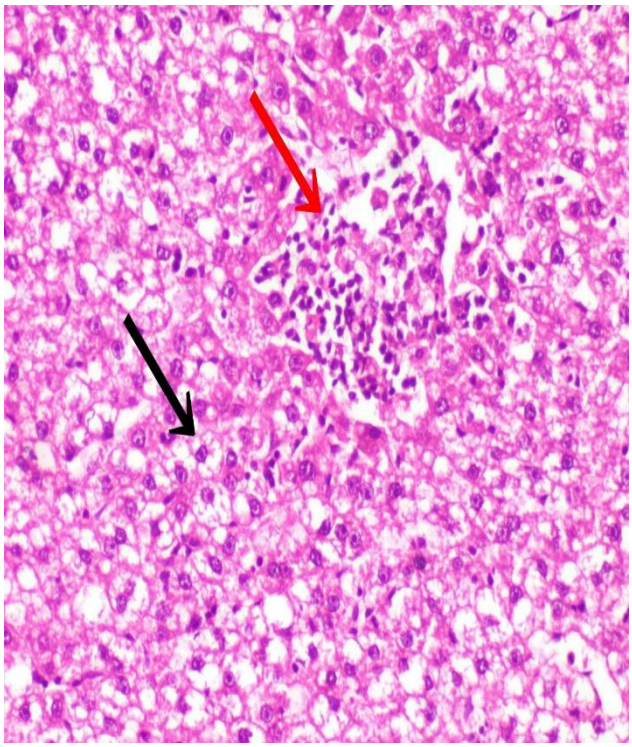

photo. (6c): Photomicrograph of liver of rat from group 6 showing vacuolar degeneration of hepatocytes (black arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (red arrow) (H and E X 400).

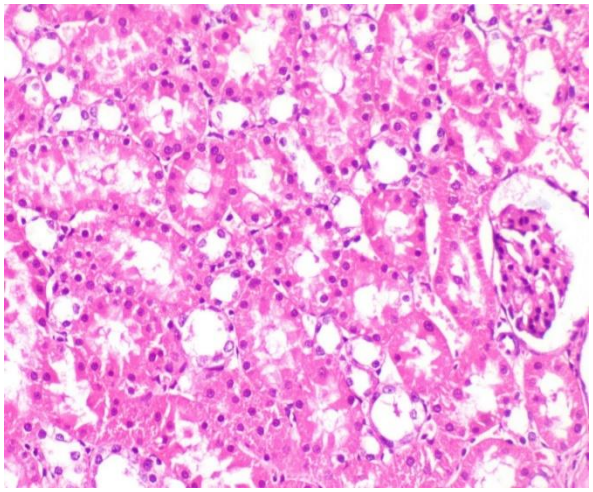

photo. (7a): Photomicrograph of kidney of rat from group 1 showing normal histological structure of renal parenchyma (H and E X 400).

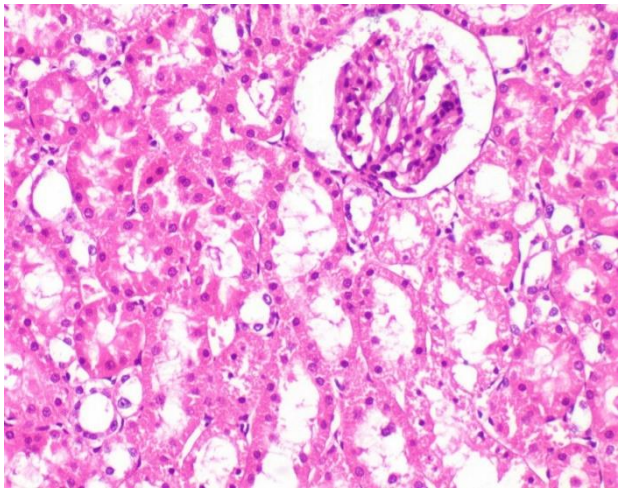

photo. (7b): Photomicrograph of kidney of rat from group 1 showing normal histological structure of renal parenchyma (H and E X 400).

