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Efficacy of Truffle on Serum Lipids and Antioxidant Status in Male Rats

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ABSTRACT

This research was conducted in order to examine the efficacy of truffle on eliminating oxidative stress of male rats. Thirty-six male rats were divided into six groups (6 rats of each) as follow; The 1st group was fed on basal diet as a negative control, the 2nd group was as the same of group 1 and was intraperitoneally injected with potassium dichromate at a dose 10mg/kg BW as a positive control, 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, respectively. At the end of experiment (after 8 weeks), rats were fasted over night before scarifying. Serum kidney and liver functions as well as lipid profile, 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 TC, TG, LDL-c, VLDL-c and MDA but significant increase in HDL-c and CAT compared to the +ve control group. Otherwise, histopathological examination showed improvement in kidney and liver of groups treated with truffle extract(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 play a therapeutic role of human beings.

Key words: Oxidative stress, Truffle, Antioxidant, HDL, CAT, potassium dichromate.

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INTRODUCTION

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**

Truffles, the symbiotic hypogenous edible fungi, have been worldwide regarded as a great delicacy because of their unique flavor and high nutritional value. By identifying their bioactive components such as phenolics, terpenoids, polysaccharides, anandamide, fatty acids, and

ergosterols, researchers have paid attention to their biological activities including antitumor, antioxidant, antibacterial, anti-inflammatory **Lee *et al.,(*2020)**

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±5g) were purchased from Helwan Farm, Cairo, Egypt.
- Basal diet constituents were purchased from El-Gomhoria Pharmaceutical Company, Cairo, Egypt.
- Soy oil, sucrose and starch were obtained from Egyptian Local Market, Cairo, Egypt.
- Potassium dichromate was purchased from El-Gomhoria Pharmaceutical Company, Cairo, Egypt.
- Truffle (Black truffle) (*Terfezia claveryi*) was brought from Sinai, Egypt.
- Biochemical Kits were purchased from Gamma Trade, Giza, Egypt.

Methods

Truffle Extract Preparation

The slices were dried then grounded to powder to increase the efficiency of extraction. The powder was twice pretreated with 70% ethanol to remove small molecular compounds, which can dissolve in ethanol. After being extracted, the truffle sample was mixed with deionized water in the ratio of (1:5 v/v). The mixture was boiled for 2 h and the extract was 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)**.Truffle proximate chemical composition was determined according to **(AOAC, 2005).** The phenolic compounds were analysed using HPLC **Hatzidimitriou *et al.*, (2007).** Free radical scavenging activity was measured 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 and was ip injected with potassium dichromate 10mg/kg BW. Groups3, 4 and 5 as the same of group 2 with 300, 400 and 500 mg/Kg BW of truffle extract, respectively. Group6, as the same of group 2 with 500 mg/Kg BW of truffle powder.

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 total cholesterol, triglyceride, HDL-cholesterol were analysed according to **Meiattini *et al.,* (1978); Fossati and Prencipe, (1982) and Grove, (1979)**, respectively**.** Whereas serum LDL-cholesterol and VLDL-cholesterol were calculated according to **Friedewald *et al.,* (1972).** 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)** that was previously reported diverse phenolics, namely gallic, homogentisic, protocatechuic, p-hydroxybenzoic, o- and p-coumaric acids and other phenolic derivatives, such as 3,4- dihydroxy benzaldehyde). Phenolic compounds including gallic acid, catechin, chlorogenic acid, rutin, p-coumaric acid, hesperidin and eugenol, were determined in *Terfezia claveryi* (**Vahdani *et al*., (2017)**.

The results displayed as the most effective methods DPPH, with IC_{50} values were 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 IC_{50} 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 agreed 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. These results agreed with the fact that free radical-scavenging activity is greatly influenced by the phenolic compounds of that shown 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**

The lipid profile of rats fed truffle extract or its powder were shown in Table 5. Data revealed that, serum of total cholesterol (TC) of the +ve control group significantly increased compared with the -ve control group with mean values 108.99±1.99vs. 51.83±1.02 u/l, respectively. While the level of TC of all treated groups which fed truffle extract or its powder were significantly decreased compared to +ve control group with mean values 94.00±1.10, 76.40±0.97, 64.00±0.86 ,57.75±0.90 vs. 108.99±1.99 mg/dl, respectively.

Regarding to the results of serum triglyceride (TG) of the +ve control group significant increases compared with the -ve control group with mean values 101.80±1.86 vs. 61.00±0.99 u/l, respectively. While the level of TG of all treated groups which fed truffle extract or its powder were significantly decreased compared to +ve control group with mean values 89.00±1.32, 74.00±1.02, 66.80±1.11 ,64.75±0.84 vs. 101.80±1.86 mg/dl, respectively.

Concerning serum of HDL-cholesterol of the +ve control group significantly decreased compared with the -ve control group with the mean values 06.11±0.06 vs. 10.53±0.04 u/l, respectively. While the level of HDL-c of all treated groups which fed truffle extract or its powder were significantly increased compared to +ve control group with mean values 07.05±0.04, 08.01±0.02, 09.56±0.01 , 09.62±0.02vs. 06.11±0.06 u/l, respectively.

Concerning serum of LDL-cholesterol of the +ve control group significant increases compared with the -ve control group with the mean values 82.52±0.22 vs. 29.10±0.92 u/l, respectively. While the level of LDL-c of all treated groups which fed truffle extract or its powder were significantly decreased compared to +ve control group with mean values 69.15±0.63, 53.59±0.74, 41.08±0.28, 35.18±0.19vs. 82.52±0.22 u/l, respectively.

Concerning serum of VLDL-cholesterol of the +ve control group significant increases compared with the -ve control group with the mean values 20.36±0.95vs. 12.20±0.66u/l, respectively. While the level of VLDL-c of all treated groups which fed truffle extract or its powder were significantly decreased when compared to +ve control group with mean values 17.80±0.10, 14.80±0.22, 13.36±0.19, 12.95±0.07vs. 20.36±0.95 u/l, respectively. The highest improvement of all lipid profile tested parameters among all treated groups was recorded in group 6 Which was fed 500mg truffle powder.

In accordance with **Wu *et al.,(* 2022)** Upon the supplementation with truffle extract, the lipid profile levels were found to decrease except HDL. The clearance of endogenous cholesterol synthesis by the black truffle extract plays a key role in suppressing total cholesterol. Overall, the effect of black truffle extract to increase insulin levels results in the decrease of endogenous cholesterol synthesis because cholesterol synthesis is inversely associated with insulin sensitivity in cells. **Moussa,(2011)** results agreed with this research results who reported That extract may exerted rapid protective effects against lipid peroxidation by scavenging of free radicals in accordance to **Table 3**. Also, this may be due to higher content of antioxidants in truffle specially vitamin E and Zinc in ST extract .

Table 6 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.**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.

Heristopathological examination of liver

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, 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).

