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Study the Histopathological Changes of Obese Rats by Chamomile and Garcinia Diets with Reference to Some Biological & Biochemical Alternations

Abstract

This study aimed to investigate the effect of chamomile (*Matricaria chamomilla*), garcinia (*Garcinia cambogia*) and mixture of both on histopathological structure along with internal organs weights and some biochemical changes of 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 Chamomile (5%). Group (4): Obese rats fed on basal diet and Garcinia (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. Overweight led to a significant ($P \leq 0.05$) decrease in the level of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) while a significant ($P \leq 0.05$) increase was recorded in leptin hormone & PFP. Obese rats treated with various diets showed the improvement in all previous parameters.

Improvement was more pronounced for garcinia compared to chamomile diets, and the best group was recorded for the mix group, indicating a synergistic action.

Keywords: Obesity- Chamomile - Garcinia.

Introduction

Chamomile (Matricaria chamomilla, synonym Matricaria recutita (L.) Rauschert) is a popular aromatic, medicinal herb mostly used in therapeutic purposes. Dried flowers and essential oil are the most widely used products mainly because it has multiple medicinal properties from anti-inflammation ,analgesic, antimicrobial and antispasmic to sedative (Žlabur *et al*., 2020) . The most common variety in use is German chamomile (Matricaria chamomilla), while from the Asteraceae family well known variety is also Roman chamomile (*Chamaemelum nobile*). Mentioned varieties strongly differs both in morphological and chemical composition primarily in the content of biologically active compounds including essential oils and several polyphenols (Raal *et al*., 2012), (Das, 2014).

In several animal studies, anxiolytic, antimutagenic and cholesterol-lowering, wound healing, and antidiabetic properties of chamomile plant were suggested. In in vitro studies, chamomile was demonstrated to possess modest antimicrobial and antioxidant properties and strong antiplatelet and anticarcinoma properties. It is able to cure skin lesions in colostomy patients and is beneficial for generalized anxiety disorders and anxiolytic activity in patients with mild to moderate generalized anxiety disorders (GAD) (Miraj and Alesaeidi, 2016).

The chemical compounds of this plant are as follows: Apigenin, apigenin-7-O-glucoside, caffeic acid, chlorogenic acid, luteolin, and luteolin-7-O-glucoside, terpene bisabolol farnesene, chamazulene, flavonoids (including apigenin, quercetin, patuletin, and luteolin), and coumarin (Avonto *et al*., 2013).

Garcinia cambogia is a herbal product derived from the fruit of the Malabar tamarind tree (also called Garcinia gummi-gutta) native to India, Nepal and Sri Lanka (Bo *et al*., 2020). The fruit rind is

used either as food preservative, flavoring agent, food-bulking agent or traditional medicine in many Asian countries (Semwal *et al*., 2015). Garcinia contains xanthones, benzophenones, amino acids and organic acids, of which hydroxy-citric acid (HCA) accounts for 10%-30% of the weight of garcinia fruit and 20%-60% of the extract (Bo *et al*., 2020).

Studies with different duration of compounds administration and doses of Garcinia cambogia or its extract, were performed both in animals and humans with conflicting results. Favorable effects of Garcinia cambogia on glucose and lipid metabolism, as well as on appetite reduction, have been reported (Haber *et al*., 2018).

In animal studies, supplementation with HCA induced energy expenditure acceleration by the activation of the adiponectin AMPK signaling pathway (Li *et al*., 2019) or through the regulation of thyroid hormone levels (Han *et al*., 2016). HCA inhibits serotonin uptake leading to satiety and reduced food intake and down-regulates ATP-citrate lyase, increasing fat oxidation and decreasing de novo lipogenesis (Fassina *et al*., 2015)..

Materials and Methods

Materials:

Chamomile (*Matricaria chamomilla)* and garcinia (*Garcinia cambogia*) were obtained dry from a herb shop in Cairo, Egypt.

Animals:

Thirty (30) adult male Sprague Dawley rats, average body weight (150 ± 10 g) were used in this study.

Methods:

High fat diet (HFD):

The experimental (HFD) were prepared, containing 14% protein from casein, 20% fat (19% saturated fat + 1% unsaturated fat), 5% cellulose, 3.5% salt mixture, 1% vitamin mixture, 10% sucrose, 0.25% choline chloride and the remainder was corn starch. The HFD used for 6 weeks to obtain obese rats (Liu *et al*., 2004).

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).

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).

Induced obesity for rats:

Rats were fed on HFD for 45 days before treating with herbs.

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 Chamomile 5% diet.

Group (4): Obese rats fed on Garcinia Cambogia 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 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). All serum samples were analyzed for determination the following parameters:

(1) Leptin hormone:

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

(2) Antioxidant enzymes:

* Superoxide dismutase (SOD) was assayed according to the method of Kakkar *et al.*, (1984).

* Glutathione peroxidase (GPx)was assayed according to the method of Habig *et al.*, (1974).
* Catalase activity (CAT) was assayed according to the method of Luck *et al.*, (1974).

The organs (liver, kidney, heart, lungs and spleen) were removed, washed in saline solution, wiped by filter paper and weighted. Liver and kidney fixed in formalin solution 10% for histological testing according to method mentioned by (Drury and Wallington, 1980) and PFP% was determined using following equation:

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

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) showed that the effect of Chamomile, Garcinia cambogia and mixture of both on organs weight (g) of obese rats.