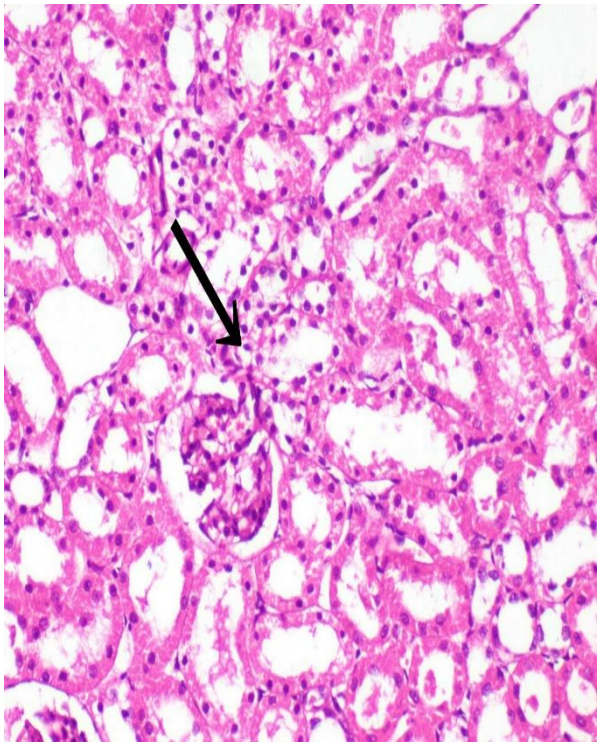

photo. (8a): Photomicrograph of kidney of rat from group 2 showing vacuolar degeneration of epithelial lining renal tubules (black arrow) (H and E X 400).

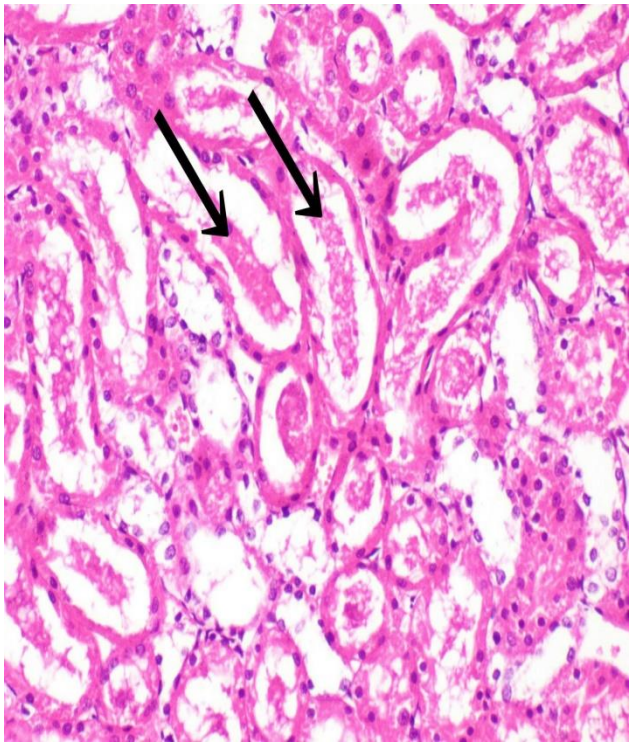

photo. (8b): Photomicrograph of kidney of rat from group 2 showing eosinophilic proteinaceous material in the lumen of renal tubules (black arrow) (H and E X 400).

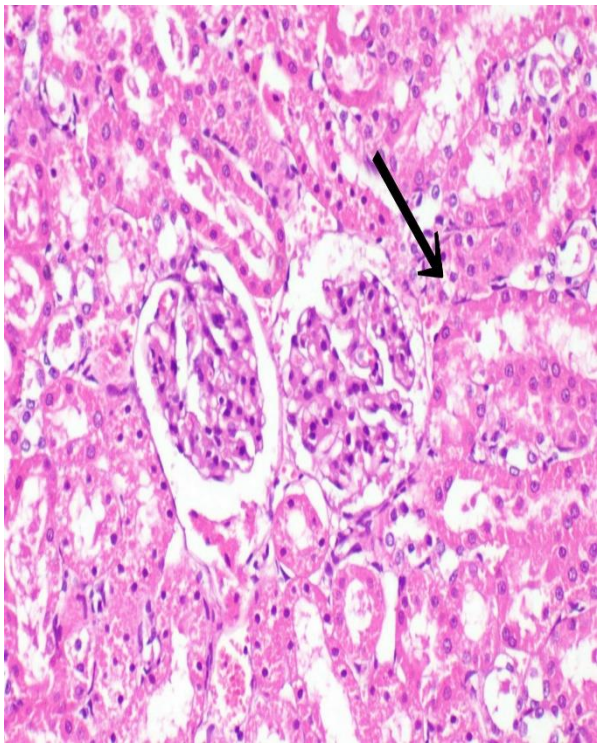

photo. (9a): Photomicrograph of kidney of rat from group 3 showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) (H and E X 400).

