

# Effects of Galangal and Phyllanthus Herbs on Carbon Tetrachloride Hepatointoxicated Rats

Amal, M. Al-Ailbban

Nutrition and Food Science (Applied Nutrition), Department  
of Family Education, Univrsity of Umm Al-Qura, Makka Al-  
Mukarama, Kingdom Saudi Arabia



## مجلة البحوث في مجالات التربية النوعية

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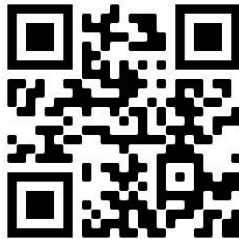
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## دراسة تأثير أعشاب الخلنجان والأمّج على سمية كبد الفئران البيضاء

### المحقونة برابع كلوريد الكربون

د/أمل محمد اللبان

كلية التغذية وعلوم الأطعمة (التغذية التطبيقية) - قسم التربية الأسرية- جامعة أمّ القري-  
مكة المكرمة- المملكة العربية السعودية

### المخلص العربي

يهدف هذا البحث إلى تقييم تأثير أعشاب الخلنجان والأمّج والخليط منهما علي سمية كبد الفئران المحقونة برابع كلوريد الكربون. تم تقسيم ثلاثون فأر من الذكور البالغين سيراغ داولي إلى خمس مجموعات. مجموعة (1): وهي المجموعة الضابطة السالبة (-) تغذت على الوجبة الأساسية ، المجموعة (2): وهي المجموعة الضابطة الموجبة (+) وهي الفئران المحقونة برابع كلوريد الكربون في الكبد وتغذت على الوجبة الأساسية. المجموعة (3): الفئران المحقونة برابع كلوريد الكربون في الكبد التي تغذت علي عشبة الخلنجان بنسبة 5%. المجموعة (4): الفئران المحقونة برابع كلوريد الكربون في الكبد التي تغذت على نبات الأمّج بنسبة 5%. المجموعة (5): الفئران المحقونة برابع كلوريد الكربون في الكبد التي تغذت على الاثتين معا بتركيز 5%.. في نهاية التجربة ، بعد 28 يوماً من التغذية ، تم تقدير الاختبارات البيوكيميائية للدم. الحقن برابع كلوريد الكربون سبب ارتفاع في الوزن المكتسب والمأخوذ من الغذاء ومستويات الجلوكوز واليوريا والكرياتينين واليوريك اسيد وAST وALT وALP ومستوى الكوليسترول الكلي وجلسريدات ثلاثية واللييبروتين منخفض الكثافة واللييبروتين منخفض الكثافة جدا والبيليروبين الكلي والبيليروبين المباشر وغير مباشر وانخفاض مستويات الليبروتين مرتفع الكثافة والبروتين الكلي والألبومين والجلوبيولين والانزيمات المضادة للأكسدة وتحسنت النتائج باستخدام الأغذية المعالجة خاصة في حالة الخلنجان.

الكلمات المفتاحية : الكبد ،عشبة الخلنجان ،عشبة الأمّج والخليط من الاثتين معا.

## Effects of Galangal and Phyllanthus Herbs on Carbon Tetrachloride Hepatointoxicated Rats

Amal, M. Al-Ailbban

Nutrition and Food Science (Applied Nutrition), Department of Family Education, Univrsity of Umm Al-Qura, Makka Al-Mukarama, Kingdom Saudi Arabia

### Abstract:

This investigation aimed to evaluate the effect of galangal, phyllanthus and mix diets on hepatointoxicated rats injected with  $CCl_4$ . 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): Hepatointoxicated rats injected with  $CCl_4$  fed on basal diet and galangal (5%). Group (4) Hepatointoxicated rats injected with  $CCl_4$  fed on basal diet and phyllanthus (5%). Group (5): Hepatointoxicated rats injected with  $CCl_4$  fed mix diets (5%). At the end of experiment, after 28 days of feeding, all serum samples were analyzed for biochemical parameters. Injected with  $CCl_4$  caused significant decreases in BWG, FI, TP, Alb, Glb, HDL and antioxidant enzymes while significant increases recorded in organs weight, TC, TG, VLDL, LDL, U.A, Creatinine, Urea, GOT, GPT, ALP, glucose Total bilirubin, Direct bilirubin (D.B) and Indirect bilirubin (I.B). Rats treated with various diets, showed the improvement in all previous parameters.

**Key words:** Liver, Galangal, Phyllanthus and Mix diets.

## Introduction:

Various plants have been used as drug and exhibit medicinal properties since ancient time. The medicinal property is contributed by the presence of secondary metabolism compounds in the plant *Alpinia galangal* (L) Wild syn. *Languas galanga* commonly known as greater galangal. *A. galangal* belongs to the kingdom Planta, family Zingiberaceae, and genus *Alpinia*. *Alpinia galangal* (L.) Also known as Galangal, a member of the ginger family and native to Southern China and Thailand. *A. galangal* is primarily used as a flavoring agent especially in the preparation of fresh Thai curry paste and Thai soup (Juntachote *et al.*, 2006; Verma *et al.*, 2011).

The major active compounds found in *A. galanga* are 1, 8-cineol,  $\alpha$ -fenchyl acetate,  $\beta$ -farnesene,  $\beta$ -bisabolene,  $\alpha$ -bergamotene,  $\beta$ -pinene and 1'-acetoxychavicol acetate. 1, 8-cineole known as marker compound for *Alpinia* spp and was reported as most abundant compound in most of the studies on *A. galanga* (Abdullah *et al.*, 2015).

*Alpinia galanga* possessed many pharmacological activities, including antibacterial, antifungal, antiviral, immunomodulatory, anti-oxidant effect, antidiabetic, antiplatelet, hypolipidemic and many other pharmacological effects (Shetty and Monisha, 2015).

*Phyllanthus* species are rich in phytochemical diversity, with compounds such as tannins, phenylpropanoids, terpenoids, phenolic compounds, flavonoids, alkaloids, saponins and many of their glycosides. Almost 81 compounds have been isolated from *Phyllanthus* spp. During 2016–2018, the majority of which were phenylpropanoids, triterpenoids, diterpenoids, and flavonoids (Nisar *et al.*, 2018).

*Phyllanthus niruri* L. (*P. niruri*) has been extensively reported in traditional and folk medication systems to treat various diseases including asthma, joint pains, loss of appetite, constipation, injuries, corneal opacity, conjunctivitis, diabetes mellitus, dropsy, gout, gonorrhoeal diseases of males and females, inflammatory diseases, skin itching, hepatic disorders, kidney stones or failures, leucorrhoea, obesity, scabies, stomach pains, tumors, typhoid fever, urinogenital disorders viral infections and many more (**Kaur et al.,2016**).

## **Materials and Methods**

### **Materials:**

Galangal and phyllanthus were obtained dry from aherb shop.

### **Chemicals:**

CCl<sub>4</sub> was a Website manufacture Product, Milan Italy.