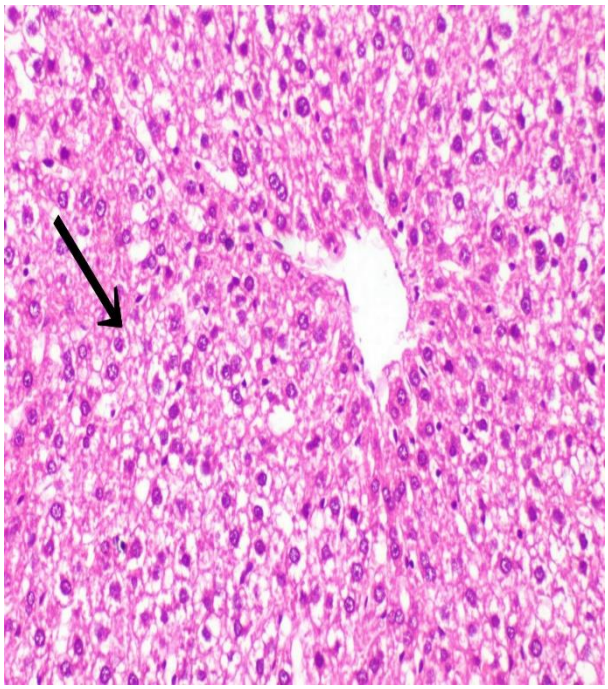

photo. (1a): Photomicrograph of liver of rat from group 1 showing hydropic degeneration of hepatocytes (black arrow) (H and E X 400).

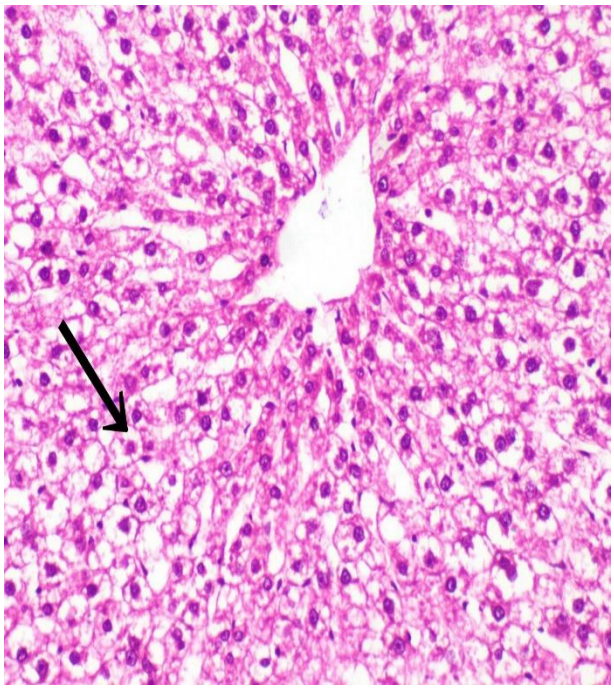

photo. (1b): Photomicrograph of liver of rat from group 1 showing hydropic degeneration of hepatocytes (black arrow) (H and E X 400).

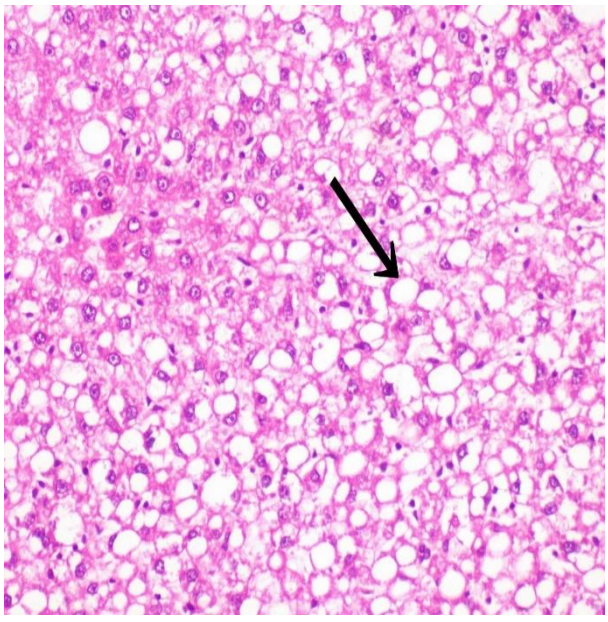

photo. (2a): Photomicrograph of liver of rat from group 2 showing hepatocellular steatosis (black arrow) (H and E X 400).

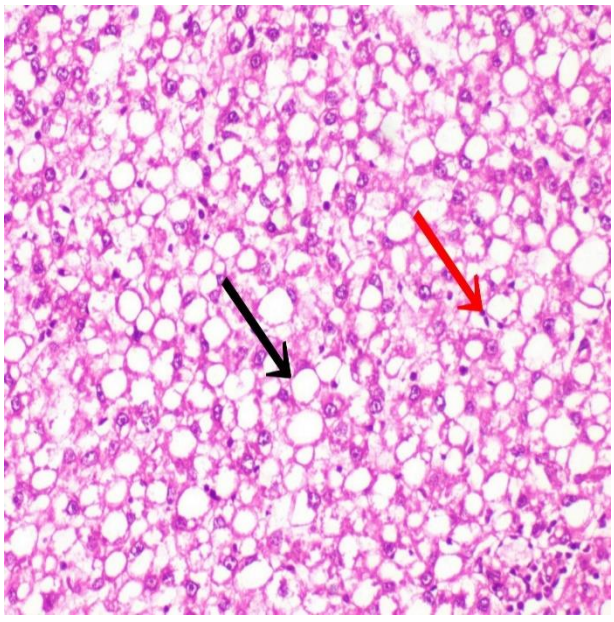

photo. (2b): Photomicrograph of liver of rat from group 2 showing hepatocellular steatosis (black arrow) and Kupffer cells activation (red arrow) (H and E X 400).

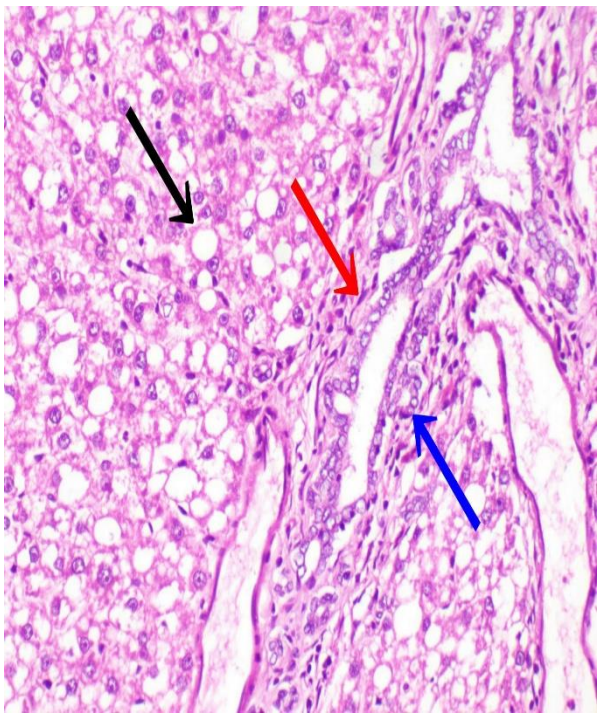

photo. (2c): Photomicrograph of liver of rat from group 2 showing hepatocellular steatosis (black arrow), fibroplasia in the portal triad (red arrow) and newly formed bile ductuoles (blue arrow) (H and E X 400).