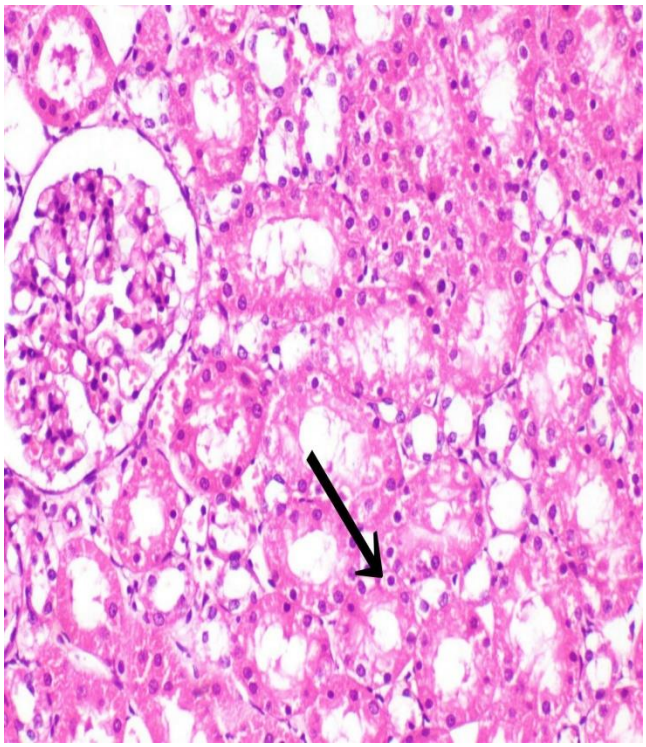

photo. (9b): Photomicrograph of kidney of rat from group 3 showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) (H and E X 400).

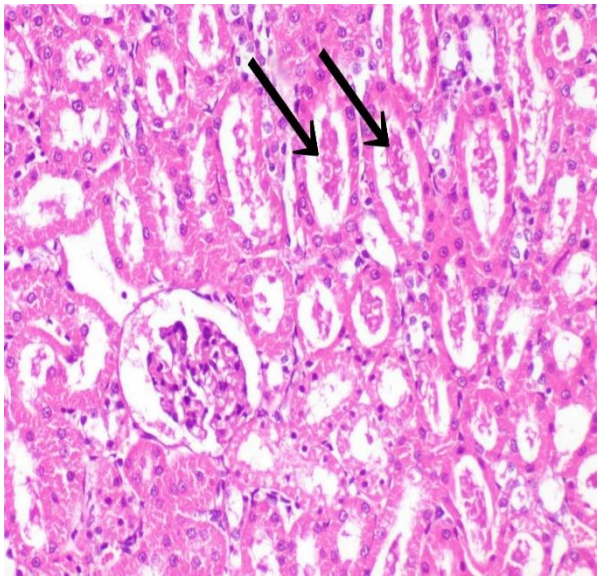

photo. (9c): Photomicrograph of kidney of rat from group 3 showing eosinophilic proteinaceous material in the lumen of renal tubules (black arrow) (H and E X 400).

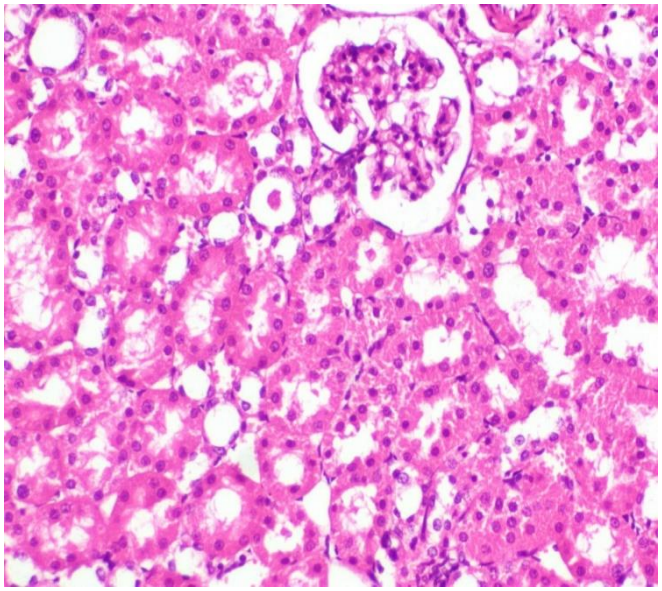

photo. (10a): Photomicrograph of kidney of rat from group 4 showing apparent normal renal parenchyma (H and E X 400).

