RESPONSE OF OVERWEIGHT RATS TO PIEPLANT AND MILK VETCH INTAKE CONSIDERING INFLAMMATION MARKERS AND SOME OBESITY EVIDENCE

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Abstract

The aim of this study was to assess the influence of Pieplant and milk vetch intake considering inflammation markers, visceral fats, Leptin hormone and antioxidant enzymes on male overweight rats. Histological changes of liver and kidneys, phenol fraction of Pieplant and milk vetch was also in the scope of this investigation. A total number of thirty five adult male rats of the strain Sprague Dawley were involved via distribution on 5 groups being: Control (-) fed on regular basal diet, Control (+) rats fed on HFD, overweight rats fed on 5% Pieplant diet, overweight rats fed on 5% milk vetch diet, and overweight rats given a diet with 5% of both plant material. After 28 days of feeding intervention rats were sacrificed. Obesity resulted in increases of visceral fats, peritoneal fats and internal organs weight. Leptin hormone, bilirubin fractions (direct and indirect) inflammation markers (Procalcitonin (PCT), C-reactive protein (CRP), and erythrocyte sedimentation rate), While antioxidant enzymes decreased and some changes occurred in the structure of liver and kidneys. Feeding on Pieplant and specially the milk vetch diets revised these parameters. This seems to be probably resulting from more phenol fraction in milk vetch compared to Pieplant diets. Combination of both plants revealed a synergism desirable action compared to each of two plants: Pieplant, milk vetch alone.

Keywords: Pieplant, milk vetch, Inflammation markers, Antioxidant enzymes Obesity markers

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1. INTRODUCTION

Actually Pieplant (famous name is rhubarb) is the name of perennial plants of the genus rheum, which have high economic value Sina Pieplant is edible **(Yi, 2010)** and also it is a purgative drug in China **(Barceloux, 2009)** since 3000 years BC.As reported by **Jiao and Du (2000)**, Pieplant suggested to show erasing several health disorders including diabetic nephropathy, chronic renal failure, gastrointestinal bleeding. Phytochemical studies on Pieplant has proved that important components were included such as the phenolic compounds **(Gao et al., 2011).**

Milk vetch (famous as astraglus) is known from ancient years in china, where many forms of things were prepared from this plant (including tea, powder capsules and others). This plant contains important beneficial components such as flavonoids, glycosides and trace elements **(Shahrajabian et al., 2019).** As reported by these authors milk vetch was used in treatment of important diseases like diabetes mellitus, kidney diseases, leukemia as well as uterine cancer. It is used also for protection against several types of diseases (physical and emotional), being of anti –aging properties. Milk vetch was thought to prevent bore less. It contains antioxidants and immune supporting agents.

Pieplant has a wide several conventional uses. Milk vetch is widely known for its antioxidant capabilities and is used to treat intestine, liver, kidney, and intestinal infections. It is also known traditionally for its restorative powers on diabetes. **(Shabeer et al., 2009).** Pieplant It also abundant in lignans, tannins, flavonoids, Coumarin, and norlignan components **(Huang et al., 1998) (Huang et al., 1996).**

2. PATIENTS AND METHODS

- **Dried Plants:**

Pieplant and milk vetch were purchased from an herb shop in Cairo.

- **Rats:**

A total number of thirty five adult Sprague Dawley male rats with a common body weight of 150±10 g and an age of about 7 weeks have been collected from the Research Institute of Ophthalmology in Giza, Egypt.

- **Standard Diet:**

The standard diet was as follows AIN (1993) containing of casein (12%), maize oil (10%), mineral and vitamin mixture (4%), cellulose (5%), choline chloride (0.2%), methionine (0.3%), and corn starch (67.5%) are all present in this composition.

for getting the fat rats feeding carried out on high-fat diet (HFD), which composed as follows **(Liu et al. 2004):** Casein (25%), maize oil (1%), saturated fat (sheep tail fat) (19%), choline chloride (0.25%), vitamin mix (1%), salt mixture (3.5%), cellulose (5%), L-casein (0.18%), and corn starch (35.07%) are the main components of fat diet.