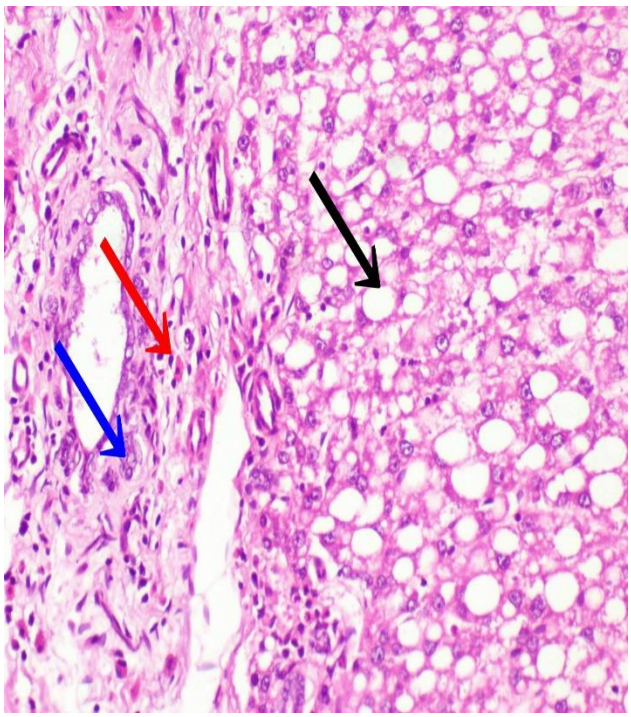

photo. (2d): Photomicrograph of liver of rat from group 2 showing hepatocellular steatosis (black arrow), fibroplasia in the portal triad (red arrow) and newly formed bile ductuoles (blue arrow) (H and E X 400).

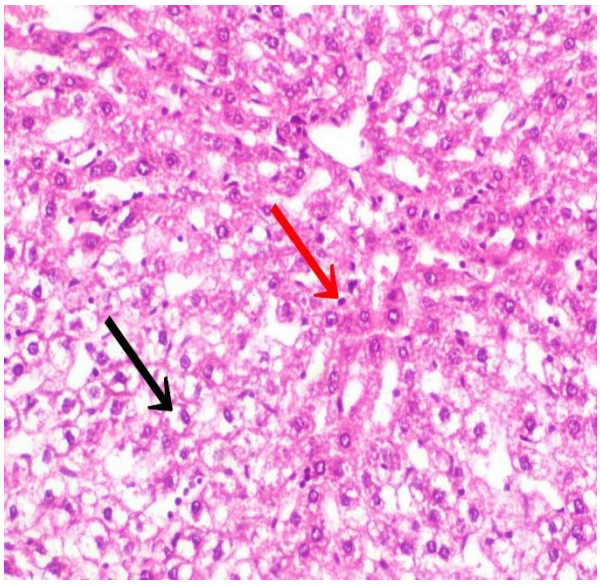

photo. (3a): Photomicrograph of liver of rat from group 3 showing vacuolar degeneration of some hepatocytes (black arrow) and slight Kupffer cells activation (red arrow) (H and E X 400).

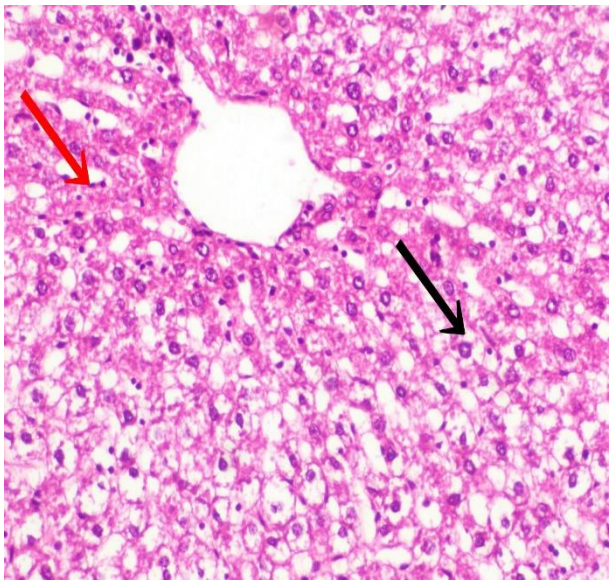

photo. (3b): Photomicrograph of liver of rat from group 3 showing vacuolar degeneration of some hepatocytes (black arrow) and slight Kupffer cells activation (red arrow) (H and E X 400).

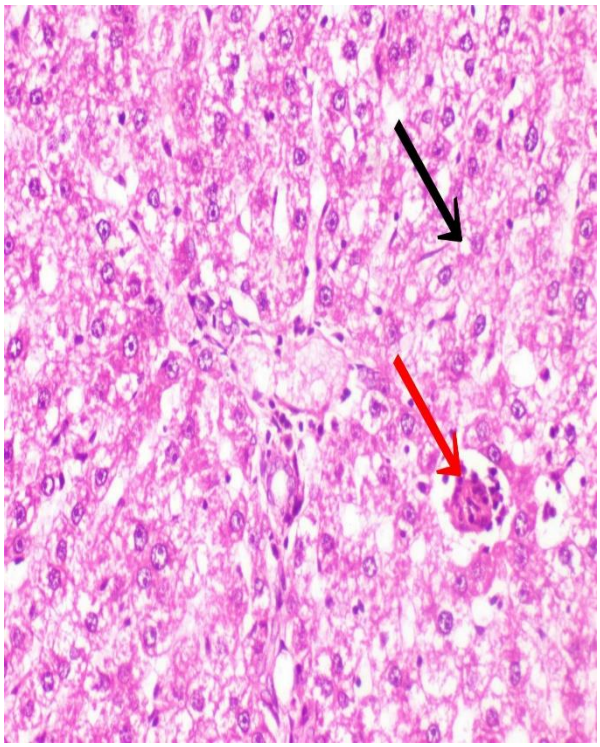

photo. (3c): Photomicrograph of liver of rat from group 3 showing vacuolar degeneration of some hepatocytes (black arrow) and small focal hepatocellular necrosis (red arrow) (H and E X 400).