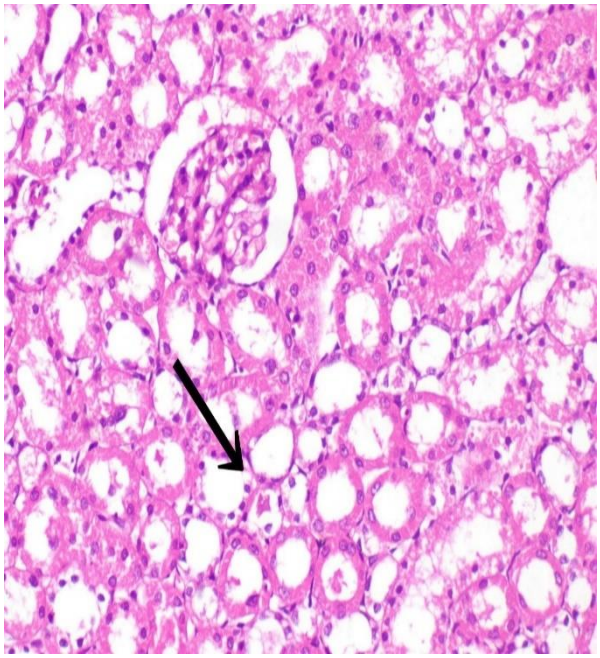

photo. (10b): Photomicrograph of kidney of rat from group 4 showing slight vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

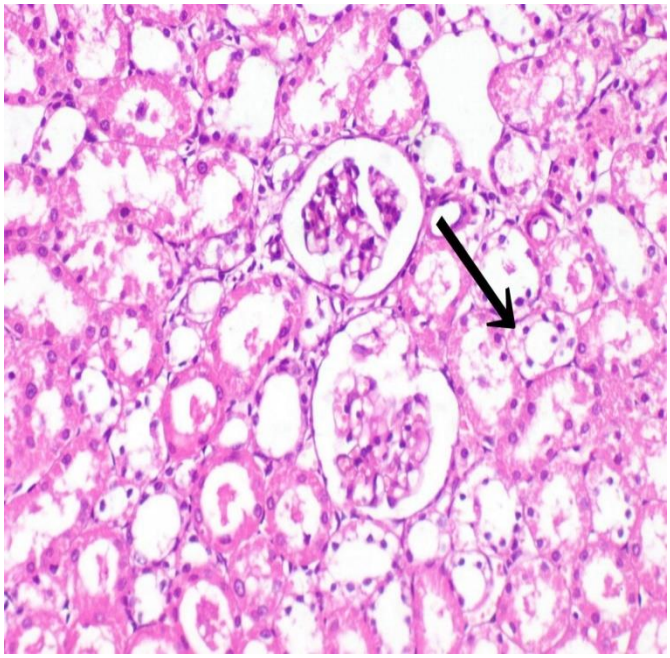

photo. (10c): Photomicrograph of kidney of rat from group 4 showing slight vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

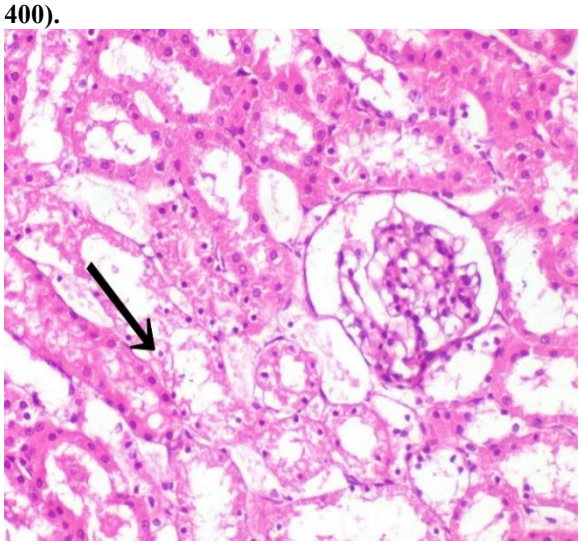

photo. (11a): Photomicrograph of kidney of rat from group 5 showing vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