The process of Herbs and Other Feed Components: All ingredients were ground into a fine powder using an electric grinder and stored in dark-stoppered glass tubes in a cool, dry environment until **(Russo, 2011).**

- **Animal Species and Experimental Layout:**

Normal and overweight rats were housed in twine cages below ordinary laboratory circumstances, and fed on basal weight loss plan for one week as acclimatisation duration at 25 Ć.

- **Grouping of rats turned into as the subsequent:**

Group (1): Control (-) where normal rats consumed the main diet.

Group (2): Control (+) where fattened rats consumed the main diet.

Group (3): Overweight rats consumed 5% Pieplant diet.

Group (4): Overweight rats consumed 5% milk vetch diet.

Group (5): Overweight rats consumed 5% combination of Pieplant and milk vetch diets.

- **Methods:**

At the end of experiment post 12 hours of fasting rats sacrificed and Receiving blood samples into clean centrifuge tubes, allowing them to clot, and then centrifuging them, peritoneal fat determined using the following equation fat.

$$\text{Peritoneal fat pad (PEP \%)} = \frac{\textit{Fat}\text{ weight}}{\textit{Final}\text{ weight}} \times 100$$

CBC was measured in laboratory at Shebin El-Kom, Minufiya (Mera Laboratory), where values included levels of Procalcitonin (PCT), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Serum direct bilirubin, indirect bilirubin and total bilirubin were determined (Chary and Sharma, 2014). SOD, CAT and GPx were assayed by the approaches used by **Habig et al. (1974), Luck (1974),** and **Kakkar et al. (1984),** respectively. According to Guillaume and Björntorp's approach, Leptin Elisakit's Leptin hormone determination was made in 1996. Phenolic compounds fractions were assessed as outlined by **Ben-Hammouda et al., (1995).**

After being sacrificed internal organs (liver, heart, lungs, spleen and kidneys) removed, washed, wiped gently to remove excess water and then weighed, Fixed liver and kidney with 10% natural formalin, embedded in paraffin wax and thin sections prepared to be examined by light microscope according to **Drury and Wallington (1980).**

ANALYTICAL STATISTICS:

(Armitage et al., 2002) Used a computerized Costat Programme to perform a one-way ANOVA with a completely randomized factorial design to statistically analyze the data. The Duncan's Multiple Range Test was used to separate the means when a substantial mean impact was found. P≤ 0.05 was used to determine whether differences between treatments were significant.

3. RESULTS AND DISCUSSION

The results of **Table (1)** show the levels of peritoneal fat pad of fat rats as influenced by feeding on pie plant and milk vetch diets.

Table (1): Visceral test of fattened rats level as responding to feeing on Pieplant and milk vetch diets.

Parameters / **Groups**	**PFP %**
Control -ve in G1.	3.20 ±0.008 [e]
Control +ve in G2.	5.04 ±0.002[a]
Pieplant (5%) in G3.	3.50±0.006 [c]
Milk vetch (5%) in G4.	3.62 ±0.003 [b]
G5: Both together (5%).	3.40 ±0.005 [d]
LSD	0.02

Each column has values that are statistically different from the others (P≤ 0.05).

It is evident that visceral fat was higher in control (+) than control (-). In fattened rats, visceral fat accumulated clearly. less the never nutritional intervention especially with milk vetch diets and in particular when fat rats received the mixture of both plants lowered significantly the level of visceral fat. This indicates a synergistic action considering the two plants diets. This refers that both plants may be of value for fat rats, possibly because of active components in the plants, as will be shown from phenolic comments analysis.

Table (2): Inflammation blood markers of overweight rats as responding to feeing on Pieplant and milk vetch diets.

