Biological and Nutraceutical Studies on Obese Rats Using Herbs of Rhubarb and Astragalus

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دراسات بيولوجية وتغذوية علاجية علي الفئران السمينة باستخدام أعشاب الراوند والقتاده

د| مي محمود خفاجي

الملخص العربى

يهدف هذا البحث إلى تقييم تأثيرأعشاب الراوند والقتاده والخليط منهما علي ذكور الفئران المصابة بالسمنة. تم تقسيم ثلاثون فأر من الذكور البالغين سبراغ داولي إلى خمس مجموعات. مجموعة (1): وهي المجموعة الضابطة السالبة (−) تغذت على الوجبة الأساسية ، المجموعة (2): وهي المجموعة الضابطة الموجبة (+) وهي الفئران المصابة بالسمنة وتغذت على الوجبة الأساسية. المجموعة (3): الفئران المصابة بالسمنة التي تغذت علي نبات الراوند بنسبة 5%. المجموعة (4): الفئران المصابة بالسمنة التي تغذت على نبات القتاده بنسبة 5 %. المجموعة (5): الفئران المصابة بالسمنة التي تغذت على الاثنين معا بتركيز 5%.. في نهاية التجربة ، بعد 28 يومًا من التغذية ، تم تقدير الاختبارات البيوكيميائية للدم. الفئران المصابة بالسمنة سببت ارتفاع مستويات وزن الأعضاء وهرمون اللبتين و PFP % وانخفاض في مستويات الانزيمات المضادة للأكسدة وتحسنت النتائج باستخدام الأغذية المعالجة.

الكلمات المفتاحية : مرض السمنة ، عشبة الراوند ، عشبة القتاده والخليط من الاثنين معا.

Biological and Nutraceutical Studies on Obese Rats Using Herbs of Rhubarb and Astragalus

Mai M. Khafagy

Abstract:

This investigation aimed to evaluate the effect of Rhubarb, Astragalus and Mixture of both on male obese rats. Thirty (30) adult male Sprague Dawley rats were divided into five groups. Group (1): Normal rats fed on basal diet as control negative (C-), Group (2): Control positive (C+) (untreated group). Group (3): Obese rats fed on basal diet and rhubarb (5%).Group (4): Obese rats fed on basal diet and astragalus (5%). Group (5): Obese rats fed on basal diet and mixture of both (5%). At the end of experiment, after 28 days of feeding, all serum samples were analyzed for biochemical parameters. Obese rats caused a significant increases in the level of PFP%, leptin hormone and organs weight (liver, heart, spleen, lungs and kidneys), while significant decreases recorded in SOD, GPX and CAT. Obese rats treated with various diets, showed the improvement in all previous parameters.

Key words: Obesity, Rhubarb, Astragalus and Mixture of both.

Introduction:

Rhubarb is a collective name of various perennial plants of the genus *Rheum L.* from Polygonaceae family. This plant has important economic value, not only referred to a few edible rhubarbs **(Yi, 2010)**, but also used as purgative drug in China since the third millennium BC **(Barceloux, 2009)**, firstly recorded in Shen Nong's Herbal Classic. Rhubarb has been suggested to exert eliminating heat, purging fire, cooling blood, dispersing blood stasis, dredging collateral antidotal and purgative effects, used to treat constipation, diabetic nephropathy, chronic renal failure, acute pancreatitis, gastrointestinal bleeding and other diseases **(Jiao and Du, 2000)**.

Phytochemical investigation on rhubarb has proved major bioactive ingredients are phenolic compounds in six skeletal type including anthraquinones (physcion، chrysophanol, emodin, aloe-emodin and rhein and their glucosides), anthocyanins (cyanidin 3-rutinoside and cyanidin 3-glucoside),flavonoids (catechin, quercetin 3-Orhamnoside, quercetin3-O galactoside, and quercetin 3- O-rutinoside), stilbene (trans-rhapontigenin and desoxyrhapontigenin (cis-rhapontigenin, resveratrol and piceatannol) **(Gao *et al*., 2011)**.

Astragalus is a medicinal herb which has been used in traditional Chinese medicine for many years. Specifically, the root of the plant is made into many different forms of supplements, including liquid extracts, capsules, powders and teas. Its root contain many active plant compounds, which are believed to be responsible for its potential benefits. Saponins, polysaccharides, amino acids, flavonoids, organic acid, glycosides, alkaloid, and trace elements **(Shahrajabian *et al*., 2019)**.