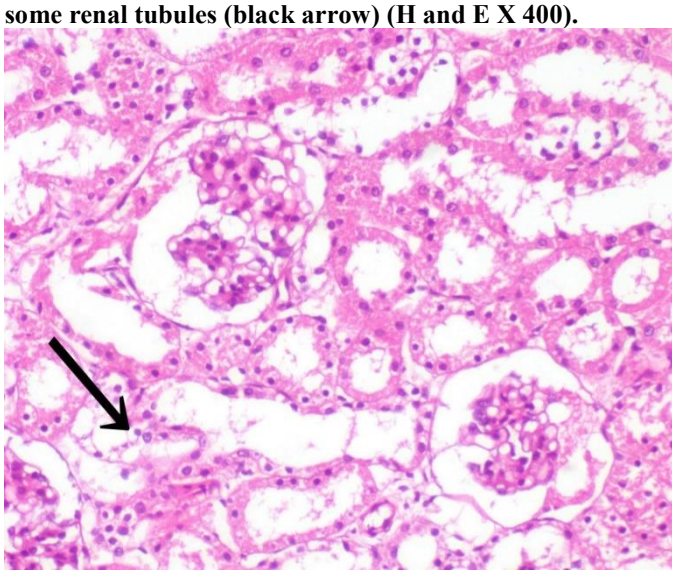

photo. (11b): Photomicrograph of kidney of rat from group 5 showing vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

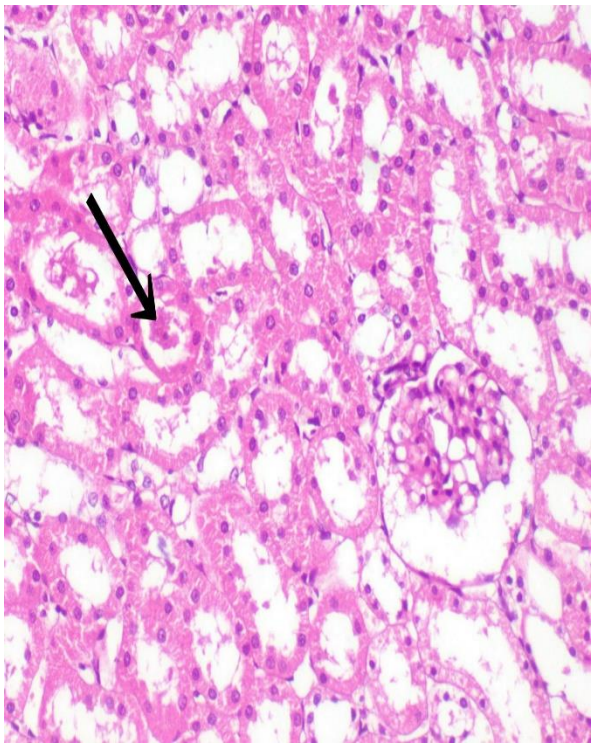

photo. (11c): Photomicrograph of kidney of rat from group 5 showing eosinophilic proteinaceous material in the lumen of sparse renal tubules (black arrow) (H and E X 400).

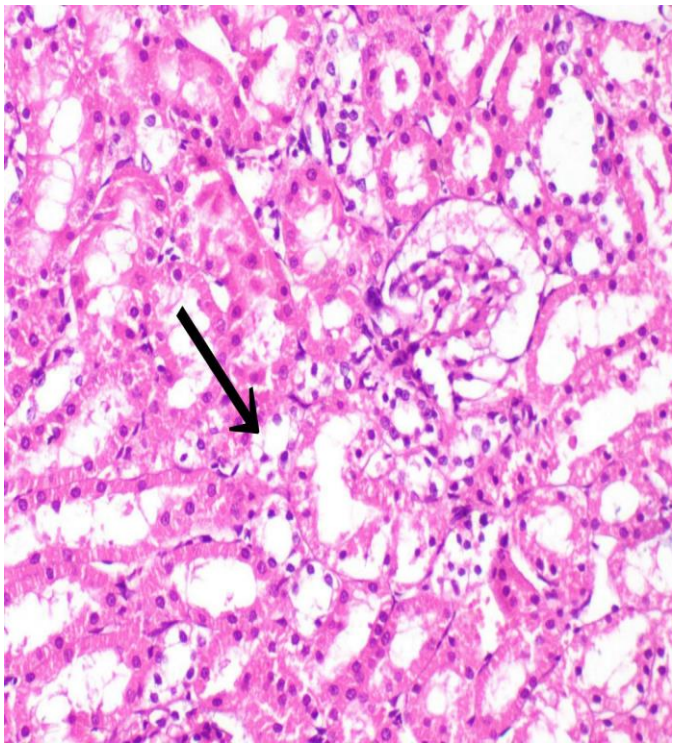

photo. (12a): Photomicrograph of kidney of rat from group 6 showing vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