### **Animals:**

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

### **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)**.

#### **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)**.

#### **Injection with CCl<sub>4</sub> in rats:**

Rats were injected by carbon tetrachloride (CCl<sub>4</sub>) at 2 ml/kg body weight, twice in week and for two weeks.

## 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<sup>0</sup>, 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 rats injected with CCl<sub>4</sub> were fed on basal diet.

**Group (3)** Rats injected with CCl<sub>4</sub> fed on galangal 5%.

**Group (4):** Rats injected with CCl<sub>4</sub> fed on phyllanthus 5%.

**Group (5):** Rats injected with CCl<sub>4</sub> fed on mix diets 5%.

## Determination of Biochemical Blood Parameters:

Blood samples were collected after 12 hours fasting at the end of experiment using the abdominal aorta. The rats firstly 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°C for biochemical analysis as described by **Schermer (1967)**. All serum samples were analyzed for determination the following parameters:

Urea was determined according to the enzymatic method of **Patton and Crouch (1977)**, creatinine was determined according to kinetic method of **Henry (1974)** and uric acid was according to the enzymatic colorimetric test of **Fossati and Prencipe (1980)**. Aspartate amino transaminase (AST) and alanine amino transferase (ALT) were carried out according to the methods of **Yound (1975) and Tietz (1976)**. Alkaline phosphatase (ALP) was determined according to **Belfield and Goldberg (1971)**. Total cholesterol (TC) was determined according to **Allain (1974)**, and high density lipoprotein cholesterol (HDL-c) according to **Lopez (1997)**. The calculation of low density lipoprotein cholesterol (LDL-c) was

carried out according to the method of **Lee and Nieman (1996)**, atherogenic index (AI) was calculated (VLDL+LDL / HDL) according to **Kikuchi et al., (1998)** and triglycerides as **Fossati and Prencipe (1982)**. Serum glucose determined according to **Kalpan (1984)**. Serum albumin was carried out to the method of **Doumas et al (1971)** and globulin was calculated as **Charry and Sharma (2004)**. Bilirubin was determined according to **Doumas et al., (1985)**, direct bilirubin and indirect bilirubin were measured according to **Sepulveda and Osterberg (1943)**. SOD was assayed according the methods of **Kakkar et al., (1984)**. Catalase activity was assayed the method of **Luck (1974)**. GPX was assayed according to the method of **Habig et al., (1974)**.

### **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  $\pm$  SD.

### **Results and Discussion:**

Data presented in table (1) illustrate the effect of galangal, phyllanthus and mix diets on BWG, FI and FER on on hepatointoxicated rats injected with  $CCl_4$ . It could be observed that the mean value of (BWG) of control (-) group was higher than control (+) group, being  $1.8 \pm 0.01$  and  $0.9 \pm 0.08$  g respectively. The best (BWG) level showed for groups 3 (rats fed on basal diet containing 5% galangal) when compared to control (+) group.

It could be noticed that the mean value of FI of control (-) group was higher than control (+) group, being  $19.1 \pm 0.08$  and  $14.3 \pm 0.02$  g respectively. The best (FI) level showed for group 3 (rats fed on basal diet + 5% galangal) when compared to control (+) group.

Also, data of table (1) observed that the mean value of (FER) of control (-) group was higher than control (+) group, being  $0.094 \pm 0.0001$  and  $0.063 \pm 0.0008$  respectively. The best FER



was shown for group 3 (rats fed on basal diet + 5% galangal) when compared to control (+) group.

**Yu et al., (2016)** reported that galangal alcohol extract increased body weight at (100, 200 mg/kg) and had describe effects on cognitive dysfunction and nerve pathological change in rats with diabetic encephalopathy.

**Table (1): Effect of galangal, phyllanthus and mix diets on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) on hepatointoxicated rats injected with CCl<sub>4</sub>**

Parameters Groups	BWG (g) Mean ± SD	FI (g) Mean ± SD	FER (%) Mean ± SD
<b>G1:</b> Control –ve	1.8 <sup>a</sup> ±0.01	19.1 <sup>a</sup> ±0.08	0.094 <sup>a</sup> ±0.0001
<b>G2:</b> Control +ve	0.9 <sup>d</sup> ±0.08	14.3 <sup>c</sup> ±0.02	0.063 <sup>c</sup> ±0.0008
<b>G3:</b> Galangal (5%)	1.6 <sup>b</sup> ±0.05	18.4 <sup>b</sup> ±0.09	0.087 <sup>b</sup> ±0.0006
<b>G4:</b> Phyllanthus (5%)	1.2 <sup>c</sup> ±0.07	16.5 <sup>d</sup> ±0.06	0.073 <sup>d</sup> ±0.0003
<b>G5:</b> Mix diets (5%)	1.3 <sup>c</sup> ±0.04	17.0 <sup>c</sup> ± 0.04	0.077 <sup>c</sup> ±0.0002
<b>LSD</b>	0.101	0.12	0.0009

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

Data presented in table (2) show the effect of galangal, phyllanthus and mix diets on organs weight on on hepatointoxicated rats injected with CCl<sub>4</sub> It could be noticed that the mean value of liver weight of control (+) group was higher than control (-) group, being 7.9±0.05 and 3.6±0.09 g respectively. The best liver weight showed for groups 3 (rats fed on basal diet containing 5% galangal) when compared to control (+) group.

It could be showed that the mean value of heart weight (g) of control (+) group was higher than control (-) group, being  $1.3\pm 0.002$  and  $0.62\pm 0.008$  g respectively. The best heart weight level showed for group 3 (rats fed on basal diet + 5% galangal) 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  $1.9\pm 0.08$  and  $1.1\pm 0.02$  respectively. The best lungs weight was shown for group 3 (rats fed on basal diet + 5% galangal) when compared to control (+) group.

Data of the same table (2) showed that the mean value of spleen weight (g) of control (+) group was higher than control (-) group, being  $1.1\pm 0.03$  and  $0.4\pm 0.09$  respectively. The best spleen weight was shown for group 3 (rats fed on basal diet + 5% galangal) when compared to control (+) group.

Also, it could be revealed that the mean value of kidneys weight (g) of control (+) group was higher than control (-) group, being  $1.6\pm 0.07$  and  $0.9\pm 0.03$  respectively. The best kidneys weight was shown for group 3 (rats fed on basal diet + 5% galangal) when compared to control (+) group.