It could be observed that the mean value of liver of control (+) group was higher than control (-) group, being 9.3±0.06 and 7.6±0.02g respectively. The best liver weight was showed for group 5 (rats fed on basal diet containing 5% mixture of both herbs) when compared to control (+) group.

It could be showed that the mean value of heart weight of control (+) group was higher than control (-) group, being 1.9±0.02 and 1.0±0.09 g respectively. The best heart weight showed for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

The same table indicated that the mean value of lungs weight of control (+) group was higher than control (-) group, being 2.1±0.05 and 1.0±0.07 g respectively. The best lungs weight showed for group 4 (rats fed on basal diet + garcinia 5%) when compared to control (+) group.

Also, data of table (1) noticed that the mean value of spleen weight of control (+) group was higher than control (-) group, being 1.9±0.07 and 0.9±0.02 g respectively. The best spleen weight was shown for group 4 (rats fed on basal diet + garcinia 5%) when compared to control (+) group.

It could be noticed that the mean value of kidneys weight of control (+) group was higher than control (-) group, being 2.9±0.07 and 2.0±0.04 g respectively. The best kidneys weight showed for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

Table (1): Effect of Chamomile, Garcinia 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^{e}±0.02	1.0^{c}±0.09	1.0^{d}±0.07	0.9^{b}±0.02	2.0^{e}±0.04
G2: Control +ve	9.3^{a}±0.06	1.9^{a}±0.02	2.1^{a}±0.05	1.9^{a}±0.07	2.9^{a}±0.07
G3: Chamomile (5%)	8.3^{b}±0.07	1.5^{b}±0.04	1.5^{b}±0.04	1.0^{b}±0.07	2.4^{b}±0.04
G4: Garcinia (5%)	8.0^{c}±0.07	1.2^{c}±0.01	1.3^{c}±0.08	0.8^{c}±0.04	2.3^{c}±0.05
G5: Mixture of both (5%)	7.9^{d}±0.02	1.1^{c}±0.2	1.4^{bc}±0.01	1.0^{b}±0.02	2.2^{d}±0.02
LSD	0.097	0.18	0.1	0.09	0.085

Values of same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

Data presented in table (2) show that the effect of Chamomile, Garcinia cambogia and mixture of both on PFP % of obese rats.

It could be observed that the mean value of PFP % of control (+) group was higher than control (-) group, being 5.90±0.002 and 3.99±0.004g respectively. The best PFP % showed for group 5 (rats fed on basal diet containing 5% mixture of both herbs) when compared to control (+) group.

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

Parameters / Groups	PFP % Mean ±SD
G1: Control –ve	3.99^{e}±0.004
G2: Control +ve	5.90^{a}±0.002
G3: Chamomile (5%)	4.56^{b}±0.005
G4: Garcinia (5%)	4.31^{c}±0.003
G5: mixture of both (5%)	4.23^{d}±0.008
LSD	0.009

Values of same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

Data presented in table (3) illustrate the effect of Chamomile, Garcinia cambogia and mixture of both on leptin hormone (ng/ml) of obese rats.

It could be noticed that the mean value of leptin hormone (ng/ml) of control (+) group was higher than control (-) group, being 28.32±0.004 and 5.68±0.003(ng/ml) respectively. The best leptin hormone showed for group 5 (rats fed on basal diet containing 5% mixture of both herbs) when compared to control (+) group.

Shelbaya (2017) indicated that chamomile powder and oil showed significant decrease in leptin hormone in rats with High Fat High Fructose diet.

Altiner *et al*., (2018) found that *Garcinia cambogia* extract decreased serum leptin levels in rats fed with the high lipid diet.

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

Parameters / Groups	Leptin hormone (ng/ml) Mean ±SD
G1: Control –ve	5.68^{e}±0.003
G2: Control +ve	28.32^{a}±0.004
G3: Chamomile (5%)	9.03^{b}±0.006
G4: Garcinia (5%)	8.40^{c}±0.005
G5: Mixture of both (5%)	8.11^{d}±0.008
LSD	0.0099

Values of same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

Data presented in table (4) show effect of Chamomile, Garcinia and mixture of both on antioxidant enzymes of obese rats.

It could be showed that the mean value of SOD of control (-) group was higher than control (+) group, being 60.45±0.009 and 39.70±0.005(nmol/min/mg protein) respectively. The best SOD showed for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

The same table indicated that the mean value of GPx of control (-) group was higher than control (+) group, being 0.78±0.003 and 0.40±0.009 (nmol/min/mg protein) respectively. The best GPx showed for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

Also, data of table (4) noticed that the mean value of CAT of control (-) group was higher than control (+) group, being 0.17±0.002 and 0.12±0.004 (U/mg) respectively. The best CAT was shown for groups 4 & 5 when compared to control (+) group.

Jabri *et al*., (2017) found that chamomile (*Matricaria recutita L*.) decoction extract increased SOD, GPx, & CAT in rat gastric mucosa which with ulcer and oxidative stress induced by ethanol.

Airaodion *et al*., (2020) indicated that ethanolic extract of *Garcinia kola* seed increased SOD, GPx, & CAT in wistar rats.