In traditional Chinese Medicine, Astragalus considered to use in the treatment of diabetes mellitus, nephritis, leukemia, uterine cancer, besides its tonic agent and diuretic effects. Astragalus polysaccharide, is the active component extracted from Astragali radix which is the root of *Astragalus membranaceus* Bunge. Some uses of astragalus are in kidney and urinary problems, digestion, liver problems, female

reproductive system problems, muscular, skin problems, cardiovascular and blood, immune and lymphatic system, nervous system, respiratory system, and for some specific disease. It helps protect the body against various types of stress such as physical and emotional stress. Astragalus root including anti-aging properties, and also helping to prevent bone loss. It contains astragalosides (antioxidants), which support the integrity of the respiratory tract. In addition, the polysaccharides found in astragalus are known for their immune supporting properties. Astragalus herb also supports deep immune function by promoting normal levels of specific immune cells and aids in their function. Astragalus appears especially effective when immune function is stressed by environmental or endogenous challenges. Astragali radix, the root of *Astragalus membranaceus* Bunge, has been reported to exert hepatoprotective effects, anti-oxidative effects,antiviral activity, anti-oxidative effects, anti-hypertensive effects, and immunostimulant properties **(Shahrajabian *et al*., 2019)**.

Materials and Methods

Materials:

Rhubarb and astragalus were obtained dry from herb shop in Cairo, Egypt.

Animals:

Thirty (30) adult male Sprague Dawley rats, average body weight (150± 10 g) about 7 weeks old, were used in this study. Rats were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Methods:

Basal Diet Composition of Tested Rats:

The basal diet in the experiment consisted of casein (12%), corn oil (10%), mineral mixture (4%), vitamin mixture (1%), cellulose (5%), chorine chloride (0.2%), methionine (0.3%) and the remained is corn starch (67.5%) according to **AIN (1993)**.

To induce obesity rats carried out via feeding on high- fat diet (HFD) for 6 weeks according to **Liu *et al*., (2004)**. The composition of HFD was as follows (%): Casein 25, Corn oil 1,

Saturated fat (shep tail fat) 19, Choline chloride 0.25, Vit- Mix 1, Salt Mix 3.5, Cellulose 5, L. cystine 0.18, Sucrose 10 and corn starch 35.07.

Preparation of Materials:

All materials were milled to soft powder by using electric grinder and kept in dusky stoppered glass bottles in a cool and dry location till use according to **Russo (2001)**.

Experimental Design and Animal Groups:

Rats were housed in wire cages under the normal laboratory condition, and were fed on basal diet for a week as an adaptation period. The rats were divided into 5 groups each of 6 rats. All groups of rats were housed in wire cages at room temperature 25 C^0, and kept under normal healthy condition. Rats were divided into the following groups:

Group (1): Control negative group (-), in which normal rats were fed on basal diet.

Group (2): Control positive group (+), in which obese rats were fed on basal diet.

Group (3): Obese rats fed on rhubarb 5% diet.

Group (4): Obese rats fed on astragalus 5% diet.

Group (5): Obese rats fed on mixture of both 5% diet.

Determination of Biochemical Blood Parameters:

Blood samples were collected after 12 hours fasting at the end of experiment using the abdominal aorta. The rats were scarified under ether anaesthesia. Blood samples were received into in clean dry centrifuge tubes, in which blood was left to clot at room temperature, and then centrifuged for 10 minutes at 3000 r.p.m to separate the serum. Serum was carefully aspirated and transferred into clean cuvette tubes and stored frozen at-20^{o}C for biochemical analysis as described by **Schermer (1967)** to determine the following parameters: SOD was assayed according the methods of **Kakkar *et al.*, (1984)**. Catalase activity was assayed by the method of **Luck (1974)**. GPX was assayed according to the method of **Hablig *et al.*, (1974)**. Leptin hormone

determined by Leptin ELISA Kit according to the method described by **Guillaume and Bjorntorp (1996)**.

Histopathological examination:

The organs: kidney and liver were removed, washed in saline solution, blotted by filter paper, weighted, and stored frozen in formalin solution 10% for histopathological testing according to method mentioned by **(Drury and Wallington, 1980)** and PFP% was determined using following equation:

$$\text{Peritoneal fat pad/ body weight \%} = \frac{\boldsymbol{Fat}\ \textbf{weight}}{\boldsymbol{Final}\ \textbf{weight}} \times 100$$

Statistical Analysis:

The data were statistically analyzed using a computerized Costat Program by one way ANOVA using a Completely Randomized Factorial Design **(SAS, 1988)** when a significant mean effect was detected, the means were separated with the Duncan's Multiple Range Test. Differences between treatments at $P \leq 0.05$ were considered significant. The results are presented as mean ± SD.

Results and Discussion:

Data presented in table (1) illustrate the effect of rhubarb, astragalus and mixture of both on organs weight of obese rats. It could be observed that the mean value of liver weight of control (+) group was higher than control (-) group, being 9.2±0.05 and 7.6±0.02 g respectively. The best liver weight showed for groups 5 (rats fed on basal diet containing 5% mixture of both) when compared to control (+) group.