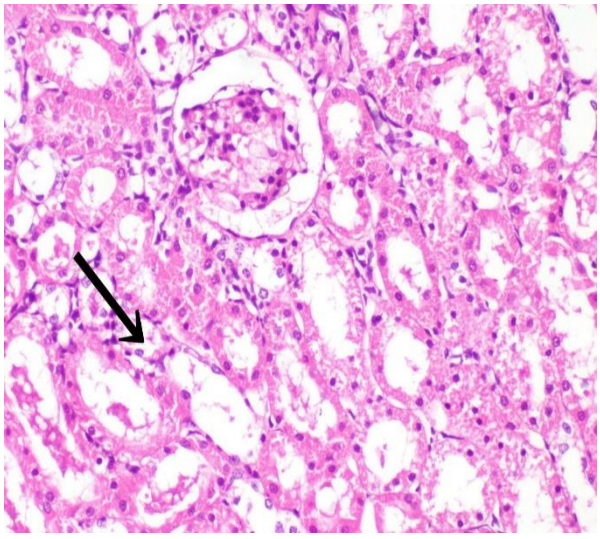

photo. (12b): Photomicrograph of kidney of rat from group 6 showing vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

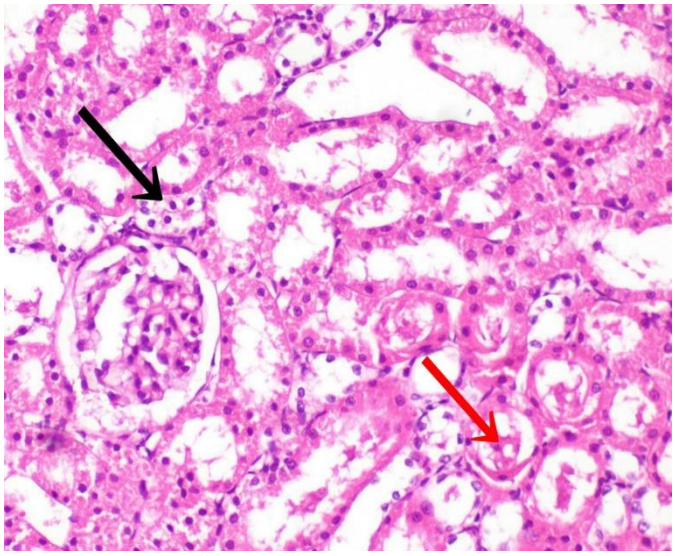

photo. (12c): Photomicrograph of kidney of rat from group 6 showing vacuolization of epithelial lining some renal tubules (black arrow) and eosinophilic proteinaceous material in the lumen of sparse renal tubules (red arrow) (H and E X 400).

Table (1): chemical composition of the truffle powder (g/100g)

Truffle composition	g/100g
Total solid	96.40
Ash	2
Fat	19
Protein	15
Fiber	5
Total carbohydrate	59
Energy	469kcal/100g

Table (2): Total phenolic content of the truffle powder and truffle extract

Phenolic compounds	Truffle powder mg/g	Truffle extract mg/g
Gallic acid	4.90	162.16
Chlorogenic acid	1.33	18.95
Catechin	6.95	0.15
Methyl gallate	0.11	0.58
Caffeic acid	0.28	0.99
Syringic acid	0.48	1.30
Rutin	0.009	0.46
Ellagic acid	0.001	0.36
Coumaric acid	0.04	0.13
Ferulic acid	0.01	0.61
Naringenin	0.006	0.41
Rosmarinic acid	0.003	0.23
Quercetin	0.01	0.28
Cinnamic acid	0.026	0.07
Kaempferol	0.002	2.91
Hesperidin	0.001	3.04

Table (3): DPPH of the truffle powder and truffle extract

DPPH scavenging%											IC_{50} µg/ml
Conc. (µg/ml)	**1.95**	**3.9**	**7.8125**	**15.635**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**	
Truffle Extract	**11.3**	**18.6**	**25.9**	**33.3**	**39.4**	**46.1**	**53.0**	**60.4**	**67.4**	**74.4**	**89.07**
Truffle powder	**28.5**	**35.9**	**41.7**	**48.5**	**55.6**	**62.6**	**69.5**	**76.6**	**83.5**	**89.4**	**17.4**

Table (4): Efficacy of Truffle on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of rats

Groups	FI(g/day)	BWG (g/day)	FER
-ve control	**18.93**	**1.45±0.018^{a}**	**0.07±0.009^{a}**
+ve control	**17.22**	**0.99±0.023^{c}**	**0.05±0.001^{b}**
300mg TE	**18.40**	**1.22±0.017^{b}**	**0.06±0.007^{a}**
400mg TE	**18.84**	**1.25±0.017^{b}**	**0.06±0.008^{a}**
500mg TE	**18.76**	**1.44±0.083^{a}**	**0.07±0.004^{a}**
500mg TP	**18.60**	**1.24±0.020^{b}**	**0.06±0.009^{a}**

TE: Truffle extract; TP: Truffle Powder,
Results presented as mean ± SE,
Different superscript litters in the same column refer to significancy ($P<0.05$).