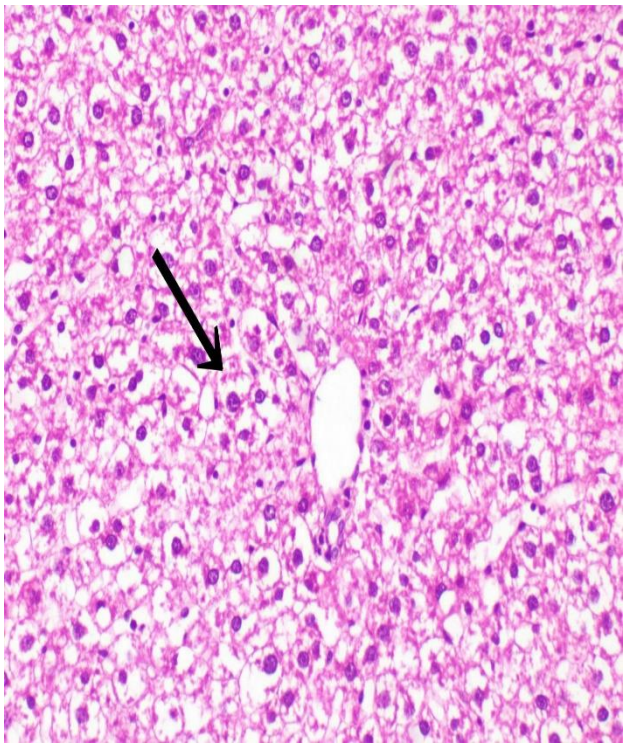

photo. (4a): Photomicrograph of liver of rat from group 4 showing hydropic degeneration of some hepatocytes (black arrow) (H and E X 400).

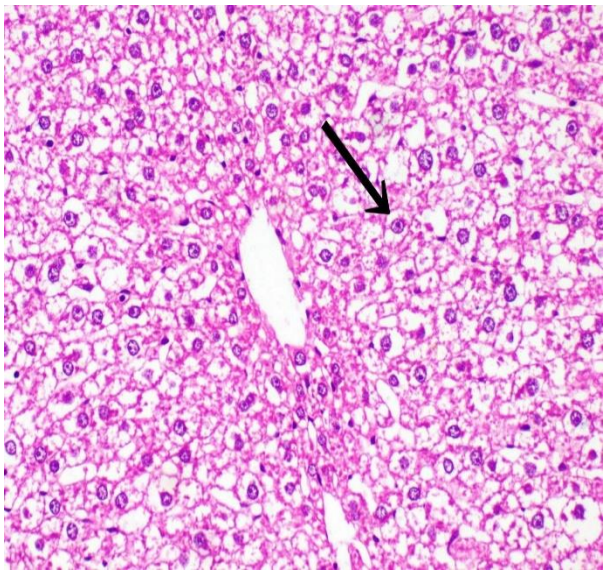

photo. (4b): Photomicrograph of liver of rat from group 4 showing hydropic degeneration of some hepatocytes (black arrow) (H and E X 400).

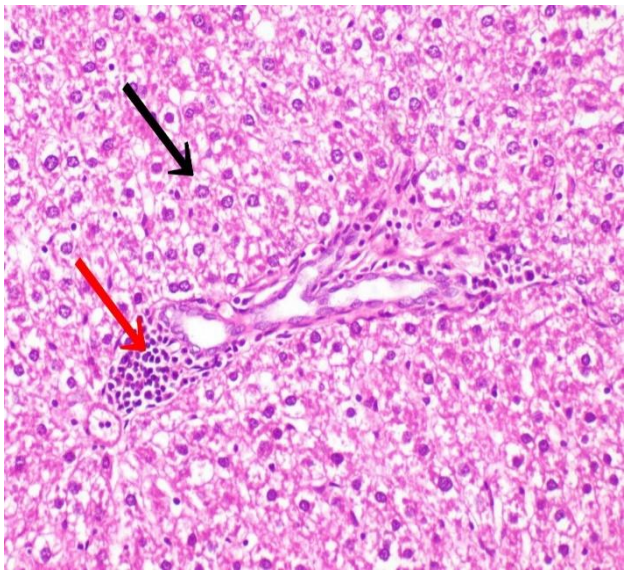

photo. (4c): Photomicrograph of liver of rat from group 4 showing hydropic degeneration of some hepatocytes (black arrow) and portal infiltration with few inflammatory cells (H and E X 400).

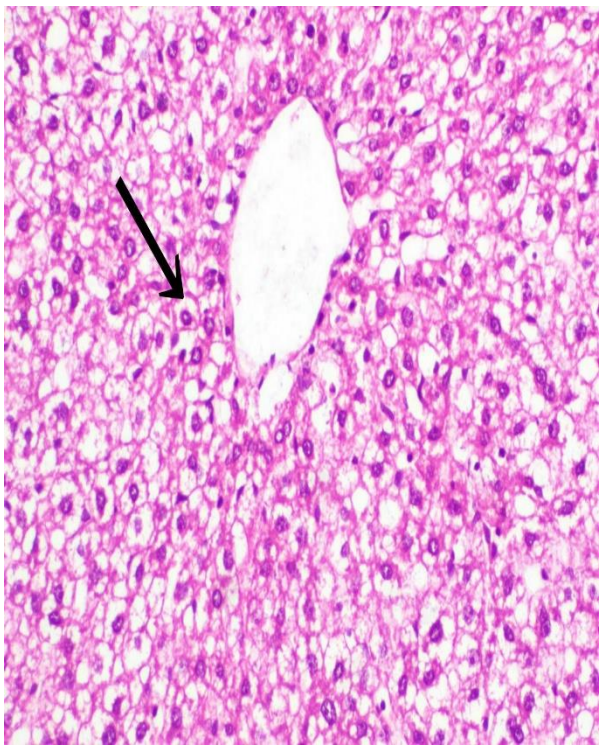

photo. (5a): Photomicrograph of liver of rat from group 5 showing vacuolar degeneration of hepatocytes (black arrow) (H and E X 400).

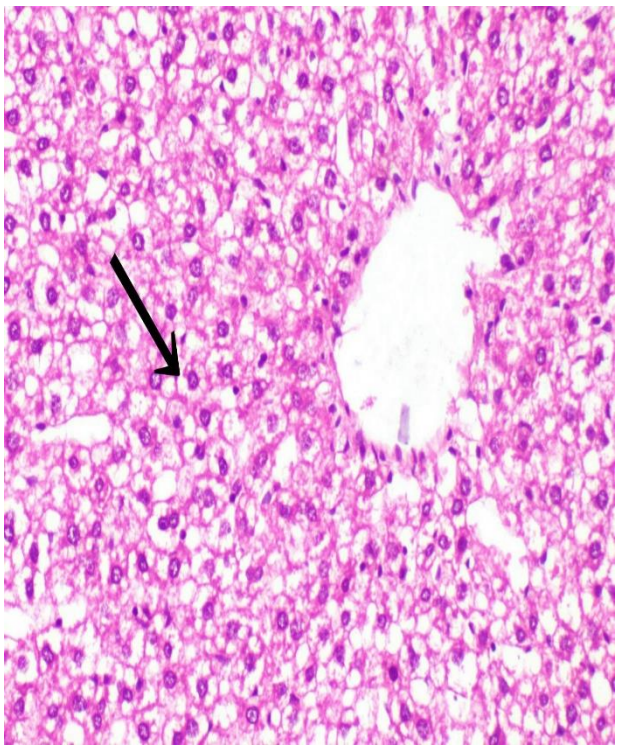

photo. (5b): Photomicrograph of liver of rat from group 5 showing vacuolar degeneration of hepatocytes (black arrow) (H and E X 400).