Parameters / Groups	CRP (ng/dl)	PCT (ng/dl)	ESR (mmol/L)
Control -ve in G1	0.6 ± 0.12 [e]	0.10 ±0.01 [d]	3.5 ± 0.8 [c]
Control +ve, G2	4.5 ± 0.2 [a]	0.40 ± 0.012 [a]	6.7 ± 0.51 [a]
Pieplant (5%) in G3.	3.6 ± 0.23 [b]	0.30 ± 0.022 [b]	5. 4 ± 0.62 [b]
Milk vetch (5%) in G4.	2.1 ± 0.31 [c]	0.15 ± 0.017 [c]	3.8 ± 0.81 [c]
G5: Both together (5%).	1.3 ± 0.32 [d]	0.10 ± 0.023 [b]	3.4 ± 0.47 [c]
LSD	0.5	0.06	1.2

Each column has values that are statistically different from the others (P≤ 0.05).

From results of **Table (2)** it is obvious that the health of rat deteriorate by fattening, while the lowers inflammation marker values found for fat rats fed on Pieplant an especially on milk vetch diets. Best treatments, however, was in case of the combination of both plants diets. It should be mentioned that according to **(Saltiel and Olefsky, 2017)** raising of inflammation markers is undesired and indicate the bad health case. According to these authors, values for normal and obese persons for CRP, PCT & ESR were 0.7- 2.6, 2-7.3 ng/l ; 0.10.9 ng/ml and ESR 0-5, 7.8 mmol/l respectively **(Barati et al., 2008).**

Table (3): Internal organs weights of overweight rats as responding to feeing on Pieplant and milk vetch diets.

Parameters / Groups	Liver (g)	Heart (g)	Lungs (g)	Spleen (g)	Kidneys (g)
Control -ve in G1	6.7 ±0.01 [c]	1.3±0.2 [c]	1.1±0.2[b]	0.8±0.06 [d]	2.0±0.2 [d]
Control +ve, G2	8.3±0.04 [a]	1.8±0.03[a]	2.3±0.08 [a]	1.7±0.02 [a]	2.8±0.03 [a]
Pieplant (5%) in G3.	7.8±0.06 [a]	1.2±0.07 [c]	1.6±0.03 [b]	1.3±0.04 [c]	2.1±0.07 [cd]
Milk vetch (5%) in G4.	8.2±0.08 [a]	1.6±0.08 [b]	2.1±0.04 [a]	1.5±0.07 [b]	2.5 ±0.05 [b]
G5: Both together (5%).	7±0.03 [b]	1.1±0.05 [c]	1.5±0.06 [b]	1 ±0.03 [c]	2.2±0.06[cd]
LSD	0.35	0.12	0.18	0.103	0.15

Each column has values that are statistically different from the others (P≤ 0.05).

The results shown that due to inflammations the internal organs weights raised. Meanwhile the reverse was found for pie plant and specially milk vetch feeding diets. All internal organs showed a considerable weight gain as compared to the control negative (-) group., however, consuming tested plant diets, in particulate the combination of both Pieplant and milk vetch improved appreciably the weight showing pounced decreases.

Table (4): Total bilirubin, direct bilirubin and indirect bilirubin of overweight rats as responding to feeing on Pieplant and milk vetch diets

Parameters / Groups	T. Bil (ng/dl)	D. Bil (ng/dl)	Ind. Bil (ng/dl)
Control -ve in: G1	2.1 ± 0.1 [c]	1. 0 ± 0.22 [d]	1.1 ± 0.06 [c]
Control +ve in: G2	5.3 ± 0.13 [a]	3.9 ± 0.10 [a]	2.4 ± 0.05 [a]
Pieplant (5%) in: G3.	3.2 ± 0.21 [b]	2.5 ± 0.31 [b]	0. 7 ± 0.06 [b]
Milk vetch (5%) in: G4.	1.9 ± 0.32 [c]	1.3 ± 0.24 [c]	0.8 ± 0.08 [b]
G5: Both together (5%).	1.2 ± 0.31 [d]	1.1 ± 0.14 [d]	0.1 ± 0.07 [d]
LSD	0.7	0.6	0.11

Each column has values that are statistically different from the others (P≤ 0.05).