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

Parameters / Groups	SOD (nmol/min/mg protein) Mean ±SD	GPx (nmol/min/mg protein) Mean ±SD	CAT (U/mg) Mean ±SD
G1: Control –ve	60.45^{a}±0.009	0.78^{a}±0.003	0.17^{a}±0.002
G2: Control +ve	39.70^{e}±0.005	0.40^{e}±0.009	0.12^{b}±0.004
G3: Chamomile (5%)	41.31^{d}±0.002	0.68^{d}±0.005	0.13^{b}±0.008
G4: Garcinia (5%)	54.20^{c}±0.007	0.73^{c}±0.008	0.16^{a}±0.009
G5: Mixture of both (5%)	59.90^{b}±0.06	0.75^{b}±0.008	0.16^{a}±0.001
LSD	0.049	0.013	0.01

Values of same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

Histopathological 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 hydropic degeneration of hepatocytes (Photo 2, 3) and focal hepatocellular necrosis associated with inflammatory cells infiltration. Rats of liver sections of group 3 chamomile diets (Photo 4) revealed small focal hepatocellular necrosis associated with inflammatory cells infiltration, and slight hydropic degeneration of hepatocytes (Photo 5). Liver of rats from obese

group fed on garcinia diet (Group 4) revealed only slight activation of Kupffer cells (Photo 6). While liver sections of mix diet groups (Photo 7) revealed no histopathological changes.

Sections of kidneys from healthy rat (control -) group 1 showed the normal histopathological structure of renal parenchyma (Photo 8). Meanwhile that of group 2 (control +) obese rats, revealed marked cytoplasmic vacuolization of epithelial lining renal tubules and endothelial lining glomerular tuft (Photo 9). Section of kidneys from chamomile diet of group 3 revealed no histopathological changes (Photo 10). Sections from garcinia and mix diets (Photo 11, 12) respectively showed no histopathological alternations. Such observations indicated that chamomile, garcinia and mixed diets more or less restored histological changes by obesity.

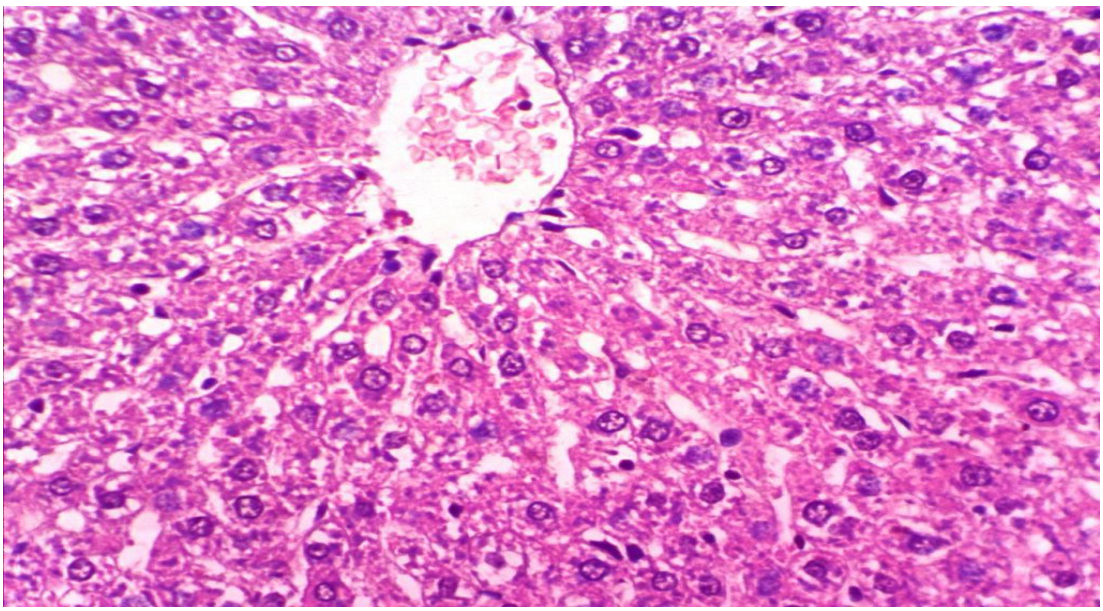

Photo (1): Liver of rat from group 1 (healthy rats) showing the normal histological structure of hepatic lobule (H & E X 400).