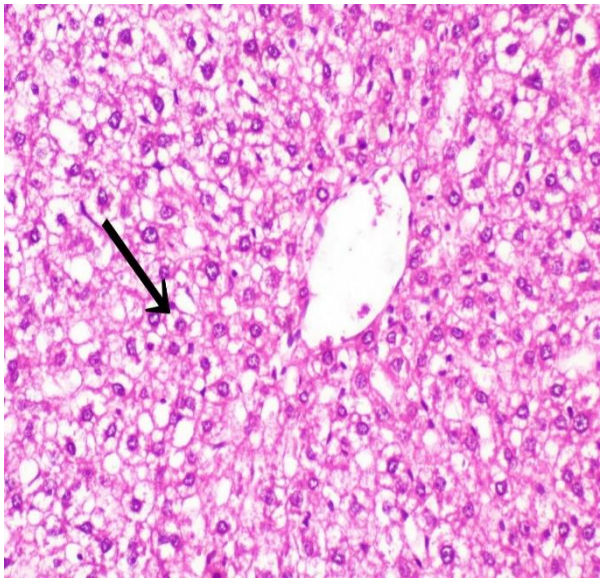

photo. (5c): Photomicrograph of liver of rat from group 5 showing vacuolar degeneration of hepatocytes (black arrow) (H and E X 400).

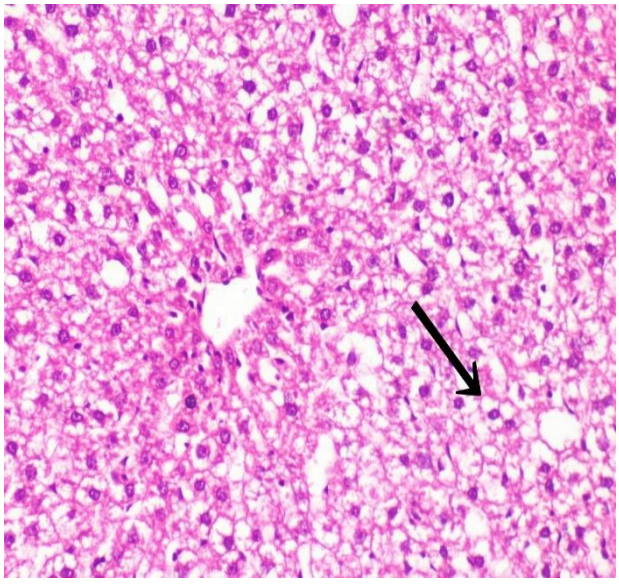

photo. (6a): Photomicrograph of liver of rat from group 6 showing vacuolar degeneration of hepatocytes (black arrow) (H and E X 400).

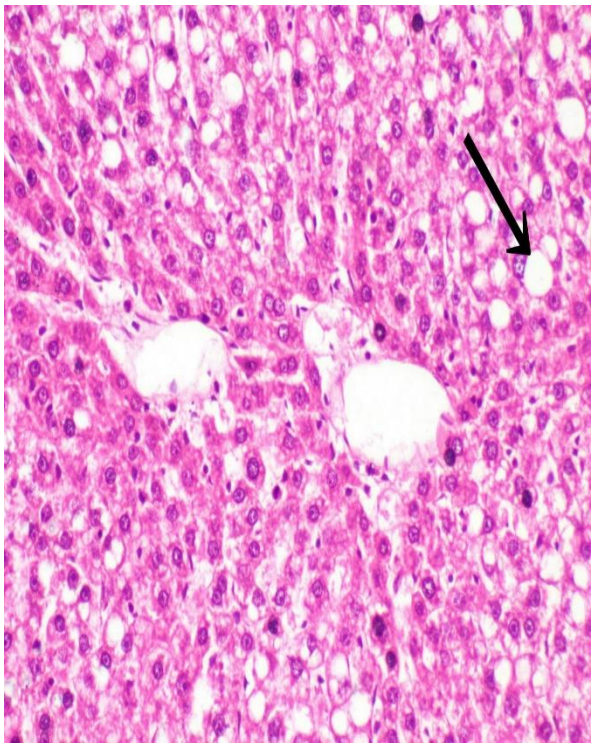

photo. (6b): Photomicrograph of liver of rat from group 6 showing hepatocellular steatosis of some hepatocytes (black arrow) (H and E X 400).

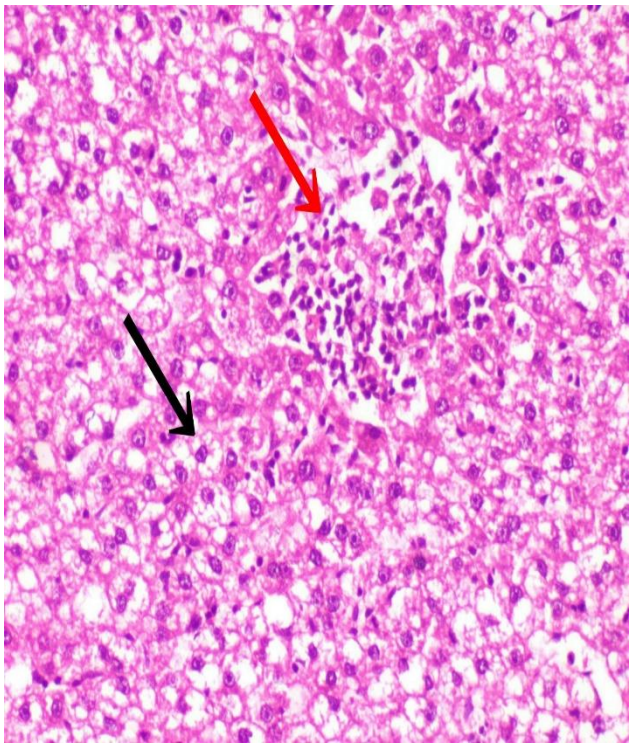

photo. (6c): Photomicrograph of liver of rat from group 6 showing vacuolar degeneration of hepatocytes (black arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (red arrow) (H and E X 400).

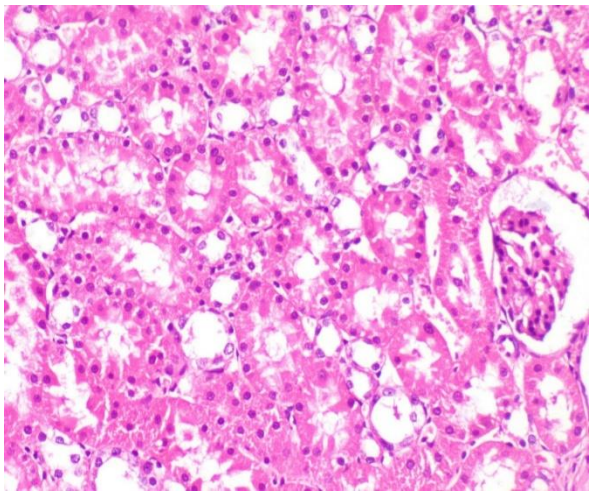

photo. (7a): Photomicrograph of kidney of rat from group 1 showing normal histological structure of renal parenchyma (H and E X 400).