Bilirubin is found when red blood cells are broken down. Bilirubin is a part of the bile, which is made in the liver and sulfed in the gallbladder. The abnormal buildup of bilirubin cause jaundice. Bilirubin is a yellowish substance made dursy the body's normal person of breathing down of recells.

If liver is healthy, it will remove most of the bilirubin from the body. Level of T. bilirubin in normal person is usually 1.2 ng/dl According to **Corash, (1983).** Fattiness raised bilirubin reactions in **(Table 4)** indicating that liver functions is affected which was eliminate via Pieplant and specially milk vetch diets, and in particular the combination of both plants.

SERUM ANTIOXIDANT ENZYMES:

Table (5): SOD, GPx and CAT enzymes of overweight rats as responding to feeing on Pieplant and milk vetch diets.

Parameters / Groups	Superoxide dismutase (mmol/L protein)	Glutathione peroxidase (mg/mL protein)	Catalase (mmol/L)
Control -ve in: G1	64.40±0.007 [a]	0.80±0.003[a]	0.18±0.002 [a]
Control +ve in: G2	31.34±0.001 [e]	0.42±0.006 [e]	0.13±0.006 [c]
Pieplant (5%) in: G3.	31.83±0.008 [d]	0.65±0.002 [d]	0.14±0.002 [bc]
Milk vetch (5%) in: G4.	37.70±0.003 [c]	0.70±0.008 [c]	0.15±0.007 [b]
G5: Both together (5%).	63.30±0.004 [b]	0.77±0.007 [b]	0.17±0.003 [a]
LSD	0.010	0.010	0.03

Each column has values that are statistically different from the others (P ≤ 0.05).

It was found that fattiness lower significantly the levels of SOD, GPX and CAT enzymes. Meanwhile, nutritional intervention using Pieplant and milk vetch diets, specially the last diet reversed such a day. Combination of both plant raised more the levels of the enzymes significantly indicating synergism action. On rats, **Jiangwei et al., (2011) and He et al., (2012)** confirmed the value of feeding on Pieplant and milk vetch for raising serum antioxidants.

Table (6): Leptin hormone of overweight rats as responding to feeing on Pieplant and milk vetch diets

Parameters / Groups	L H (ng/ml)
Control -ve in : G1	3.45 ±0.002 [e]
Control +ve in : G2	28.30 ±0.007 [a]
Pieplant (5%) in: G3.	7.30 ±0.002 [c]
Milk vetch (5%) in: G4.	7.40 ±0.004 [b]
G5: Both together (5%).	7.01 ±0.006 [d]
LSD	0.008

Each column has values that are statistically different from the others (P≤ 0.05). LH=Leptin hormone.

The results of this study of **Table (6)** show that due to fattiness Leptin hormone was increased. Meanwhile by feeding on pie plant diet and specially the milk vetch diets Leptin hormone reduced. Maximum decrease recorded for the combination diets of both to lower. The last diet revealed very close value of Leptin hormone in comparison with normal rats. The value of feeding on Pieplant and milk vetch plants supported by conclusions of **Yu et al., (2003)** and **Huang et al., (2017)** working on fat rats.

Most of side effects due to fattiness are due to the oxidation stress and less of immunity Fernandez et al., (2011). At the same time phenolic compounds may reverse such a change because of the ant oxidation effect of mentioned compounds Savini et al., (2013). Therefore, in measuring phenolic compounds were determined in Pieplant and milk vetch plants **(Table 7).**

Table (7): Phenolic compounds of Pieplant and milk vetch (ppm).