**Table (2): Effect of Galangal, Phyllanthus and mix diets on organs weight (g) on hepatointoxicated rats injected with CCl<sub>4</sub>**

Parameters Groups	Liver (g) Mean ±SD	Heart (g) Mean ±SD	Lungs (g) Mean ±SD	Spleen (g) Mean ±SD	Kidneys (g) Mean ±SD
<b>G1:</b> Control -ve	3.6 <sup>d</sup> ±0.09	0.62 <sup>e</sup> ± 0.008	1.1 <sup>e</sup> ±0.02	0.4 <sup>c</sup> ±0.09	0.9 <sup>d</sup> ±0.03
<b>G2:</b> Control +ve	7.9 <sup>a</sup> ±0.05	1.3 <sup>a</sup> ± 0.002	1.9 <sup>a</sup> ±0.08	1.1 <sup>a</sup> ±0.03	1.6 <sup>a</sup> ±0.07
<b>G3:</b> Galangal (5%)	3.5 <sup>d</sup> ±0.07	0.65 <sup>d</sup> ± 0.009	1.2 <sup>d</sup> ±0.04	0.5 <sup>c</sup> ±0.07	1 <sup>cd</sup> ±0.09
<b>G4:</b> Phyllanthus (5%)	7.4 <sup>b</sup> ±0.02	0.90 <sup>b</sup> ± 0.004	1.6 <sup>b</sup> ±0.07	0.8 <sup>b</sup> ±0.02	1.2 <sup>b</sup> ±0.05
<b>G5:</b> mix diets (5%)	5.7 <sup>c</sup> ±0.06	0.81 <sup>c</sup> ± 0.006	1.4 <sup>c</sup> ±0.03	0.7 <sup>b</sup> ±0.04	1.1 <sup>bc</sup> ±0.08
<b>LSD</b>	0.18	0.01	0.09	0.102	0.12

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

Data presented in table (3) illustrate the effect of galangal, phyllanthus and mix diets on total cholesterol and triglycerides on hepatointoxicated rats injected with CCl<sub>4</sub>. It could be observed that the mean value of total cholesterol (TC) of control (+) group was higher than control (-) group, being 129±1.89 and 96±1.25 mg/dl respectively. The best serum (TC) level showed for groups 3 (rats fed on basal diet containing 5% galangal) when compared to control (+) group.

It could be noticed that the mean value of triglycerides TG of control (+) group was higher than control (-) group, being

131±1.74 and 112±1.09 mg/dl respectively. The best serum (TG) level was showed for group 3 (rats fed on basal diet + 5% galangal) when compared to control (+) group.

**Maruthappan and Shree (2010)** reported that the aqueous extract of *Phyllanthus reticulatus* (250 mg and 500 mg/kg) produced significant reduction ( $P < 0.05$ ) in triglycerides and total cholesterol in atherogenic diet-induced hypercholesterolemic rats.

**Kaushik et al., (2013)** found that the alcoholic extract of the rhizomes of *Alpinia galangal* (100 and 200 mg kg<sup>-1</sup> respectively) reduced total cholesterol and triglycerides of diabetes induced nephropathy in rats.

**Table (3): Effect of galangal, phyllanthus and mix diets on total cholesterol (TC) and triglycerides (TG) on hepatointoxicated rats injected with CCl<sub>4</sub>**

Parameters	TC (mg/dl) Mean ± SD	(TG mg/dl) Mean ± SD
<b>Groups</b>		
<b>G1:</b> Control -ve	96 <sup>d</sup> ±1.25	112 <sup>e</sup> ±1.09
<b>G2:</b> Control +ve	129 <sup>a</sup> ±1.89	131 <sup>a</sup> ±1.74
<b>G3:</b> Galangal (5%)	92 <sup>e</sup> ±1.68	116 <sup>d</sup> ±1.13
<b>G4:</b> Phyllanthus (5%)	109 <sup>b</sup> ±1.58	125 <sup>b</sup> ±1.45
<b>G5:</b> Mix diets (5%)	100 <sup>c</sup> ±1.31	120 <sup>c</sup> ±1.62
<b>LSD</b>	2.84	2.50

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

Data presented in table (4) show the effect of galangal, phyllanthus and mix diets on VLDL<sub>c</sub>, HDL<sub>c</sub>, LDL<sub>c</sub> & AI on hepatointoxicated rats injected with CCl<sub>4</sub>.

It could be observed that the mean value of (VLDL<sub>c</sub>) of control (+) group was higher than control (-) group, being 26.2±0.05 and 22.4±0.09 mg/dl respectively. The best serum

VLDLc was shown for group 3 (rats fed on basal diet + 5% galangal) when compared to control (+) group.

It could be showed that the mean value of (HDLc) of control (-) group was higher than control (+) group, being  $51\pm0.58$  and  $36\pm0.01$  mg/dl respectively. The best serum HDLc was shown for group 3 (rats fed on basal diet containing 5% galangal) when compared to control (+) group.

The same table indicated that the mean value of (LDLc) of control (-) group was lower than control (+) group, being  $22.6\pm1.29$  and  $66.8\pm1.17$  mg/dl respectively. The best serum LDLc was shown for group 3 (rats fed on basal diet +5% galangal) when compared to control (+) group.

Also, data of table (4) observed that the mean value of (AI) of control (+) group was higher than control (-) group, being  $2.58\pm0.008$  and  $0.88\pm0.001$  respectively. The best AI was shown for group 3 (rats fed on basal diet + 5% galangal) when compared to control (+) group.

**Maruthappan and Shree (2010)** reported that the aqueous extract of *phyllanthus reticulatus* (250 mg and 500 mg/kg) produced significant reduction ( $P < 0.05$ ) in VLDL-cholesterol, LDL-cholesterol while increased HDL-cholesterol in atherogenic diet-induced hypercholesterolemic rats.

**Kaushik et al., (2013)** found that the alcoholic extract of the rhizomes of *Alpinia galangal* (100 and 200 mg kg<sup>-1</sup> respectively) reduced low density lipoprotein and increased high density lipoprotein of diabetes induced nephropathy in rats

**Table (4): Effect of galangal, phyllanthus and mix diets on (VLDLc), (HDLc), (LDLc) (mg/dl) and Atherogenic index (AI) on hepatointoxicated rats injected with CCl<sub>4</sub>**

Parameters Groups	VLDL (mg/dl) Mean ± SD	HDL (mg/dl) Mean ± SD	LDL (mg/dl) Mean ± SD	AI Mean ± SD
<b>G1:</b> Control -ve	22.4 <sup>e</sup> ±0.09	51 <sup>a</sup> ±0.58	22.6 <sup>d</sup> ±1.29	0.88 <sup>e</sup> ±0.001
<b>G2:</b> Control +ve	26.2 <sup>a</sup> ±0.05	36 <sup>e</sup> ±0.01	66.8 <sup>a</sup> ±1.17	2.58 <sup>a</sup> ±0.008
<b>G3:</b> Galangal (5%)	23.2 <sup>d</sup> ±0.04	46 <sup>b</sup> ±0.08	22.8 <sup>d</sup> ±1.42	1.00 <sup>d</sup> ±0.005
<b>G4:</b> Phyllanthus (5%)	25 <sup>b</sup> ±0.06	41 <sup>d</sup> ±0.04	43 <sup>b</sup> ±1.76	1.66 <sup>b</sup> ±0.007
<b>G5:</b> Mix diets (5%)	24 <sup>c</sup> ±0.08	43 <sup>c</sup> ±0.05	33 <sup>c</sup> ±1.82	1.33 <sup>c</sup> ±0.002
<b>LSD</b>	0.12	0.64	2.75	0.009

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

Data of table (5) indicate the effect of galangal, phyllanthus and mix diets on serum levels of AST, ALT, ALP enzymes & (AST/ALT) ratio on hepatointoxicated rats injected with CCl<sub>4</sub>.