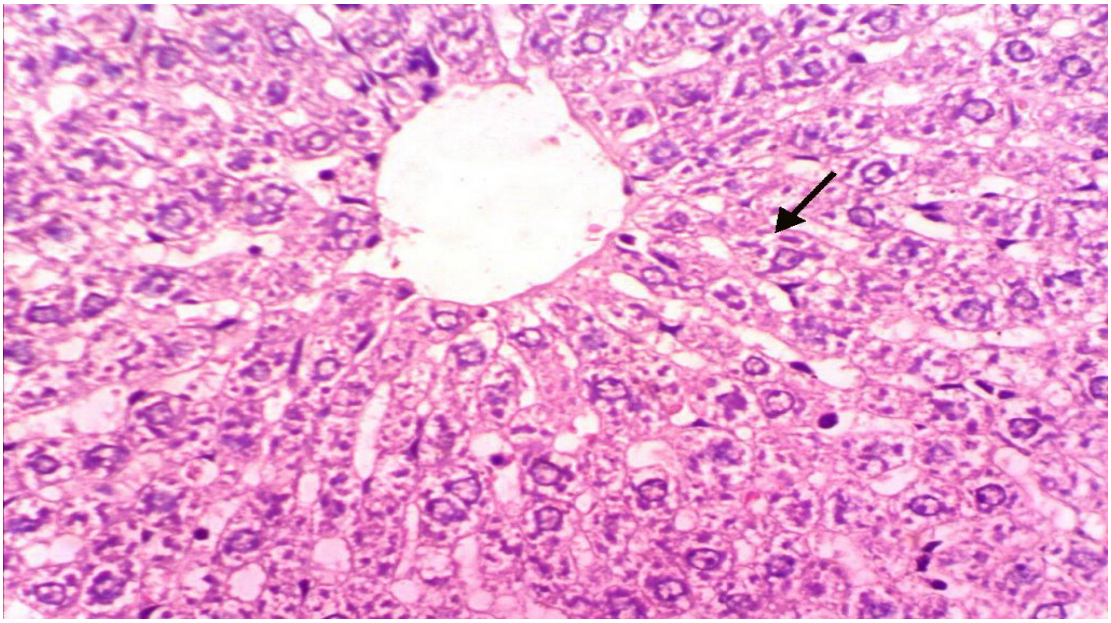

Photo (2): Liver of rat from group 2 (obese rats) showing slight hydropic degeneration of hepatocytes (H & E X 400).

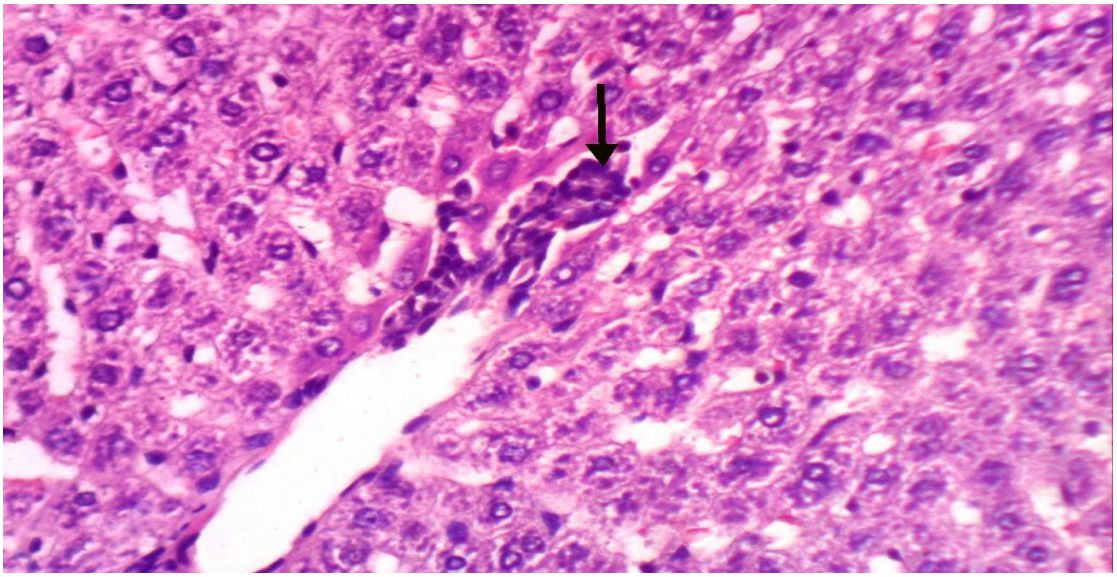

Photo (3): Liver of rat from group 2 (obese rats) showing small focal hepatocellular necrosis associated with inflammatory cells infiltration (H & E X 400).

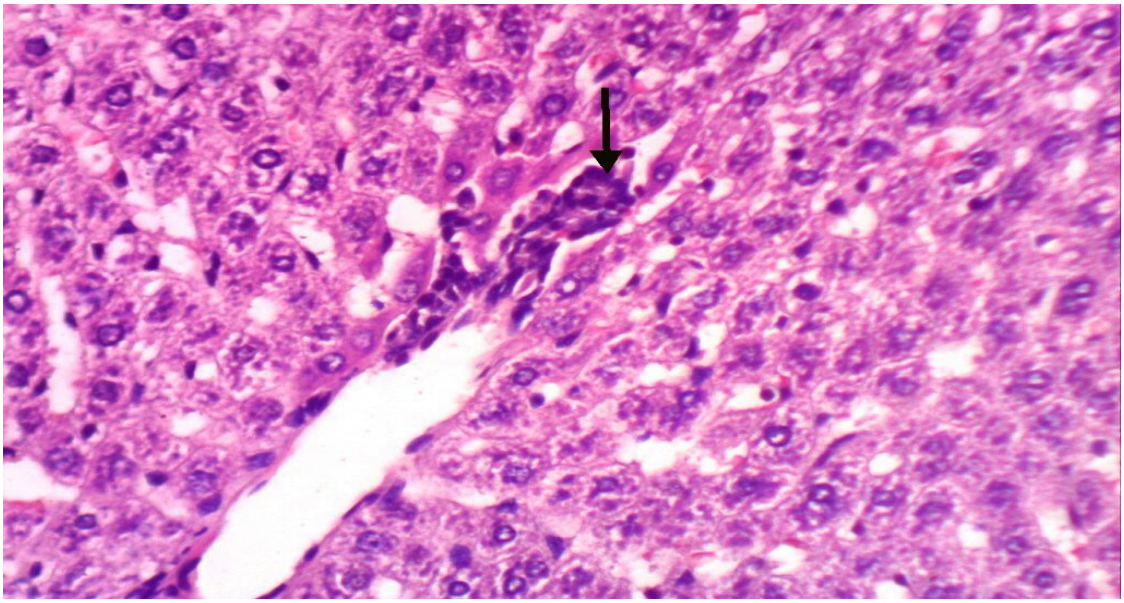

Photo (4): Liver of rat from group 3 (chamomile 5%) showing small focal hepatocellular necrosis associated with inflammatory cells infiltration (H & E X 400).