It could be noticed that the mean value of heart weight (g) of control (+) group was higher than control (-) group, being 1.9±0.04 and 1±0.1 g respectively. The best heart weight level showed for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

It could be observed that the mean value of lungs weight (g) of control (+) group was higher than control (-) group, being 2.1±0.09 and 1±0.2 respectively. The best lungs weight was

shown for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

Data of the same table (1) showed that the mean value of spleen weight (g) of control (+) group was higher than control (-) group, being 1.9±0.01 and 0.9±0.07 respectively. The best spleen weight was shown for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

Also, it could be reveled that the mean value of kidneys weight (g) of control (+) group was higher than control (-) group, being 2.9±0.04 and 2±0.1 respectively. The best kidneys weight was shown for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

Table (1): Effect of Rhubarb, Astragalus and mixture of both on organs weight (g) of obese rats

Parameters / Groups	Liver (g) Mean ±SD	Heart (g) Mean ±SD	Lungs (g) Mean ±SD	Spleen (g) Mean ±SD	Kidneys (g) Mean ±SD
G1: Control –ve	7.6^{c}±0.02	1^{d}±0.1	1.0^{c}±0.1	0.9^{d}±0.07	2.0^{d}±0.1
G2: Control +ve	9.2^{a}±0.05	1.9^{a}±0.04	2.1^{a}±0.09	1.9^{a}±0.01	2.9^{a}±0.04
G3: Rhubarb (5%)	8.9^{a}±0.07	1.3^{c}±0.06	1.7^{b}±0.04	1.2^{c}±0.05	2.2^{c}±0.08
G4: Astragalus (5%)	9.1^{a}±0.09	1.7^{b}±0.08	2^{a}±0.05	1.6^{b}±0.08	2.6^{b}±0.05
G5: mixture of both (5%)	8^{b}±0.04	1.2^{c}±0.05	1.6^{b}±0.07	1.1^{c}±0.04	2.1^{cd}±0.07
LSD	0.34	0.13	0.19	0.101	0.13

Values in each coloum with different letters are significantly different ($P<0.05$).

Data presented in table (2) show the effect of rhubarb, astragalus and mixture of both on PFP% of obese rats. It could be

observed that the mean value PFP% of control (+) group was higher than control (-) group, being 6.02±0.001 and 4.15±0.009 respectively. The best PEP% level was showed for groups 5 (rats fed on basal diet containing 5% mixture of both) when compared to control (+) group.

Table (2): Effect of Rhubarb, Astragalus and mixture of both on PFP % of obese rats

Parameters / Groups	PFP % Mean ±SD
G1: Control –ve	4.15^{e}±0.009
G2: Control +ve	6.02^{a}±0.001
G3: Rhubarb (5%)	4.48^{b}±0.007
G4: Astragalus (5%)	4.41^{c}±0.004
G5: mixture of both (5%)	4.38^{d}±0.006
LSD	0.01

Values in each coloum with different letters are significantly different (P<0.05).

Data presented in table (3) illustrate the effect of rhubarb, astragalus and mixture of both on leptin hormone of obeses rats.

It could be noticed that the mean value of leptin hormone (ng/ml) of control (+) group was higher than control (-) group, being 27.05±0.008 and 4.86±0.001 (ng/ml) respectively. The best leptin hormone was shown for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

Yu *et al.*, (2003) found that oral administration of complex prescription of rhubarb for month reduced the levels of leptin in obese rats.

Huang *et al.*, (2017) reported that *Astragalus membranaceus* polysaccharides reduced leptin level on metabolically stressed transgenic mice.

Table (3): Effect of Rhubarb, Astragalus and mixture of both on leptin hormone (ng/ml) of obese rats

Parameters / Groups	Leptin hormone (ng/ml) Mean ±SD
G1: Control –ve	4.86±0.001
G2: Control +ve	27.05^{a}±0.008
G3: Rhubarb (5%)	6.51^{b}±0.003
G4: Astragalus (5%)	6.40^{c}±0.005
G5: mixture of both (5%)	6.01^{d}±0.007
LSD	0.009

Values in each coloum with different letters are significantly different ($P<0.05$).

Data of table (4) indicate the effect of rhubarb, astragalus and mixture of both on serum levels of antioxidants enzymes (SOD (nmol/min/mg), GPX (nmol/min/mg) and CAT (U/mg)) of obese rats.

It could be observed that the mean value of SOD enzyme of control (-) group was higher than control (+) group, being 66.40±0.008 and 35.34±0.002 (nmol/min/mg) respectively. The best treatment was observed for group 5 (basal diet containing 5% mixture of both) when compared to control (+) group.

It could be noticed that the mean value of GPX enzyme of control (-) group was higher than control (+) group, being 0.80±0.001 and 0.42±0.007 (nmol/min/mg) respectively. The best treatment was observed for group 5 (basal diet containing 5% mixture of both) when compared to control (+) group.