It could be observed that the mean value of AST enzyme of control (+) group was higher than control (-) group, being 98±1.4 and 55±1.2 (U/L) respectively. The best treatment was observed for group 3 (basal diet containing 5% galangal) when compared to control (+) group.

It could be noticed that the mean value of ALT enzyme of control (+) group was higher than control (-) group, being 37±0.8 and 23±0.1 (U/L) respectively. The best treatment was observed for group 3 (basal diet containing 5% galangal) when compared to control (+) group.

Data of the same table (5) show the mean value of ALP enzyme of control (+) group was higher than control (-) group, being 239±3.8 and 142±2.1 (U/L) respectively. Group 3 showed

the lowest mean value of ALP enzyme level as compared to control (+) group which and recorded the best result.

It could be noticed that the mean value of (AST/ALT) of control (+) group was higher than control (-) group, being  $2.65 \pm 0.009$  and  $2.39 \pm 0.006$  respectively. The best treatment was observed for group 3 when compared to control (+) group.

**Manjrekar et al., (2008)** found that activities of alanine transaminase, aspartate transaminase and alkaline phosphatase enzymes were significantly reduced in the curative group (*P. niruri* treatment after CCl<sub>4</sub> injection).

**Negm and Ragheb, (2019)** reported that *Alpinia officinarum* (5, 7.5&10%) significantly decreased ( $P < 0.05$ ) the mean activities of serum liver enzymes (AST, ALT & ALP) compared to the control positive group in rats.

**Table (5): Effect of galangal, phyllanthus and mix diets on GOT, GPT, GOT/GPT and ALP (U/L) on hepatointoxicated rats injected with CCl<sub>4</sub>**

Parameters Groups	AST (U/L) Mean $\pm$ SD	ALT (U/L) Mean $\pm$ SD	AST/ALT Mean $\pm$ SD	ALP (U/L) Mean $\pm$ SD
<b>G1:</b> Control -ve	55 <sup>c</sup> $\pm$ 1.2	23 <sup>d</sup> $\pm$ 0.1	2.39 <sup>b</sup> $\pm$ 0.006	142 <sup>c</sup> $\pm$ 2.1
<b>G2:</b> Control +ve	98 <sup>a</sup> $\pm$ 1.4	37 <sup>a</sup> $\pm$ 0.8	2.65 <sup>a</sup> $\pm$ 0.009	239 <sup>a</sup> $\pm$ 3.8
<b>G3:</b> Galangal (5%)	58 <sup>d</sup> $\pm$ 1.8	26 <sup>c</sup> $\pm$ 0.9	2.23 <sup>c</sup> $\pm$ 0.001	148 <sup>d</sup> $\pm$ 2.5
<b>G4:</b> Phyllanthus (5%)	69 <sup>c</sup> $\pm$ 1.3	30 <sup>b</sup> $\pm$ 0.2	2.30 <sup>d</sup> $\pm$ 0.005	190 <sup>b</sup> $\pm$ 2.4
<b>G5:</b> Mix diets (5%)	72 <sup>b</sup> $\pm$ 1.9	31 <sup>b</sup> $\pm$ 0.3	2.32 <sup>c</sup> $\pm$ 0.008	169 <sup>c</sup> $\pm$ 2.3
<b>LSD</b>	2.81	1.02	0.01	4.89

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

Data presented in table (6) show the effect of galangal, phyllanthus and mix diets on serum glucose on hepatointoxicated rats injected with CCl<sub>4</sub>. It could be noticed that the mean value of glucose of control (+) group was higher than control (-) group, being  $184 \pm 1.28$  and  $106 \pm 1.14$  (mg/dl) respectively. The best

serum glucose was observed for group 3 (basal diet containing 5% galangal) when compared to control (+) group.

**Okoli et al., (2010)** reported that the antidiabetic potentials of methanol extract (ME) of aerial parts of *Phyllanthus niruri L* (Euphorbiaceae) reduced blood glucose in diabetic rats.

**Ragab (2018)** indicated that diets containing 10% rhizome, 5% rhizome and 200ppm ethanol extract of galangal decreased the serum glucose level in rats.

**Table (6): Effect of galangal, phyllanthus and mix diets on serum glucose (mg/dl) on hepatointoxicated rats injected with CCl<sub>4</sub>**

Parameters Groups	Glucose (mg/dl) Mean ± SD
G1: Control -ve	106 <sup>e</sup> ±1.14
G2: Control +ve	184 <sup>a</sup> ±1.28
G3: Galangal (5%)	110 <sup>d</sup> ±1.34
G4: Phyllanthus (5%)	130 <sup>b</sup> ±1.49
G5: Mix diets (5%)	124 <sup>c</sup> ±1.94
LSD	2.46

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

Results of table (7) show the mean value of serum creatinine, urea and uric acid (mg/dl) on hepatointoxicated rats injected with CCl<sub>4</sub> fed on various diets.

It could be observed that the mean value of uric acid of control (+) group was higher than control (-) group, being 5.16±0.005 and 3.98±0.007 mg/dl respectively. Group 3 (basal diet containing 5% galangal) recorded the best result as compared to control (+) group.

The same table (7) results illustrate that mean value of creatinine of control (+) group was higher than control (-) group, being 1.22±0.008 and 0.93±0.002 mg/dl respectively. In concern to creatinine the best treatment was recorded for the group 3 (rats fed on basal diet +5% galangal) when compared to control (+) group.



It could be noticed that the mean value of urea of control (+) group was higher than control (-) group, being  $59 \pm 1.12$  and  $33 \pm 0.75$  mg/dl respectively. Group 3 (rats fed on basal diet +5% galangal) recorded the best result as compared to control (+) group.

**Kaushik et al., (2013)** suggested that orally administered alcoholic extract of *Alpinia galanga* (50, 100 and 200 mg kg<sup>-1</sup>), once daily for 40 days reduced creatinine of diabetes-induced nephropathy in rats.

**Giribabu et al., (2017)** found that *P. niruri* leaves aqueous extract (PN) reduced serum urea and uric acid of diabetic rats.