Groups	Pieplant	Milk vetch
Syringic	2.9	2.01
Pyrogallol	500.26	398.15
Gallic	12.15	36.81
Protocathechias	25.60	138.55
Catechol	17.32	55.03
4-Aminobenzoic	13.78	28.6

Catechein	18.00	30.15
Chlorogenic	17.82	80.27
P-OH-Benzoic	86.62	130.14
Epicatechen	76.19	86.51
Caffeic	7.84	21.30
Vanellic	25.17	33.16
Ferulic	4.26	7.24
Caffeine	140.31	150.32
Benzoic	68.54	80.41
Ellagic	167.11	247.11
Coumarin	12.17	18.34
Cinnamic	2.36	7.01
Total	1198.42	1551.15

From **table (7)'s** results, it was conceivable to see that the phenolic compounds was much more in milk vetch (about 30% more) as compared with that of Pieplant which was in line with the more pronounced effect of milk vetch considering biochemical results of present work.

Histopathological Alterations:
In a microscopic slice of the healthy (control -) group 1 liver, the hepatic lobule's typical structure could be seen (Photo 1). However, the livers of the rats in group 2 (control + fat) exhibited symptoms of hepatocyte vacuolar degeneration (Photos 2 & 3). With the exception of a minimal central vein constriction and a mild swelling of the hepatocytes, rats fed group 3 Pieplant meals in their liver segments did not show any abnormalities (Photos 4 and 5). Additionally, rats in the Milk vetch diet-fed fat group (group 4) displayed modest hydropic hepatocyte degeneration and slight hepatic sinusoids congestion in the liver (Photos 6 and 7). Hydropic hepatocyte degeneration, a small hepatic sinusoidal congestion, and a slight activation of Kupffer cells (Photo 9) were also seen.
It is clear that the Pieplant, Milk vetch, and particularly the different meals benefited. The histological form of the kidney and liver, resulting in was changed by weight gain and happened at the same time as the biochemical changes that occurred.

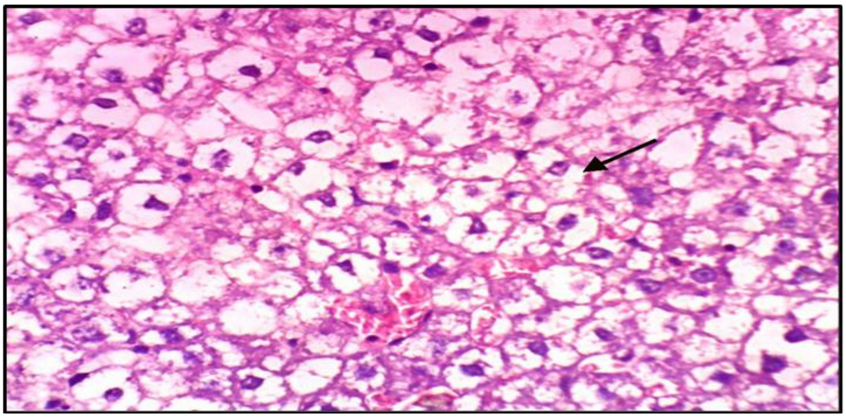


Photo (1): shows the typical histological structure of hepatic lobules in the liver of a group 1 (healthy rats) rat.

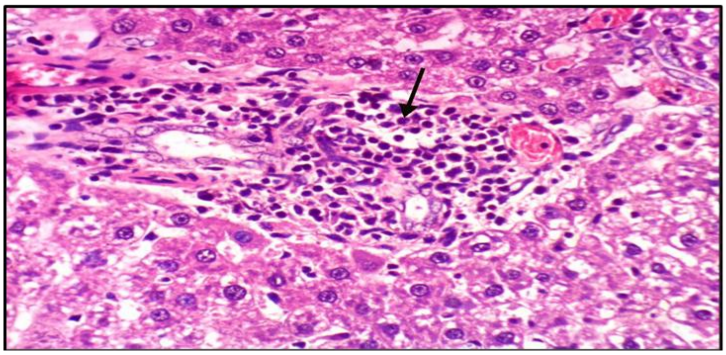


Photo (2): Hepatocyte vacuolar degeneration is visible in the rat liver from group 2 (fat rats)