Data of the same table (4) show the mean value of CAT enzyme of control (-) group was higher than control (+) group, being 0.18±0.002 and 0.13±0.008 (U/mg) respectively. Group 5 showed the highest mean value of CAT enzyme level as compared

to control (+) group which and recorded the best result**.**

Jiangwei *et al*., (2011) found that *Astragalus mongholicus* extract as a dietary supplement on hyperlipidemia and oxidative stress in rats maintained on a high- cholesterol diet increased superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase in rats.

He *et al*., (2012) indicated that rhubarb choleretic capsule (RCC) increased enzyme SOD on alcoholic fatty liver in rats.

Table (4): Effect of Rhubarb, Astragalus and mixture of both on antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) of obese rats

Parameters / Groups	SOD (mmol/L protein) Mean ±SD	GPx (mg/mL protein) Mean ±SD	CAT (mmol/L) Mean ±SD
G1: Control –ve	66.40^{a}±0.008	0.80^{a}±0.001	0.18^{a}±0.002
G2: Control +ve	35.34^{e}±0.002	0.42^{e}±0.007	0.13^{c}±0.008
G3: Rhubarb (5%)	35.83^{d}±0.009	0.65^{d}±0.003	0.14^{bc}±0.004
G4: Astragalus (5%)	39.70^{c}±0.004	0.70^{c}±0.009	0.15^{b}±0.009
G5: mixture of both (5%)	65.30^{b}±0.005	0.77^{b}±0.006	0.17^{a}±0.001
LSD	0.011	0.011	0.01

Values in each coloum with different letters are significantly different (P<0.05).

Histopathololgical changes:

Microscopically section of liver from healthy (control -) group 1revealed the normal structure of hepatic lobule (Photo 1).However, liver from group 2 (control +) obese rats showed vacuolar degeneration of hepatocytes (Photos 2& 3). Rats of liver sections of group 3 rhubarb diets showed no changes except slight

hydropic degeneration of hepatocytes and slight congestion of central vein (Photos 4&5). Moreover, liver of rats from obese group fed on astragalus diet (group 4) showed slight hydropic degeneration of hepatocytes and slight congestion of hepatic sinusoids (Photos 6&7).Also liver sections of mix diet group revealed mild changes described as hydropic degeneration of hepatocytes and slight congestion of hepatic sinusoids (Photo 8) as well as slight activation of Kupffer cells (Photo 9).

Sections of kidneys from healthy rat (control -) group 1 showed the normal histopathological structure of renal parenchyma (Photo 10).Meanwhile that of group 2 (control +) obese rats, revealed congestion of renal blood vessels (Photo 11). Section of kidneys from rhubarb diet of group 3, astragalus and mix diets (Photos 12, 13&14) respectively showed no histopathological alternations.

It is evident that rhubarb, astragalus and especially mix diets improved the histopathological structure of both liver and kidney which was changed by obesity, and this coincidred with the biochemical changes.

Su *et al*., (2013) found that rhe extracts from rhubarb improved renal function and significantly reduced renal fibrosis and interstitial inflammation in chronic allograft nephropathy (CAN) in rats.

Qin *et al*., (2014) reported that rhubarb anthraquinones and tannins have a biphasic effect on liver, protection and damage. Anthraquinones showed stronger improvement on liver fibrosis and liver cell injury than tannins, and high dose tannins may injury liver in rats.

Hamid *et al*., (2017) indicated that selenizing astragalus polysaccharides (sAPS) could increase the activities of Astragalus polysaccharides and sodium selenite to protect the liver from damage by attenuating hepatic inflammation, oxidative stress, fibrogenesis, and induces apoptosis and cell cycle arrest in hepatic stellate cells in rats.

Meng *et al*., (2020) found that renal damage in the astragalus polysaccharide groups (all doses) was less severe compared that in the model group. Partial glomerular enlargement, mesangial cell proliferation, and mild tubular stenosis were

observed, although these signs were milder than in the model group and relieved to a greater degree in the higher dose group than in the lower dose groups in rats.