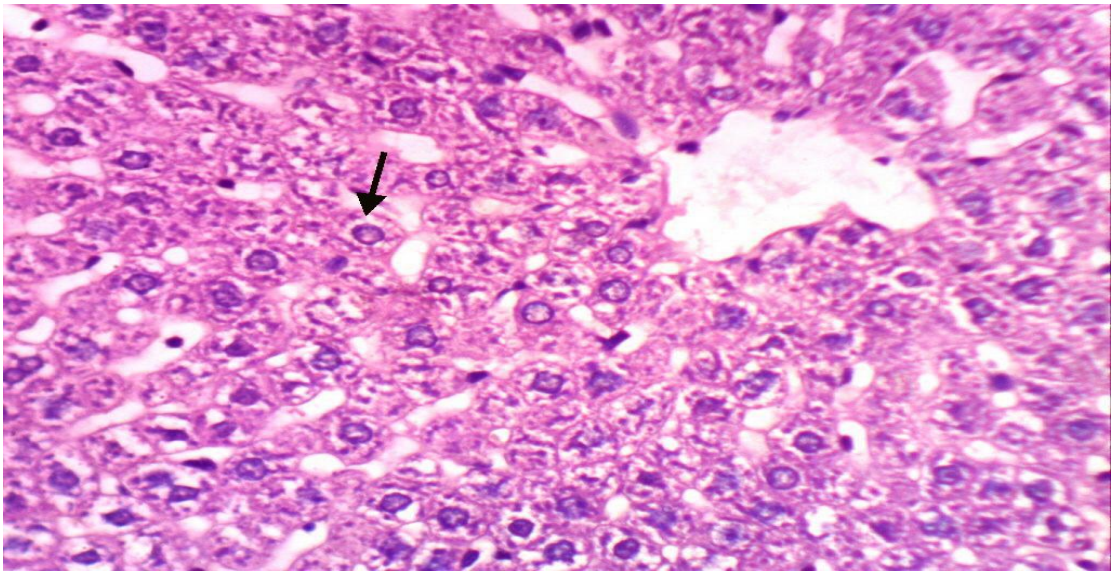

Photo (5): Liver of rat from group 3 (chamomile 5%) showing slight hydropic degeneration of hepatocytes (H & E X 400).

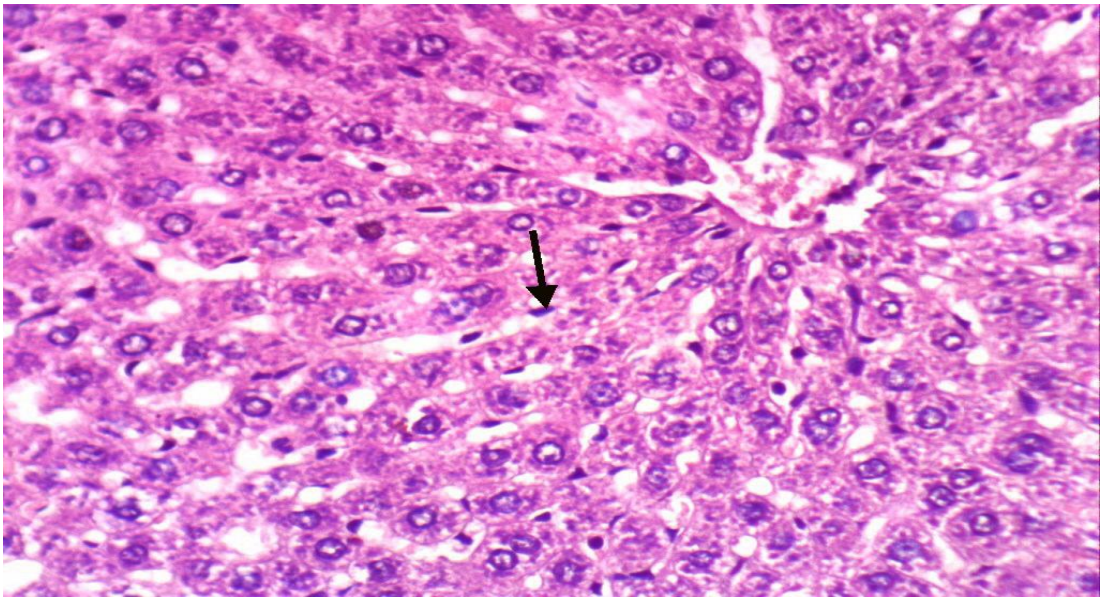

Photo (6): Liver of rat from group 4 (garcinia 5%) showing slight activation of Kupffer cells (H & E X 400).