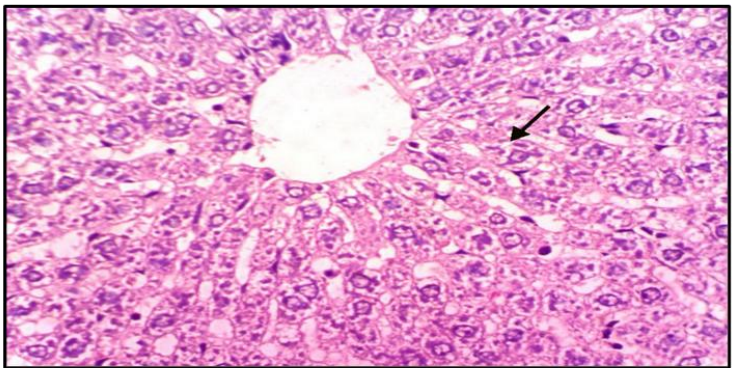


Photo (3): Rat liver from group 2 (fat animals) demonstrating inflammatory cell infiltration in the portal triad and vacuolar degeneration of hepatocyte

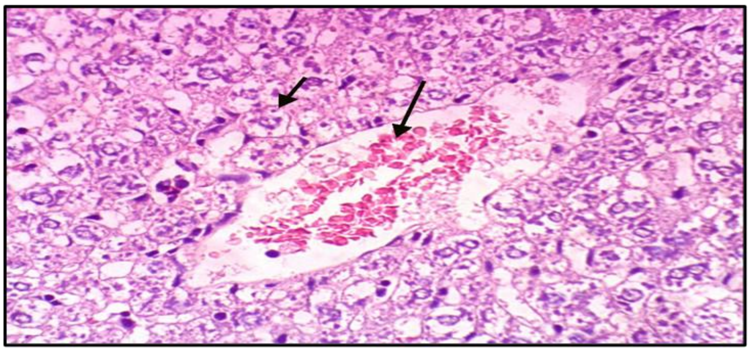


Photo (4): Hepatocytes are mildly hydropically degenerating in the liver of a group 3 (5% Pieplant) rat

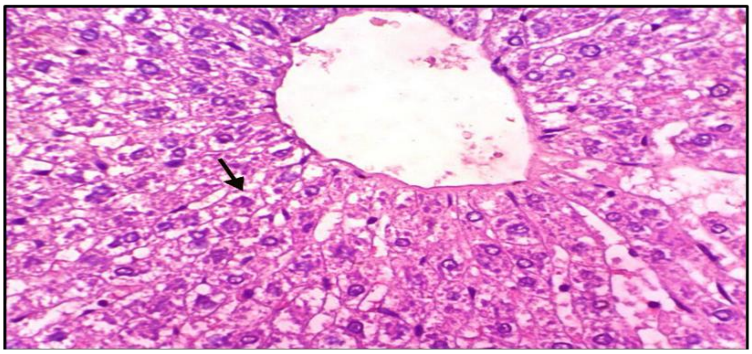


Photo (5): Rat liver from group 3 (5% Pieplant) demonstrating minor hydropic hepatocyte degeneration and slight central venous congestion

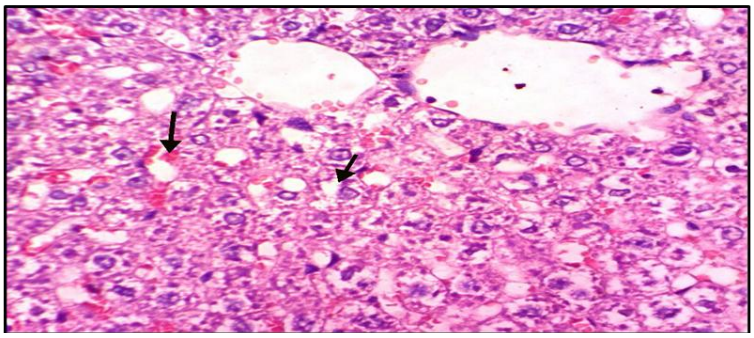


Photo (6): Rat liver from group 4 (milk vetch 5%) demonstrating mild hydropic hepatocyte degeneration.