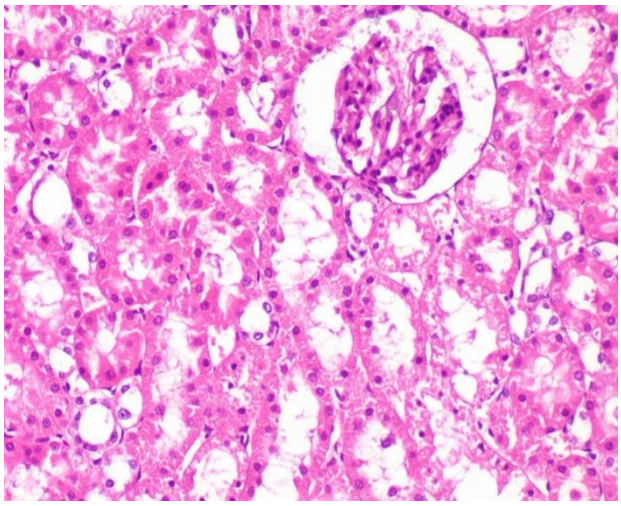

photo. (7b): Photomicrograph of kidney of rat from group 1 showing normal histological structure of renal parenchyma (H and E X 400).

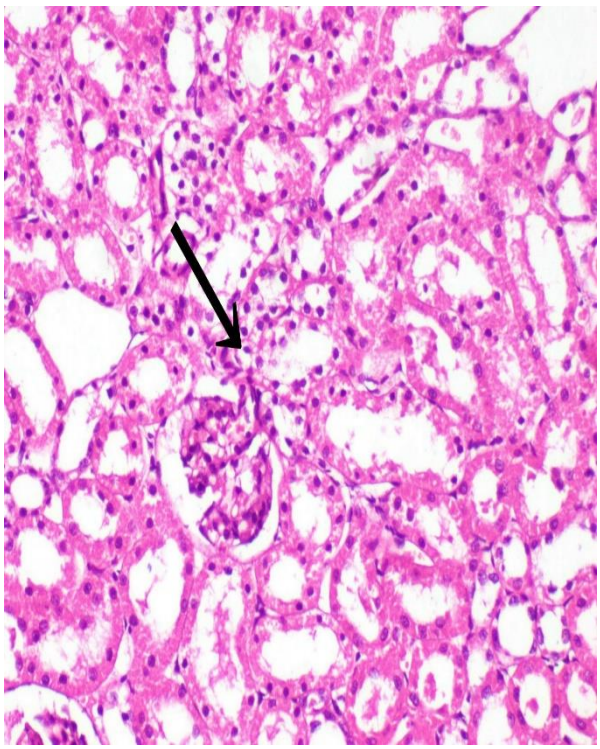

photo. (8a): Photomicrograph of kidney of rat from group 2 showing vacuolar degeneration of epithelial lining renal tubules (black arrow) (H and E X 400).

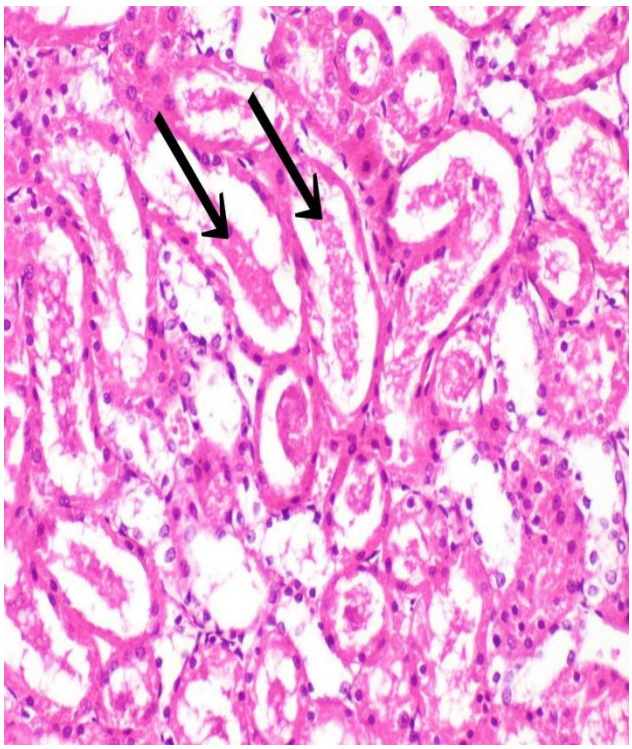

photo. (8b): Photomicrograph of kidney of rat from group 2 showing eosinophilic proteinaceous material in the lumen of renal tubules (black arrow) (H and E X 400).

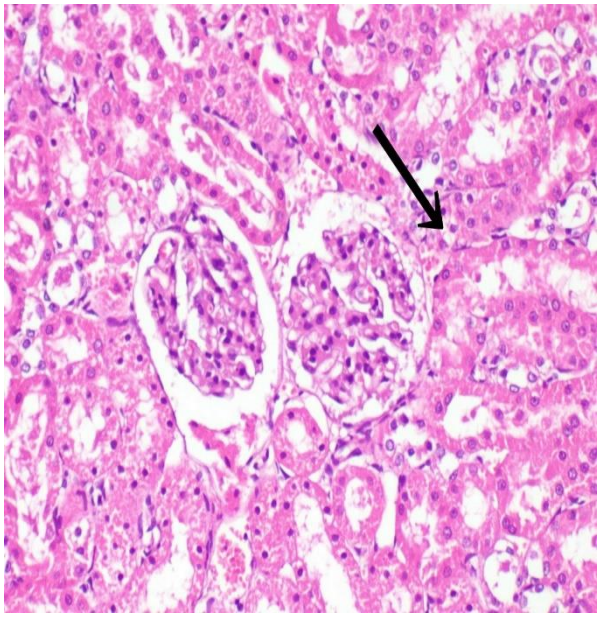

photo. (9a): Photomicrograph of kidney of rat from group 3 showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) (H and E X 400).

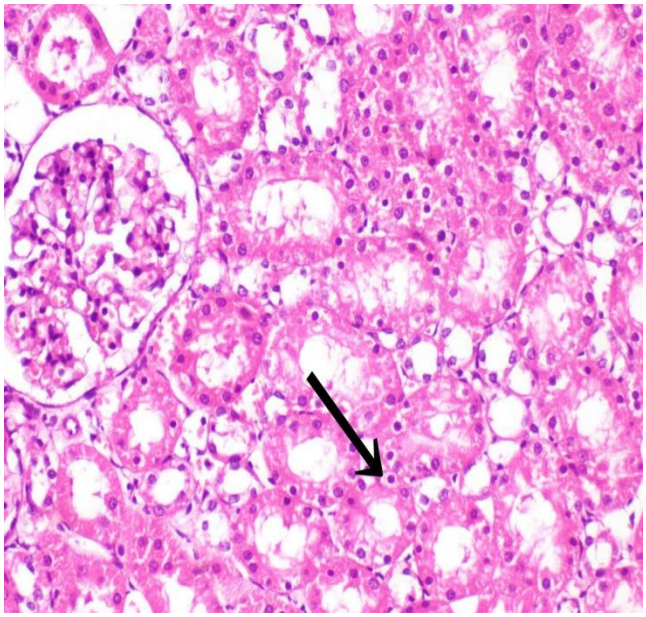

photo. (9b): Photomicrograph of kidney of rat from group 3 showing vacuolar degeneration of epithelial lining some renal tubules (black arrow) (H and E X 400).