Table (5): Efficacy of truffle on serum creatinine (CR) and serum urea on rat

Groups	**Malondialdehyde (nmol/ml)**	**Catalase (u/l)**
-ve control	0.88^{e} ±0.01	15.73^{a} ±1.07
+ve control	2.01^{a} ±0.07	12.85^{e} ±1.01
300mg TE	1.79^{b} ±0.04	13.66^{d} ±0.09
400mg TE	0.93^{d} ±0.02	15.08^{b} ±1.10
500mg TE	0.90^{d} ±0.02	15.34^{b} ±1.05
500mg TP	0.98^{c} ±0.01	14.50^{c} ±0.99

TE: Truffle extract; TP: Truffle Powder,
Results presented as mean ± SE,
Different superscript litters in the same column refer to significancy (P<0.05)

Table (6): Efficacy of Truffle on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of rats

Groups	**Creatinine**	**Urea**
	mg/dL	
-ve control	**0.32±0.008^{e}**	**13.74± 01.84^{e}**
+ve control	**0.59±0.003^{a}**	**17.08 ±00.82^{a}**
300mg TE	**0.50 ±0.005^{b}**	**16.03 ±01.54^{b}**
400mg TE	**0.46 ±0.004^{c}**	**15.11±00.96^{c}**
500mg TE	**0.35 ±0.006^{d}**	**14.42±00.72^{d}**
500mg TP	**0.34±0.006^{d}**	**14.15±00.51^{d}**

TE: Truffle extract; TP: Truffle Powder,
Results presented as mean ± SE,
Different superscript litters in the same column refer to significancy (P<0.05)

Table (7): Efficacy of truffle on serum malondialdehyde (MDA) and catalase (CAT) on rats

Groups	sAST	sALT	sALP
	IU/L		
-ve control	121.17±3.20[f]	52.83±1.67[e]	83.00±0.89[f]
+ve control	178.00±2.99[a]	92.20±1.800[a]	142.80±0.95[a]
300mg TE	164.20 ±3.06[b]	80.80±2.00[b]	109.20±1.01[b]
400mg TE	151.60±1.97[c]	73.40 ±1.49[c]	95.00±1.11[c]
500mg TE	133.00±0.99[d]	59.80±0.89[d]	87.60 ±0.79[d]
500mg TP	145.33±1.55[e]	63.00 ±1.02[d]	91.33 ±1.00[e]

TE: Truffle extract; TP: Truffle Powder,
Results presented as mean ± SE,
Different superscript litters in the same column refer to significancy (P<0.05).

REFERENCES

Althobaiti, N., Alhasani, R., BinMowyna, M., Albalawi, A., and Alshari, I. (2022): *Terfezia claveryi* chatin: Anti-tutor effects against Ehrlich solid tumour in mice. Pharmacognosy Magazine, 18 (79): 651-658.

Anita, K., Constance, B., Clement, Y., Vema, V.R. and Paul B. (2009): Oxidative Stress, DNA Damage, and Antioxidant Enzyme Activity Induced by Hexavalent Chromium in Sprague-Dawley Rats, *Environmental Toxicology*, 24(1): 66 - 73.

Ayed, H. Khalaf, A. and Mohammed, T. (2021): Effect of Aqueous Extract of Truffle on Some Antioxidants in Rats Exposed to Oxidative Stress induced by Hydrogen peroxide. In IOP Conference Series: Earth and Environmental Science, 735 (1): 12-19.

Bancroft J and Stevens A. (1996): Theory and practice of Histological Techniques. 4th edn. Churchill and Livingstone. London.

Burtis C. and Ashwood E. (1999): Tietz Textbook of clinical chemistry. 3rd Ed Saunders. Philadelphia; 652 – 657.

Chapman D, Castillo R, and Campbell J. (1959): Evaluation of protein in foods: 1. A method for the determination of protein efficiency ratios. Canadian Journal of Biochemistry and Physiology, 37(5), 679-686.

Chemists, A. O. A., and Cunniff, P. (1990):Official methods of analysis of the Association of Official Analytical Chemists. Washington, DC.

Claiborne A (1985): Catalase activity. In: Greenwald RA (ed) Handbook of methods for oxygen research. CRC Press, Boca Raton, FL; 283–284.

Dyary, H. (2020): Subacute toxicity of brown truffle (*Terfezia claveryi*) on Sprague-Dawley rats. *The Iraqi Journal of Veterinary Medicine*, 44(2): 103-112.