**Table (7): Effect of galangal, phyllanthus and mix diets on uric acid (U.A), creatinine and urea (mg/dl) on hepatointoxicated rats injected with CCl<sub>4</sub>**

Parameters Groups	U.A (mg/dl) Mean $\pm$ SD	Creatinine (mg/dl) Mean $\pm$ SD	Urea (mg/dl) Mean $\pm$ SD
G1: Control -ve	$3.98^c \pm 0.007$	$0.93^c \pm 0.002$	$33^c \pm 0.75$
G2: Control +ve	$5.16^a \pm 0.005$	$1.22^a \pm 0.008$	$59^a \pm 1.12$
G3: Galangal (5%)	$4.11^d \pm 0.008$	$0.95^d \pm 0.004$	$36^d \pm 0.84$
G4: Phyllanthus (5%)	$4.52^b \pm 0.009$	$1.10^b \pm 0.006$	$45^b \pm 0.32$
G5: Mix diets (5%)	$4.30^c \pm 0.001$	$1.05^c \pm 0.007$	$41^c \pm 0.48$
LSD	0.01	0.01	1.37

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

Data presented in table (8) indicate the effect of galangal and phyllanthus on total protein (TP), albumin (Alb.), Globulin (Glob.) and albumin/globulin (A/G) on hepatointoxicated rats injected with CCl<sub>4</sub>.

It could be observed that the mean value of (TP) of control (+) group was lower than control (-) group, being  $5.1 \pm 0.08$  and  $7.2 \pm 0.02$  g/dl respectively. The best serum TP showed for group 3

(rats fed on basal diet +5% galangal) when compared to control (+) group.

It could be showed that the mean value of (Alb.) of control (+) group was lower than control (-) group, being  $1.6\pm 0.07$  and  $3.0\pm 0.01$  g/dl respectively. The best serum Alb was showed for group 3 (rats fed on basal diet + 5% galangal) when compared to control (+) group.

The same table indicated that the mean value of (Glob.) of control (+) group was lower than control (-) group, being  $3.9\pm 0.08$  and  $4.2\pm 0.04$  g/dl respectively. The best serum Glb Showed for group 3 (rats fed on basal diet containing 5% galangal) when compared to control (+) group.

Also, data of table (8) noticed that the mean value of (A/G) of control (-) group was higher than control (+) group, being  $0.71\pm 0.001$  and  $0.31\pm 0.005$  respectively. The best A/G showed for group 3 (rats fed on basal diet containing 5% galangal) when compared to control (+) group.

**Srividya et al., (2010)** found that antioxidant and anti-diabetic activity of *A. galangal* showed that this extract could increase total protein and albumin level in treated group when compared to diabetic control in rats.

**Peters and Omeodu (2015)** reported that ethanolic leaves extract of *Phyllanthus amarus* on carbon tetrachloride (CCl<sub>4</sub>) induced hepatotoxicity in albino rats increased total protein and albumin.

**Table (8): Effect of galangal, phyllanthus and mix diets on total protein (T.P), albumin (Alb.), globulin (Glob.) and albumin/globulin (A/G) on hepatointoxicated rats injected with CCl<sub>4</sub>**

Parameters Groups	T.P (g/dl) Mean ± SD	Alb. (g/dl) Mean ± SD	Glob. (g/dl) Mean ± SD	A/G Mean ± SD
Control -ve	7.2 <sup>a</sup> ±0.02	3.0 <sup>a</sup> ±0.01	4.2 <sup>a</sup> ±0.04	0.71 <sup>a</sup> ±0.001
Control +ve	5.1 <sup>c</sup> ±0.08	1.6 <sup>d</sup> ±0.07	3.9 <sup>b</sup> ±0.08	0.31 <sup>c</sup> ±0.005
Galangal 5%	6.3 <sup>b</sup> ±0.05	2.4 <sup>b</sup> ±0.09	3.9 <sup>b</sup> ±0.01	0.29 <sup>c</sup> ±0.006
Phyllataus 5%	5.9 <sup>d</sup> ±0.03	2.2 <sup>c</sup> ±0.04	3.7 <sup>c</sup> ±0.05	0.60 <sup>bc</sup> ±0.008
Mix diets 5%	6.1 <sup>c</sup> ±0.09	2.3 <sup>bc</sup> ±0.03	3.8 <sup>bc</sup> ±0.07	0.61 <sup>b</sup> ±0.004
LSD	0.11	0.11	0.11	0.009

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

Data presented in table (9) show the mean value of total bilirubin (TB), direct bilirubin (DB) & indirect bilirubin (IB) on hepatointoxicated rats injected with CCl<sub>4</sub> fed on galangal and phyllataus.

It could be noticed that the mean value of TB of control (-) group was lower than control (+) group, being 0.32±0.001 and 1.26±0.009 mg/dl respectively. Injected rats with CCl<sub>4</sub> fed on basal diet containing 5% galangal (group 3) showed the best treatment of TB when compared to control (+) group.

According data presented in the same table (9) it could be revealed that the mean value of DB of control (-) group was lower than control (+) group, being 0.025±0.0009 and 0.096±0.0002 mg/dl respectively. The best DB recorded for group 3 (basal diet containing 5% galangal) when compared to control (+) group.

The same table observed that the (IB) of control (-) group was lower than control (+) group, being 0.295±0.0002 and 1.164±0.0006 mg/dl respectively. Injected rats with CCl<sub>4</sub> fed on

basal diet containing 5% galangal (group 3) showed the best IB as compared to control (+) group.

**Manjrekar et al., (2008)** found that aqueous extract of *P. niruri* reduced total bilirubin of CCl<sub>4</sub> induced hepatotoxic rats.

**Srividya et al., (2010)** studied the antioxidant and anti-diabetic activity of *A. galangal* extraction showed that this extract could decrease total bilirubin and direct bilirubin level in treated group when compared to diabetic control in rats.

**Table (9): Effect of galangal, phyllanthus and mix diets on total bilirubin (T.B), direct (D.B) & indirect (I.B) bilirubin on hepatointoxicated rats injected with CCl<sub>4</sub>**

Parameters Groups	T.B (mg/dl) Mean ± SD	D.B (mg/dl) Mean ± SD	I.B (mg/dl) Mean ± SD
Control -ve	0.32 <sup>d</sup> ± 0.001	0.025 <sup>e</sup> ± 0.0009	0.295 <sup>d</sup> ± 0.0002
Control +ve	1.26 <sup>a</sup> ± 0.009	0.096 <sup>a</sup> ± 0.0002	1.164 <sup>a</sup> ± 0.0006
Galangal 5%	0.30 <sup>e</sup> ± 0.004	0.041 <sup>d</sup> ± 0.0005	0.259 <sup>e</sup> ± 0.0009
Phyllanthus 5%	0.46 <sup>b</sup> ± 0.006	0.052 <sup>b</sup> ± 0.0008	0.408 <sup>b</sup> ± 0.0004
Mix diets 5%	0.38 <sup>c</sup> ± 0.002	0.047 <sup>c</sup> ± 0.0003	0.333 <sup>c</sup> ± 0.0003
LSD	0.009	0.001	0.0009

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

Data of table (10) indicate the effect of galangal, phyllanthus and mix diets on serum levels of antioxidants enzymes (SOD (nmol/min/mg), GPX (nmol/min/mg) and CAT (U/mg)) on hepatointoxicated rats injected with CCl<sub>4</sub>.