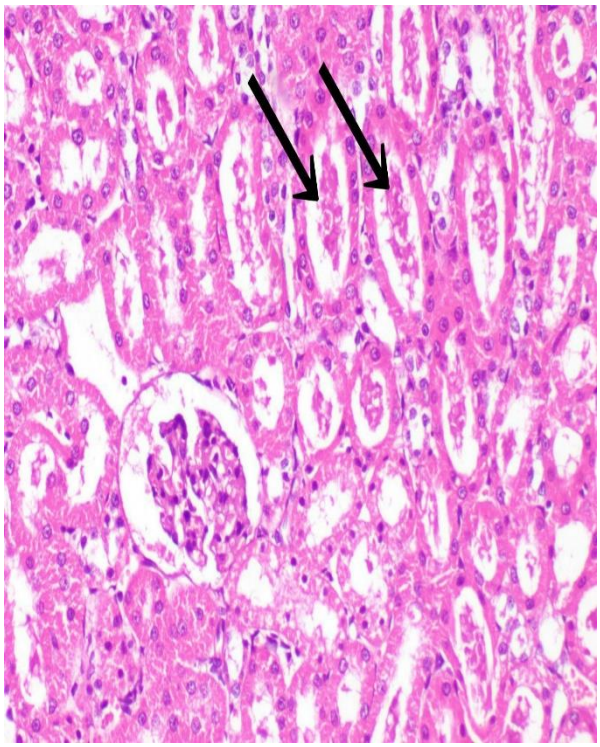

photo. (9c): Photomicrograph of kidney of rat from group 3 showing eosinophilic proteinaceous material in the lumen of renal tubules (black arrow) (H and E X 400).

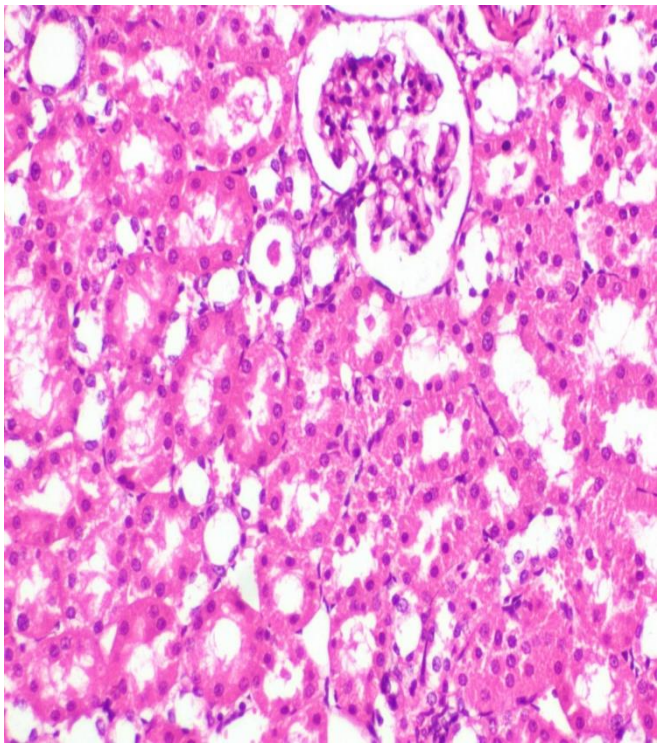

photo. (10a): Photomicrograph of kidney of rat from group 4 showing apparent normal renal parenchyma (H and E X 400).

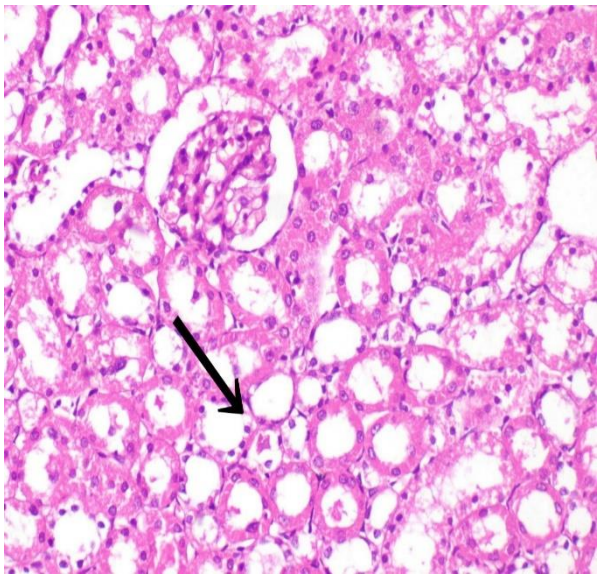

photo. (10b): Photomicrograph of kidney of rat from group 4 showing slight vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

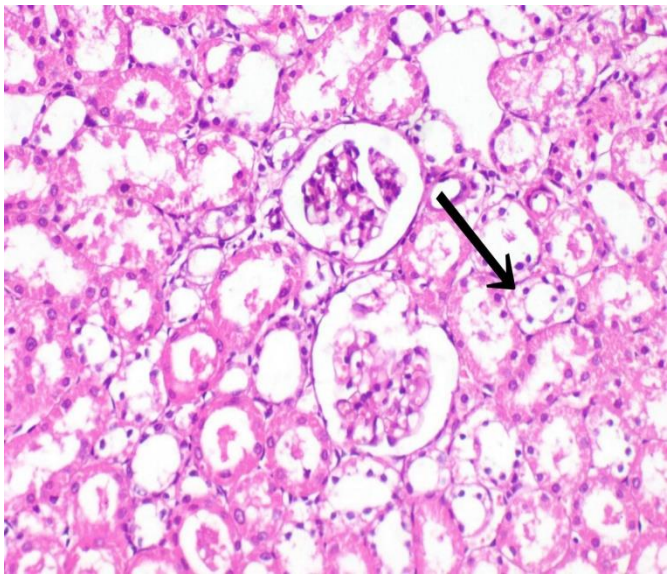

photo. (10c): Photomicrograph of kidney of rat from group 4 showing slight vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

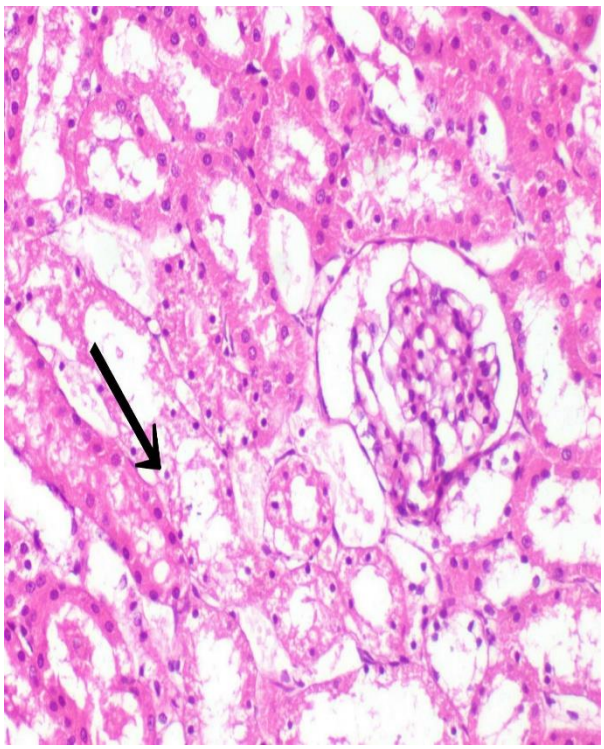

photo. (11a): Photomicrograph of kidney of rat from group 5 showing vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