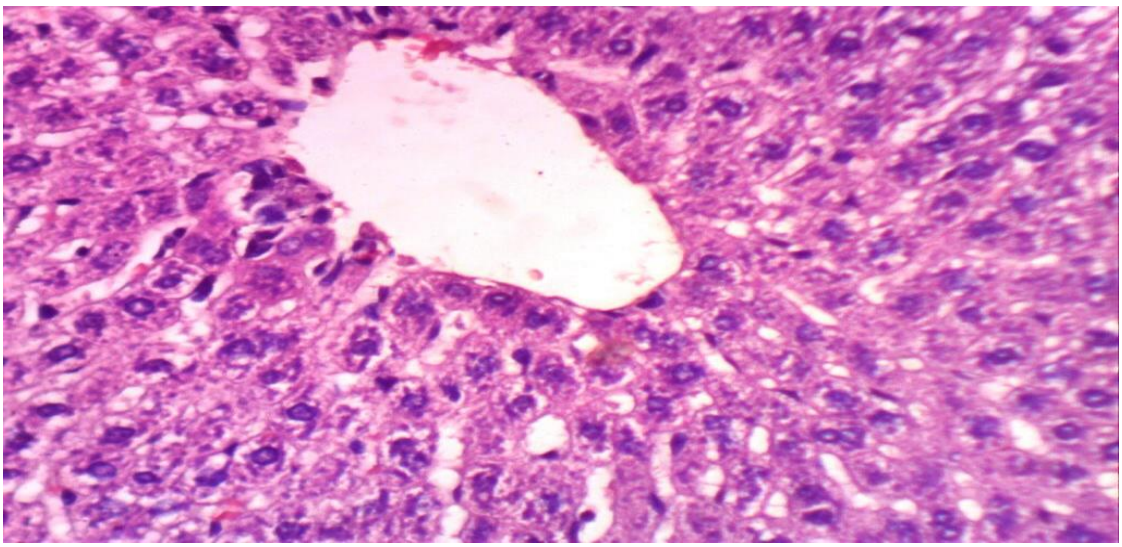

Photo (7): Liver of rat from group 5 (mix diets) showing no histopathological changes (H & E X 400).

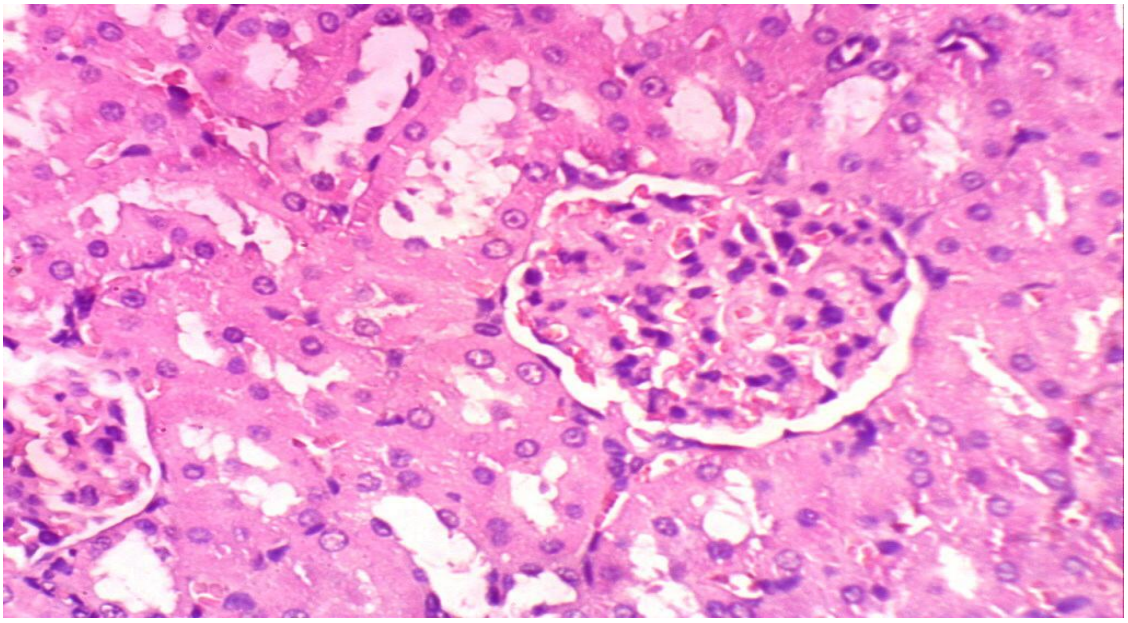

Photo (8): photomicrograph of kidney of rat from group 1(healthy rats) showing the normal histological structure of renal parenchyma (H & E X 400).