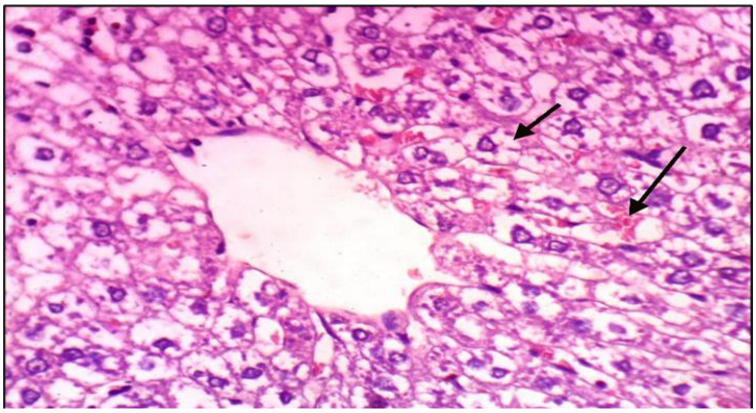


Photo (7): Rat liver from group 4 (milk vetch 5%) with mild hydropic hepatocyte degeneration and mild hepatic sinusoidal congestion

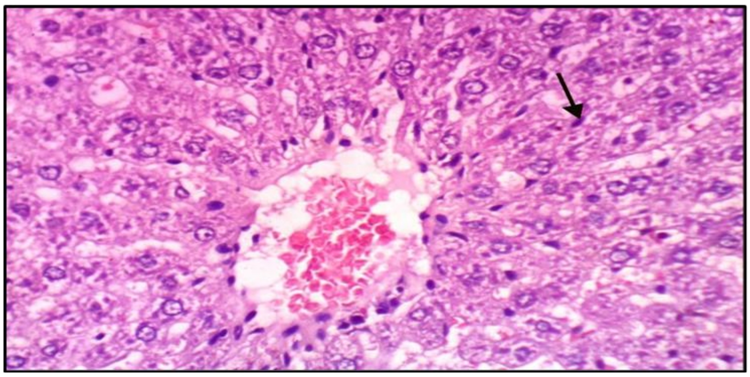
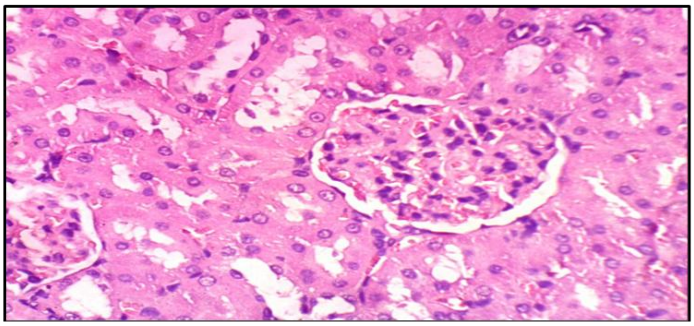


Photo (8): Rat liver from group 5 (mixed diets), showing hydropic hepatocyte degeneration and mild hepatic sinusoidal congestion



Liver of a rat from group 5 (mixed diets) demonstrating mild Kupffer cell activation in photo (9)