It could be observed that the mean value of SOD enzyme of control (-) group was higher than control (+) group, being 64.15±1.15 and 33.40±0.15 (nmol/min/mg) respectively. The best treatment was observed for group 3 (basal diet containing 5% galangal) 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

0.82±0.008 and 0.36±0.006 (nmol/min/mg) respectively. The best treatment was observed for group 3 (basal diet containing 5% galangal) when compared to control (+) group.

Data of the same table (10) show the mean value of CAT enzyme of control (-) group was higher than control (+) group, being 0.213±0.0001 and 0.101±0.0009 (U/mg) respectively. Group 3 showed the highest mean value of CAT enzyme level as compared to control (+) group which and recorded the best result.

**Kaushik et al., (2013)** suggested that orally administered alcoholic extract of *Alpinia galanga* (50, 100 and 200 mg kg<sup>-1</sup>), once daily for 40 days increased superoxide dismutase (SOD) and catalase (CAT) of diabetes-induced nephropathy in rats.

**Rekha et al., (2016)** found that aqueous extract of *Phyllanthus niruri* increased superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in rats.

**Table (10): Effect of galangal, phyllanthus and mix diets on antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) on hepatointoxicated rats injected with CCl<sub>4</sub>**

Parameters Groups	SOD (nmol/min/mg protein) Mean ±SD	GPx (nmol/min/mg protein) Mean ±SD	CAT (U/mg) Mean ±SD
G1: Control -ve	64.13 <sup>a</sup> ±1.15	0.82 <sup>a</sup> ±0.008	0.213 <sup>a</sup> ±0.0001
G2: Control +ve	33.40 <sup>c</sup> ±0.15	0.36 <sup>c</sup> ±0.006	0.101 <sup>c</sup> ±0.0009
G3: Galangal (5%)	62.08 <sup>b</sup> ±1.26	0.79 <sup>b</sup> ±0.007	0.210 <sup>b</sup> ±0.0002
G4: Phyllanthus (5%)	51.64 <sup>d</sup> ±0.54	0.63 <sup>d</sup> ±0.003	0.182±0.0008
G5: Mix diets (5%)	59.80 <sup>c</sup> ±0.42	0.74 <sup>c</sup> ±0.002	0.190 <sup>c</sup> ±0.0005
LSD	1.65	0.01	0.001

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

### **Histopathological examination of liver:**

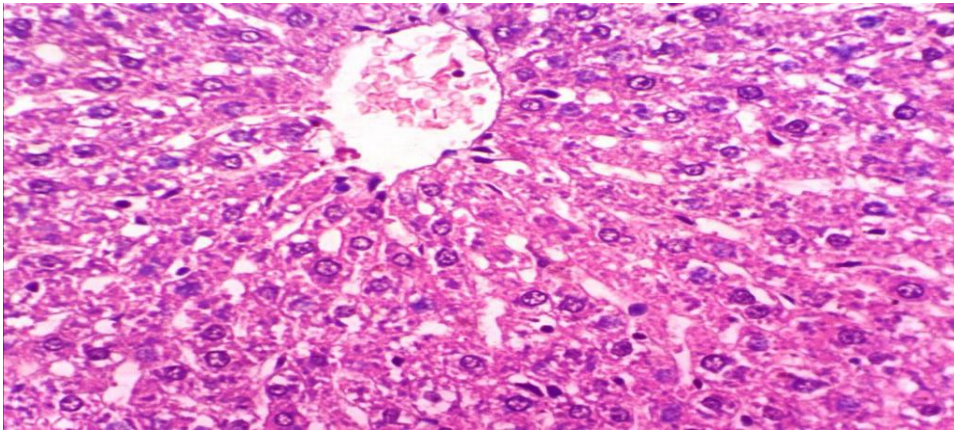
Microscopic section of liver from healthy (control -) group 1 revealed the normal structure of hepatic lobule (Photo 1). Liver section of control (+) group (heparointoxicated rats) revealed that portal areas showed severe edema, fibrous tissue proliferation and marked proliferation of the bile duct epithelium with multiple newly formed bile ducteols as well as periductal fibrosis and mild inflammatory cells infiltration (Photo 2), and severe diffuse degeneration and necrosis of the hepatic cells (Photo 3). Also, control (+) group liver revealed marked hepatocellular vacuolation with an obvious cell lysis, necrosis and nuclear pyknosis (Photo 4). Liver section of (galangal diet), showed good restoration of the hepatic parenchyma with scattered necrotic ones and few vacuolated hepatocytes (Photo 5), in other areas mild degenerated and few scattered necrotic hepatocytes were observed with mild activated Kupffer cells and very few apoptotic bodies (Photo 6), but restoration of the original structure was in general good. Liver section of rats treated with (mix diets) (Photo 7) revealed mild vacuolar degeneration and necrosis of the hepatic cells. Also, liver sections of (mix diets) showed mild hepatocellular necrobiotic changes (and slight activation of Kupffer cells (Photo 8). Liver sections of rats fed on (phyllanthus diets) showed showing good restoration of the hepatic parenchyma with scattered necrotic cells (Photo 9). Other areas showed vacuolation of scattered hepatic cells and some necrotic ones (Photo 10).

### **Histopathological examination of kidney:**

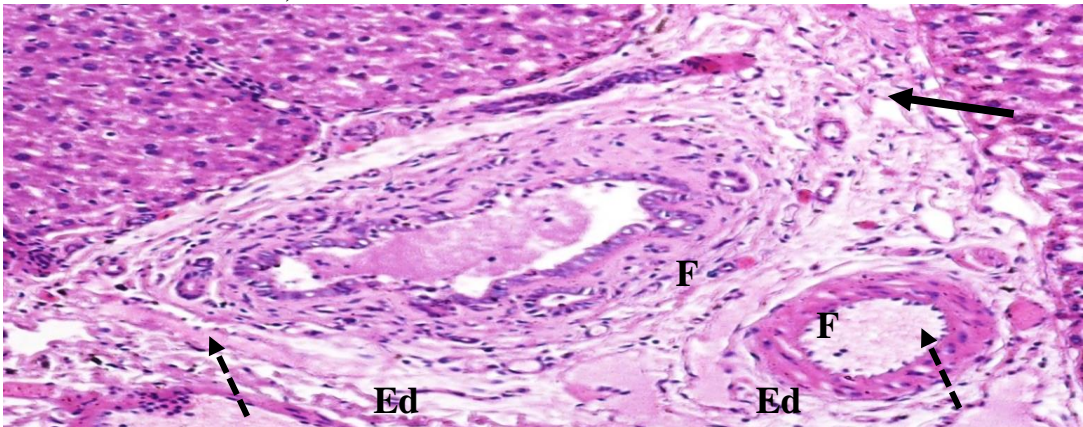
Kidneys of control (-) group revealed the normal structure of renal parenchyma (Photo 11). Kidneys tissue of control (+) rats showed severe diffuse degeneration and necrosis of the tubular epithelial linings with marked granular to homogenous eosinophilic cast formation (Ct) in the lumen of most of the renal tubules (Photo 12). Kidneys of control (+) group showed diffuse granular and vacuolar degeneration, necrosis, nuclear pyknosis and desquamation with granular cast formation (Photo 13). Also,