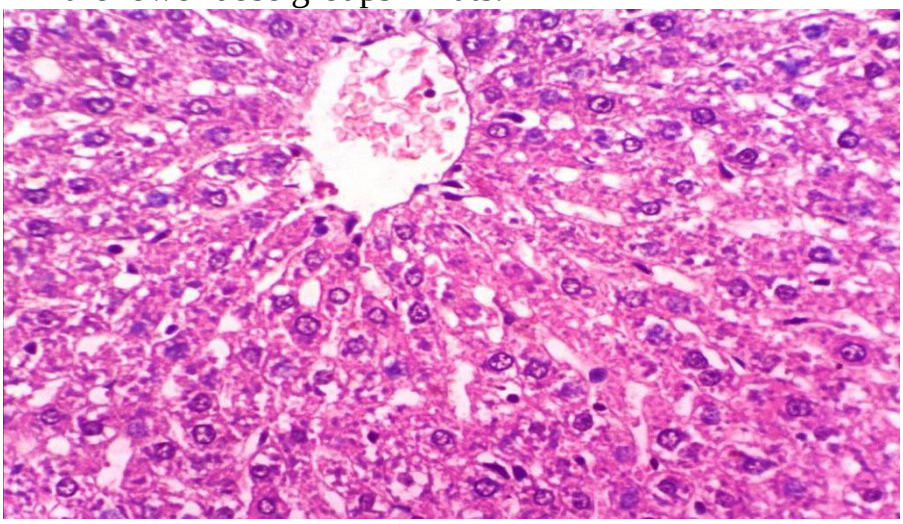

Photo (1): Liver of rat from group 1 (healthy rats) showing the normal histological structure of hepatic lobule (H & E X 400).

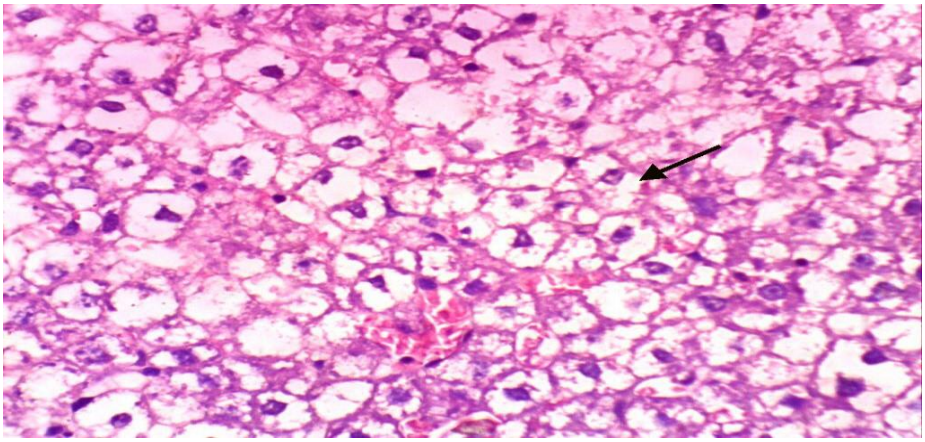

Photo (2): Liver of rat from group 2 (obese rats) showing vacuolar degeneration of hepatocytes (H & E X 400)

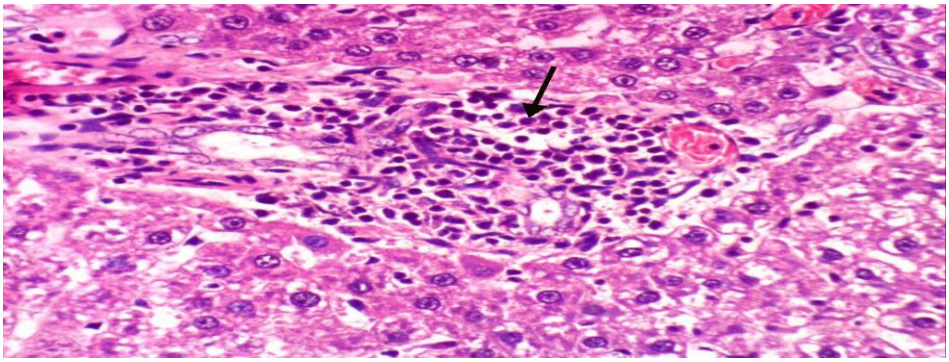

Photo (3): Liver of rat from group 2 (obese rats) showing vacuolar degeneration of hepatocytes and inflammatory cells infiltration in the portal triad (H & E X 400).

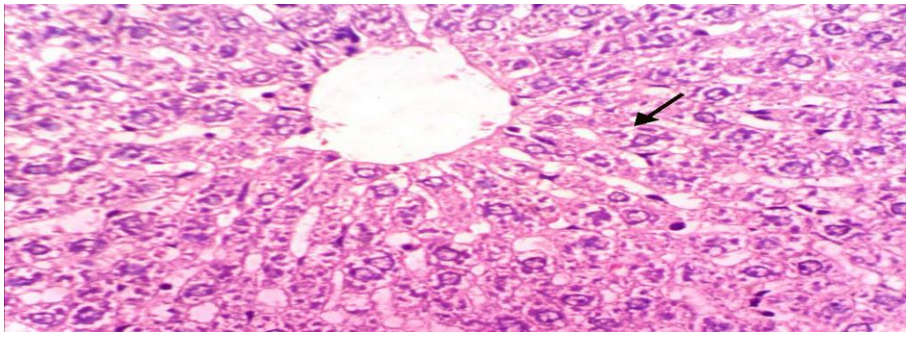

Photo (4): Liver of rat from group 3 (rhubarb 5%) showing slight hydropic degeneration of hepatocytes (H & E X 400).