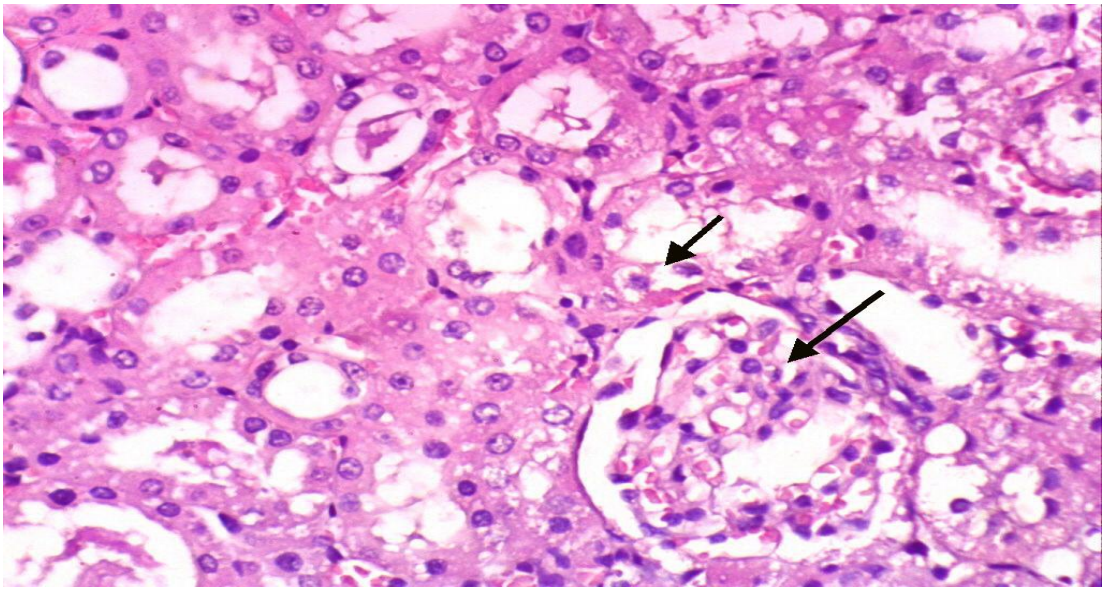

Photo (9): Kidney of rat from group 2 (obese rats) showing marked cytoplasmic vacuolization of epithelial lining renal tubules and endothelial lining glomerular tuft (H & E X 400).

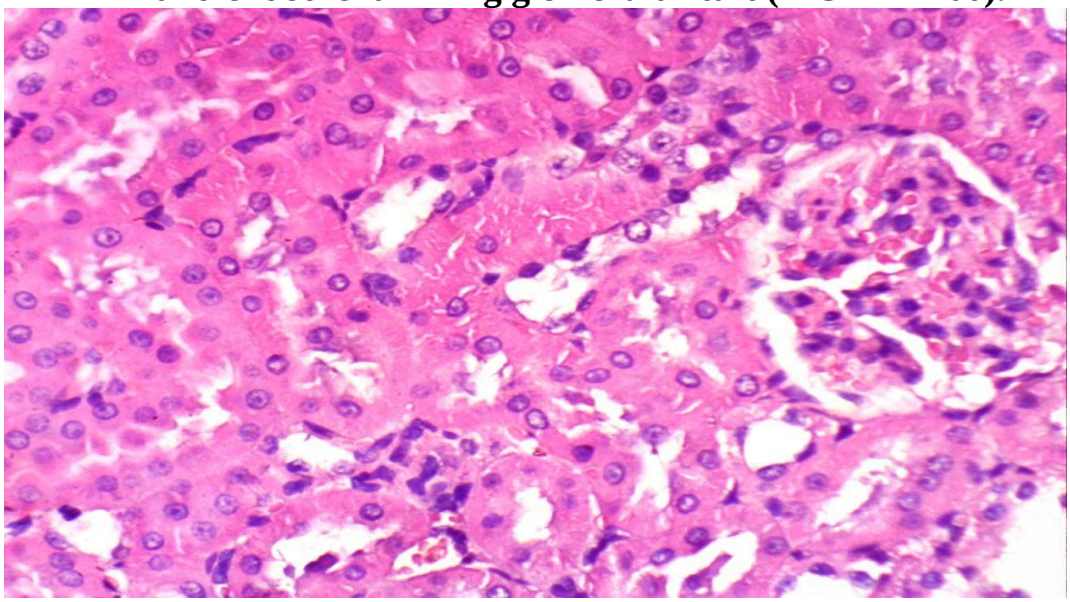

Photo (10): Kidney of rat from group 3 (chamomile 5%) showing no histopathological alterations (H & E X 400).