Elkhateeb, W., Daba, G., Somasekhar, T., and Gundoju, N. (2024): The Precious Truffles: Bioactive Compounds as a Source of Various Biological Activities. Environmental Science, 3(2), 40-50.

GSCC, (1972): German society of clinical chemistry standard method for determination alkaline phosphatase activity. *J. clin. chem. clin. Biochem*.10:182-184.

Hamza, A., Zouari, N., Zouari, S., Jdir, H., Zaidi, S., Gtari, M., and Neffati, M. (2016): Nutraceutical potential, antioxidant and antibacterial activities of *Terfezia boudieri* Chatin, a wild edible desert truffle from Tunisia arid zone. Arabian Journal of Chemistry, 9(3), 383 389.

Hatzidimitriou, E., N., N. and Tsimidou, M:(2007): Changes in the catechin and epicatechin content of grape seeds on storage under different water activity (aw) conditions. Food Chemistry, 105(4), 1504-1511.

Ichikawa, H. (2025): Oxidative Stress and Antiaging Medicine: Oxidative Stress Response and Antiaging. In Anti-Aging Medicine: Basics and Clinical Practice (pp. 119-121). Singapore: Springer Nature Singapore.

IFCC, (1976): Expert panel of enzymes of the international federation of clinical chemistry, clin. chem. Acta .70:19-42.

IFCC, (1980): Expert panel of enzymes of the international federation of clinical chemistry, clin. chem. Acta .105:147-172.

Janakat, S., and Nassar, M. (2010): Hepatoprotective activity of desert truffle (*Terfezia claveryi)* in comparison with the effect of Nigella sativa in the rat. Pakistan Journal of Nutrition, 9(1), 52-56.

Khan, F., Hussain, I., Akram, M., and Owaid, M. (2022): Truffles: The Cultivation and Health Benefits. In Biology, Cultivation and Applications of Mushrooms (pp. 285-300). Singapore: Springer Singapore.

Khlaif, D., Kadum, H., and Abod, N. (2021): Nutritional and chemical compositions of the desert Truffle (*Terfezia claveryi)* in Samawa city of Iraq. In IOP Conference Series: Earth and Environmental Science, 923: 1-7.

Leaves, L., and Leaves, L. (2014): Antioxidant activity by DPPH radical scavenging method of ageratum conyzoides. American Journal of Ethnomedicine, 1(4), 244-249.

Ljubojević, S., Vasilišin, L., Vučić, G., and Velemir, A. (2023): Chemical composition of summer truffle (*Tuber aestivum Vittad*.) from Bosnia and Herzegovina. EUREKA: Life Sciences, (6), 20-27.

Mohamed, H, Mahdi, M, Elmosallamy, S. and Abdellatif, H. (2022): Biological Activities of Black Truffle (*Terfezia Claveryi*) Against Dietary Acrylamide-Induced Toxicity in Rats Liver: Biochemical and Histopathological Study. Egyptian Academic Journal of Biological Sciences, D. Histology and Histochemistry, 14(2), 147-163.

Moussa, E. (2011): Estimation the chemical content of the Syrian truffles (*Tuber brumale vittadini* and study their effect on some blood parameters and some organs of the diabetes swiss albino mice. Journal of plant protection and pathology,2(11),981-1003.

Ohkawa H, Ohishi N, and Yagi K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry, 95(2), 351-358.

Reeves, P., Nielsen, F., and Fahey, G. (1993): AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. The Journal of nutrition, 123(11), 1939-1951.

Sies, H. (2020): "Oxidative stress: Concept and some practical aspects." *Antioxidants*, 9: 1-6.

SPSS, (1986):"Statistical package for social science", version 11. SPSS Inc., II. U.S.A.

Tongze, Z., Muthkumaran, J., Kumar, G., and Baojun, X. (2018): Black Truffle Aqueous Extract Attenuates Oxidative Stress and Inflammation inSTZ-Induced Hyperglycemic Rats via Nrf2 andNf-kB Pathways, front. Pharmocol.9:1257,doi:10.3389/fphar.2018.01257..

Villares, A., García-Lafuente, A., Guillamón, E. and Ramos, Á. (2012): Identification and Quantification of Ergosterol and Phenolic Compounds Occurring in Tuber Spp. Truffles. J. Food Composition Anal., 26:177-182.

Wu, Z., Jayachandran, M., Cheang, W. and Xu, B. (2022): Black Truffle Extract Exerts Antidiabetic Effects through Inhibition of Inflammation and Lipid Metabolism Regulation. Oxidative Medicine and Cellular Longevity,2022(1):1-10.