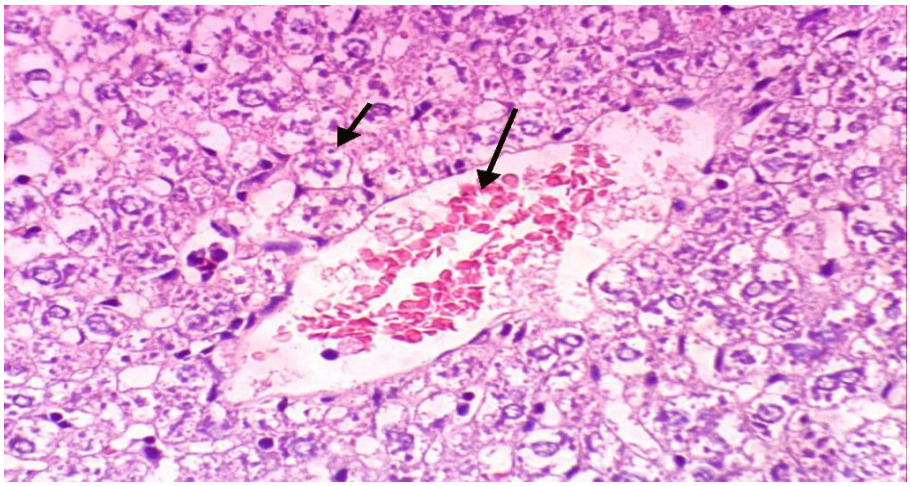

Photo (5): Liver of rat from group 3 (rhubarb 5%) showing 3 showing slight hydropic degeneration of hepatocytes and slight congestion of central vein (H & E X 400).

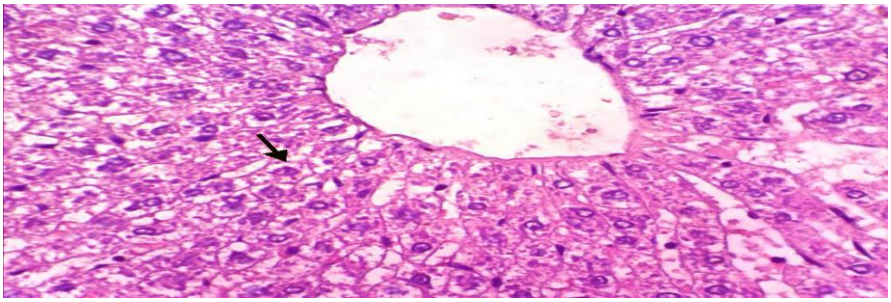

Photo (6): Liver of rat from group 4 (astragalus 5%) showing slight hydropic degeneration of hepatocytes (H & E X 400).

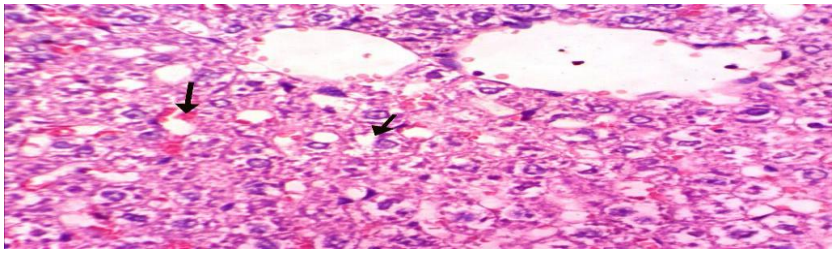

Photo (7): Liver of rat from group 4 (astragalus 5%) showing slight hydropic degeneration of hepatocytes and slight congestion of hepatic sinusoids (H & E X 400).

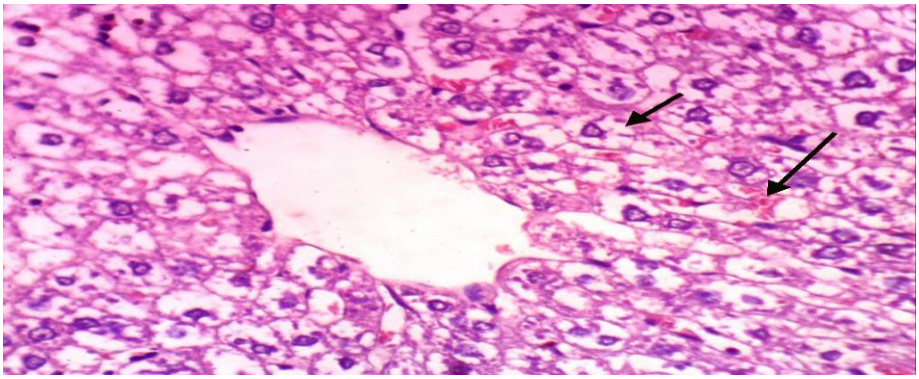

Photo (8): Liver of rat from group 5 (mix diets) showing showing hydropic degeneration of hepatocytes and slight congestion of hepatic sinusoids (H & E X 400).