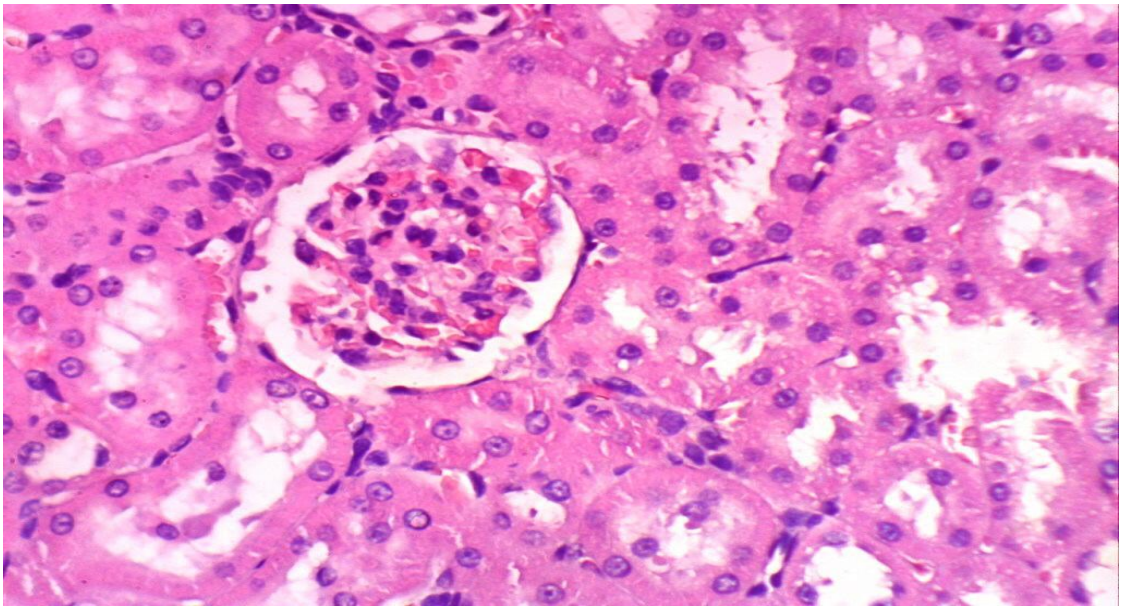

Photo (11): Kidney of rat from group 4 (garcinia 5%) showing no histopathological alterations (H & E X 400).

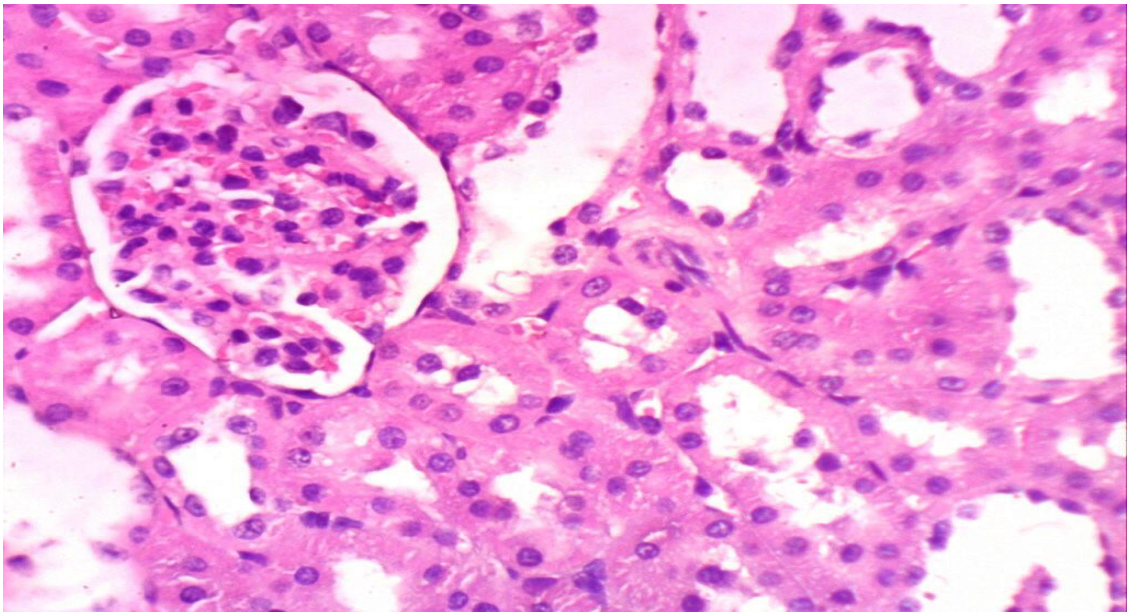

Photo (12): Kidney of rat from group 5 (mix diets) showing no histopathological alterations (H & E X 400).

Conclusion

Improvement was more pronounced for garcinia compared to chamomile diets, and the best group was recorded for the mix group, indicating a synergistic action.

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دراسة التغيرات الهستوباثولوجية فى الفئران البيضاء المصابة بالسمنة باستخدام البابونج والجارسينيا مع تناول أوزان الأعضاء الداخلية وبعض التغيرات الكيماوية الحيوية

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يهدف هذا البحث إلى تقييم تأثير أعشاب البابونج (كاموميل) والجارسينيا والخليط منهما علي التغيرات الهستوباثولوجية بالإضافة الى تغيرات أوزان الأعضاء الداخلية وبعض التغيرات الكيماوية الحيوية لدى ذكور الفئران المصابة بالسمنة. تم استخدام ثلاثين فأر من الذكور البالغين سبراغ داولي وتغذيتهم على نظام غذائى عالى الدهون لمدة 30 يوما ثم تقسيمهم 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 البابونج بنسبة 5%. المجموعة (4): الفئران المصابة بالسمنة التي تغذت على نبات الجارسينيا 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. وكذلك تم حساب وزن الاعضاء (الكبد، القلب، الكلي، الرئتين، الطحال). الاصابة بالسمنة سببت ارتفاع في مستوي هرمون الليبتين وطبقة الدهون على الغشاء البريتونى PFP وانخفاض مستويات الانزيمات المضادة للأكسدة وتشمل: (فوق أكسيد الديسموتيز SOD، جلوتاثيون بيروكسيداز GPx، الكاتاليز CAT) في الفئران المصابة بالسمنة وتحسنت النتائج باستخدام الأغذية المعالجة.

لوحظ أن التحسن كان اكبر فى حالة غذاء الجارسينيا بالمقارنة بغذاء الكاموميل وأحسن المعاملات كانت فى حالة الغذاء الخليط مما يدل على وجود التآزر.

الكلمات المفتاحية: السمنة – البابونج – الجارسينيا.