sections of control (+) group revealed granular cast (Gc) in the Bowmans' space with congested glomerular capillaries and mesangial hyalinization (Photo 13). Control (+) rats showed kidneys sections with esinophilic granular cast (Gc) in the Bowmans' space with focal hyalinization of the glomerular tuft, tubular epithelial vacuolar degeneration, necrosis, widespread nuclear pyknosis and luminal cast formation (Photo 14). Kidneys of rats treated with (galangal diet) revealed showing good protection of the renal tissue, with some congested interstitial vessels, mild degenerative and necrotic changes of the tubular epithelium and some regenerated tubules (Photo 15). But mostly kidneys rats of (galangal diet) showed near to normal appearance of the renal glomeruli with mild endothelial vacuolation and mild to moderate degree of degeneration and necrosis of the tubular epithelium with some cast formation (Photo 16). Kidneys rats of mix diets showed near to normal appearance of the renal parenchyma with very mild necrobiotic changes of the tubular epithelial linings and scattered congested inter-tubular vessels (Photo 17). Moreover kidneys of rats treated with mix diets showed mild granular degeneration and scattered necrosis of the renal tubular epithelial linings (Photo 18). Kidneys of rats treated with (phyllanthus diets) showed moderate tubular epithelial swelling, degeneration, necrosis and some desquamation, notice the congested interstitial vessels (Photo 19). This diet, however, revealed more or less restoration of the renal tissue with mild necrobiotic changes of the renal tubular epithelial linings and few cast formation (Photo 20).

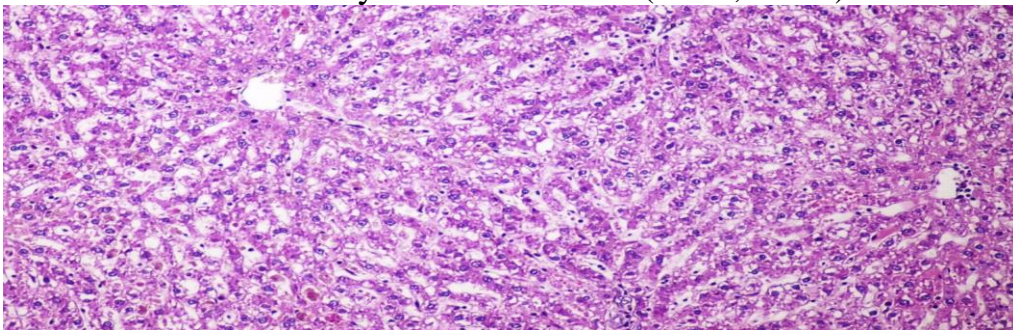
Finally histopathological results of hepatic liver and kidneys, revealed that galangal, mix diets and phyllanthus diets connected the damage of these organs specially the galangal diets, which was in line with biological and biochemical parameters changes.



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

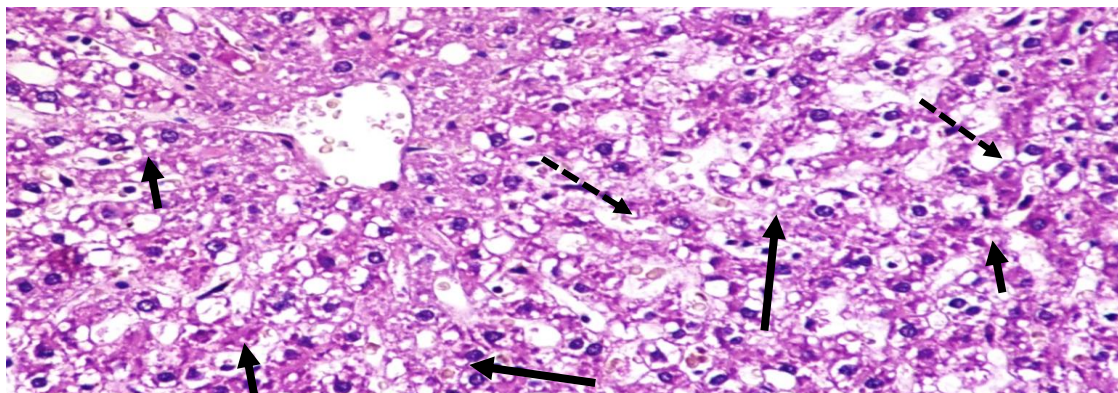


**Photo (2):** Portal area in liver of control positive rat showing severe edema (Ed), fibrous tissue proliferation (arrow) and marked proliferation of the bile duct epithelium with multiple newly formed bile ductules (dashed arrow) as well as periductal fibrosis (F) and mild inflammatory cells infiltration. (H&E, X200).