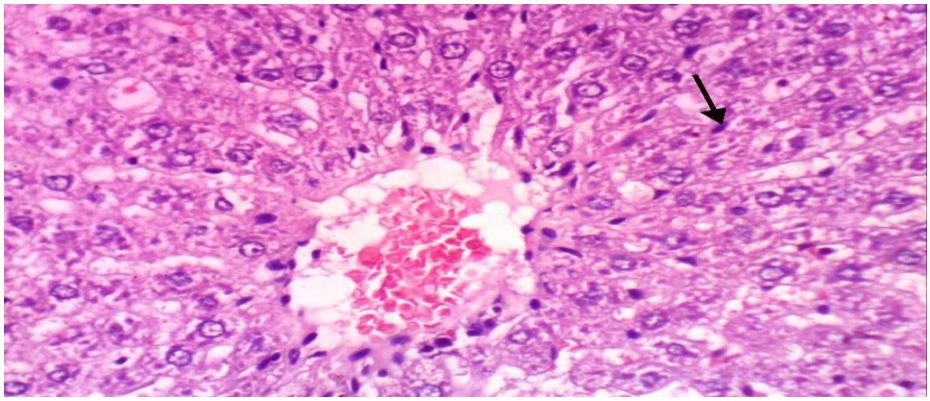

Photo (9): Liver of rat from group 5 (mix diets) showing slight activation of Kupffer cells (H & E X 400).

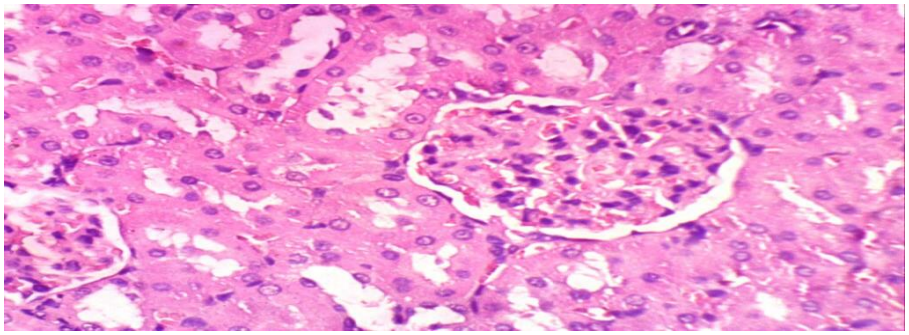

Photo (10): Photomicrograph of kidney of rat from group 1(healthy rats) showing the normal histological structure of renal parenchyma (H & E X 400).

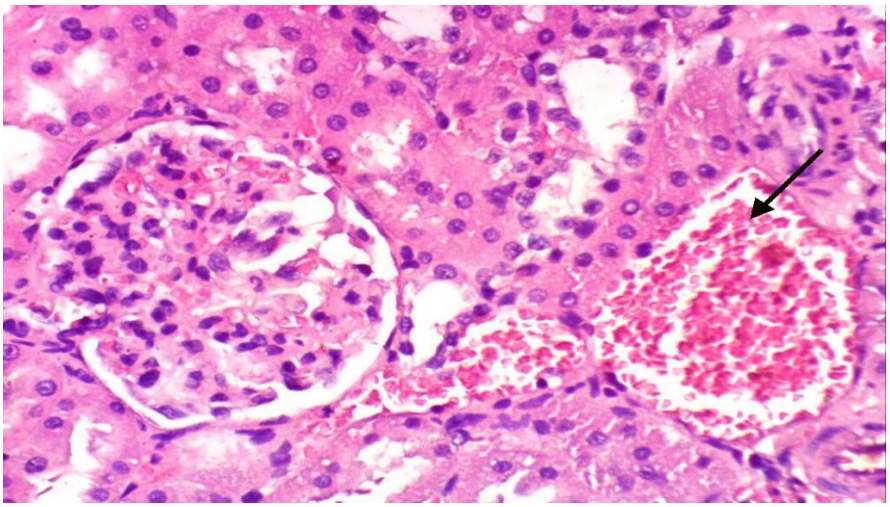

Photo (11): Kidney of rat from group 2 (obese rats) showing congestion of renal blood vessels (H & E X 400).