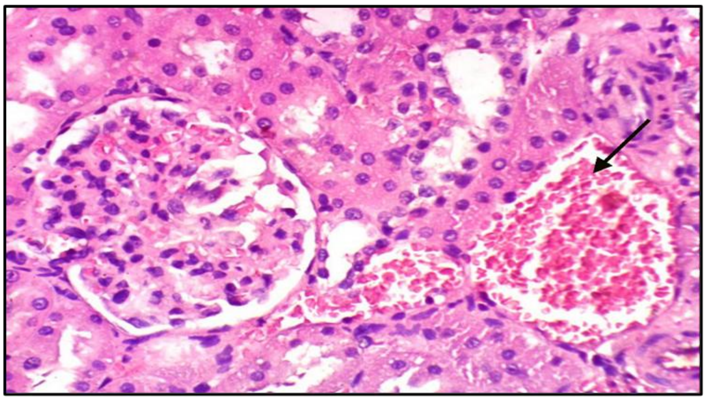


Photo (10): Photomicrograph of the renal parenchyma of a healthy rat from group 1 exhibiting the typical histological structure.

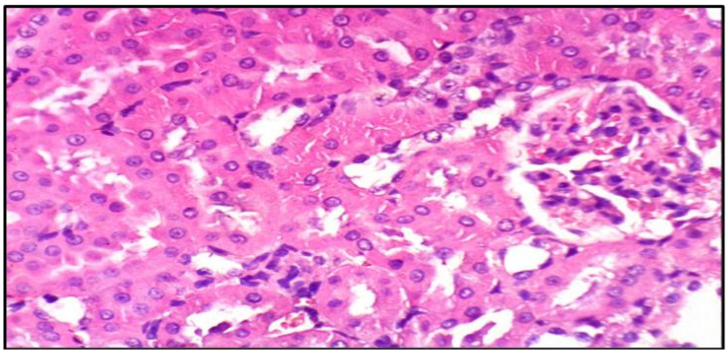


Photo (11): Congestion of renal blood vessels in a rat kidney from group 2 (overweight animals) (H & E X 400).

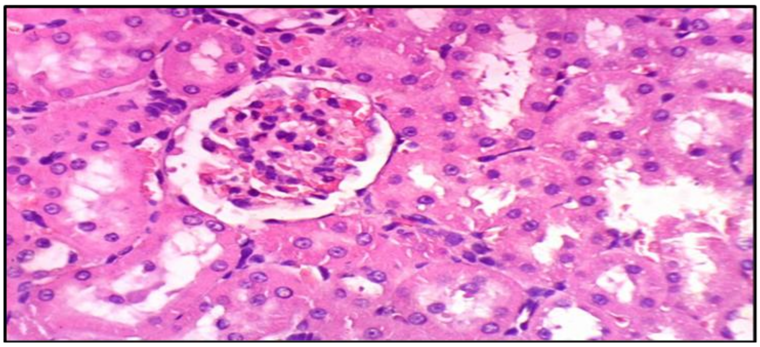
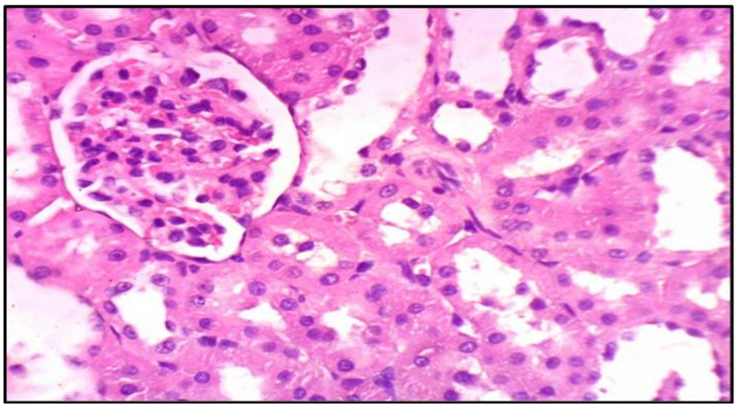


Photo (12): A rat kidney from group 3 (5% Pieplant) that has not had any histological changes (H & E X 400).



Rat kidney from group 4 (milk vetch 5%) in image 13 is shown without any histopathological changes (H & E X 400).

Rat kidney from group 5 (mixed diets), shown in photo (14) with no histopathological changes (H & E X 400).

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