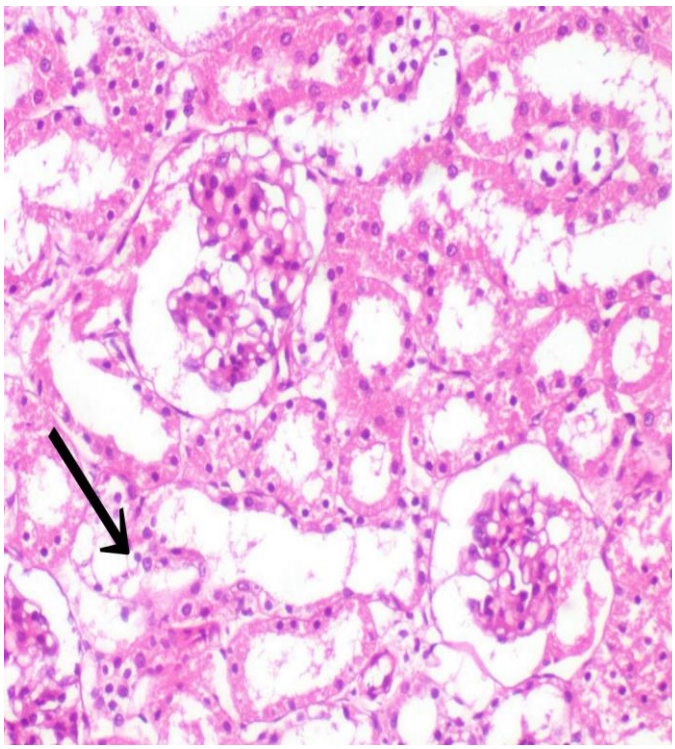

photo. (11b): Photomicrograph of kidney of rat from group 5 showing vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

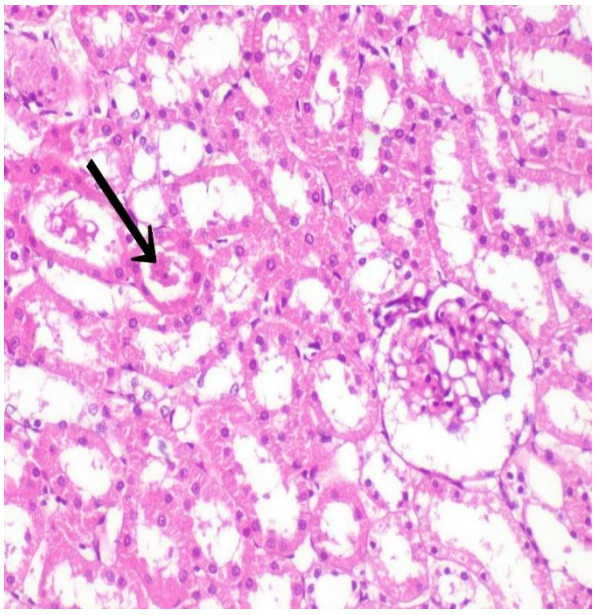

photo. (11c): Photomicrograph of kidney of rat from group 5 showing eosinophilic proteinaceous material in the lumen of sparse renal tubules (black arrow) (H and E X 400).

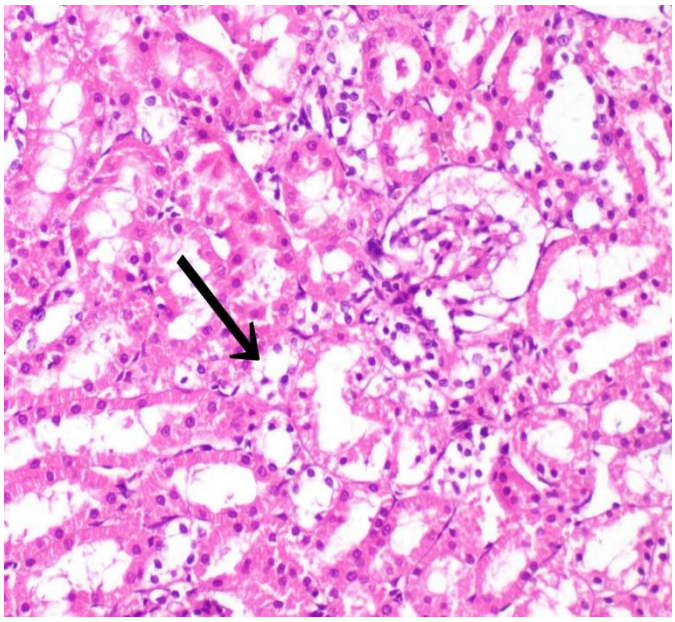

photo. (12a): Photomicrograph of kidney of rat from group 6 showing vacuolization of epithelial lining some renal tubules (black arrow) (H and E X 400).

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 extract truffle **(mg/g)**

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 extract truffle

DPPH scavenging%											IC_{50} ug/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 total cholesterol (TC), triglyceride (TG), HDL-cholesterol, LDL- cholesterol and VLDL-cholesterol of rats (mg/dl)

Groups	TC	TG	HDL	LDL	VLDL
- ve control	51.83 ± 1.02^{f}	61.00 ± 0.99^{e}	10.53 ± 0.04^{a}	29.10 ± 0.92^{f}	12.20 ± 0.66^{e}
+ve control	108.99 ± 1.99^{a}	101.80 ± 1.86^{a}	06.11 ± 0.06^{e}	82.52 ± 0.22^{a}	20.36 ± 0.95^{a}
300mg TE	94.00 ± 1.10^{b}	89.00 ± 1.32^{b}	07.05 ± 0.04^{d}	69.15 ± 0.63^{b}	17.80 ± 0.10^{b}
400mg TE	76.40 ± 0.97^{c}	74.00 ± 1.02^{c}	08.01 ± 0.02^{c}	53.59 ± 0.74^{c}	14.80 ± 0.22^{c}
500mg TE	64.00 ± 0.86^{d}	66.80 ± 1.11^{d}	09.56 ± 0.01^{b}	41.08 ± 0.28^{d}	13.36 ± 0.19^{d}
500mg TP	57.75 ± 0.90^{e}	64.75 ± 0.84^{d}	09.62 ± 0.02^{b}	35.18 ± 0.19^{e}	12.95 ± 0.07^{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 (6): Efficacy of truffle on serum malondialdehyde (MDA) and catalase (CAT) on rats

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)

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