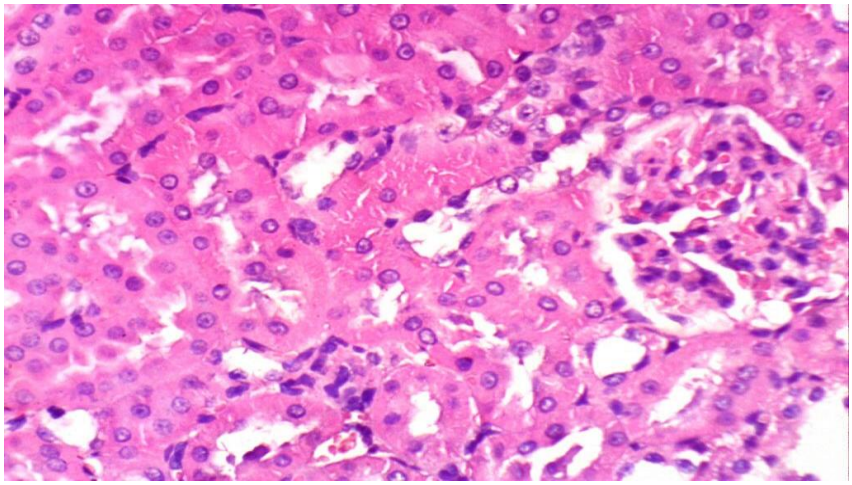

Photo (12): Kidney of rat from group 3 (rhubarb 5%) showing no histopathological alterations (H & E X 400).

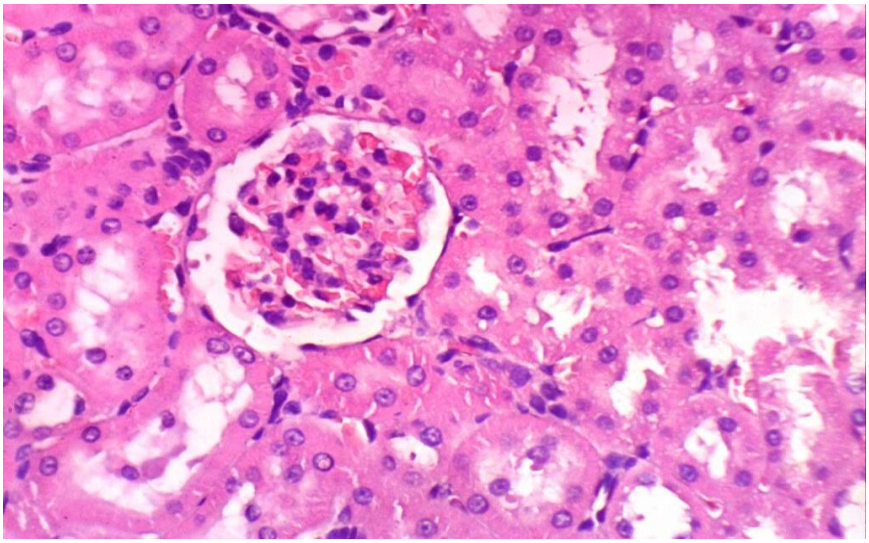

Photo (13): Kidney of rat from group 4 (astragalus 5%) showing no histopathological alterations (H & E X 400).

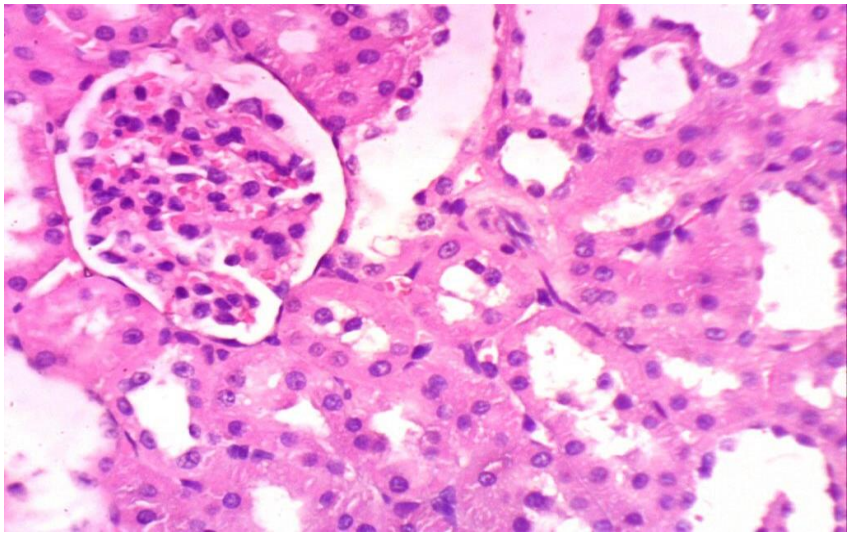

Photo (14): Kidney of rat from group 5 (mix diets) showing no histopathological alterations (H & E X 400).

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