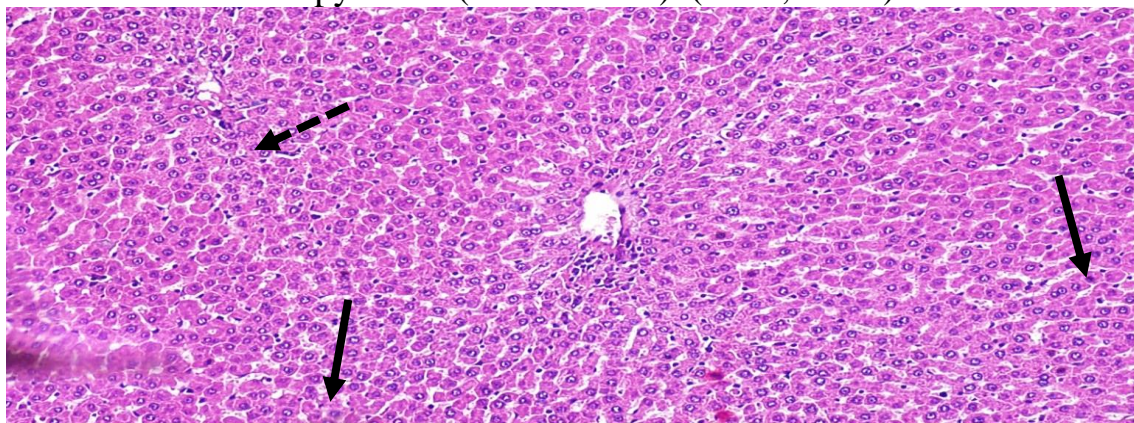




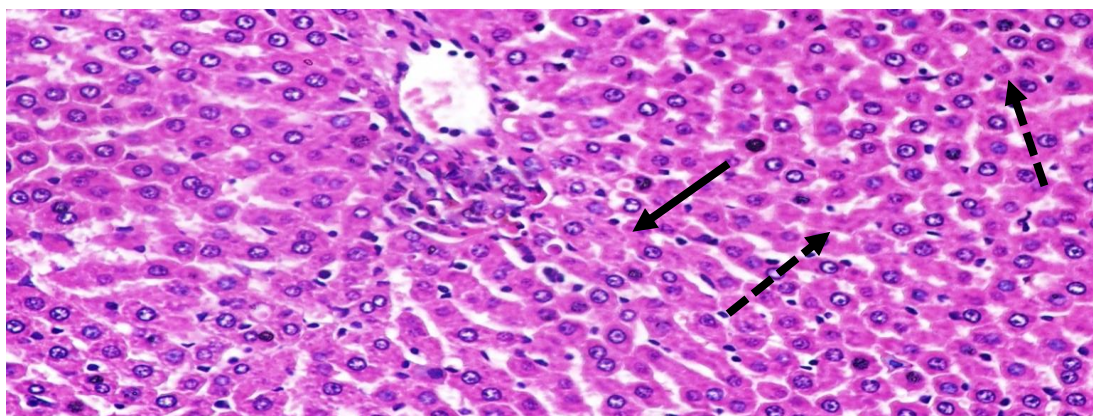
**Photo (3):** Liver of control positive rat showing severe diffuse vacuolar degeneration (arrow) and necrosis (dashed arrow) of the hepatic cells. (H&E, X200).



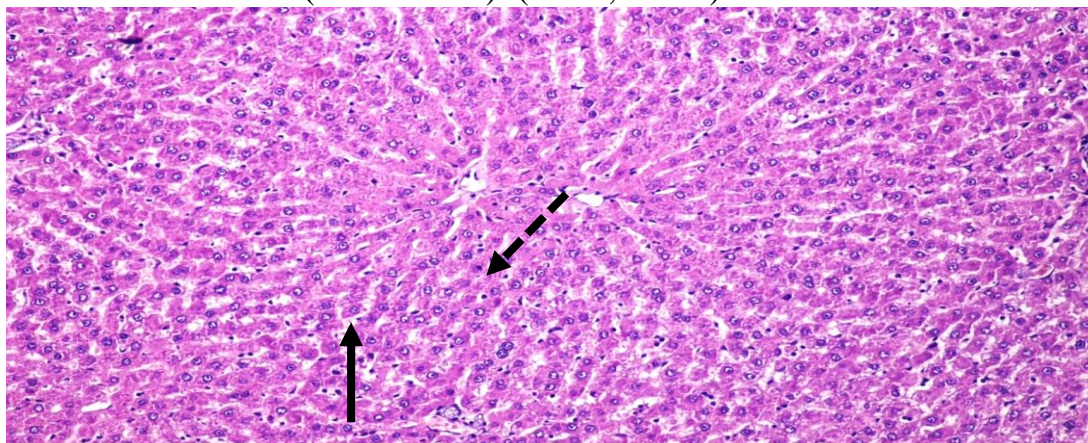
**Photo (4):** Liver of control positive rat showing marked hepatocellular vacuolation (short arrow) with an obvious cell lysis (arrow), necrosis (dashed arrow), nuclear pyknosis (dotted arrow). (H&E, X400).



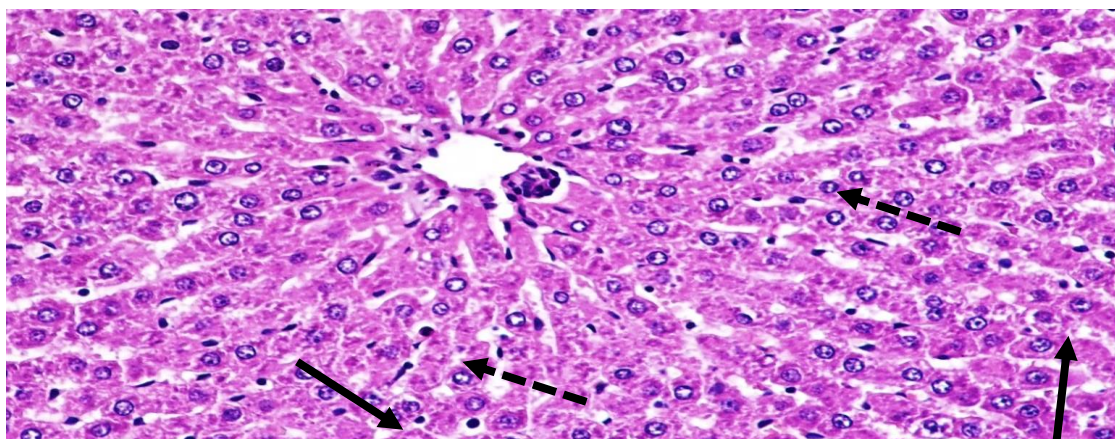
**Photo (5):** Liver of control positive rat which treated with galangal diet showing good restoration of the hepatic parenchymal cells with scattered necrotic ones (arrow) and few vacuolated hepatocytes (dashed arrow). (H&E, X200).



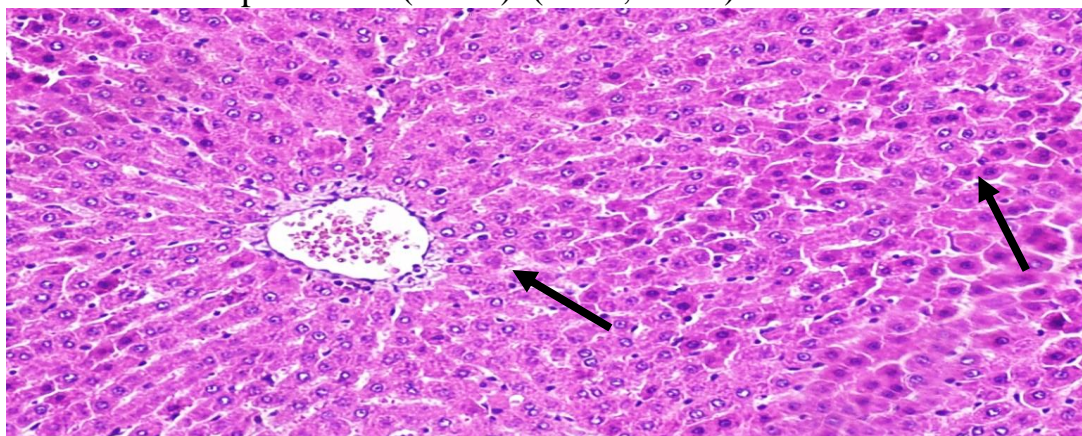
**Photo (6):** Liver of control positive rat which treated with galangal diet showing mild degenerated and few scattered necrotic hepatocytes, with mild activated Kupffer cells (dashed arrow) and very few apoptotic bodies (short arrow). (H&E, X400).



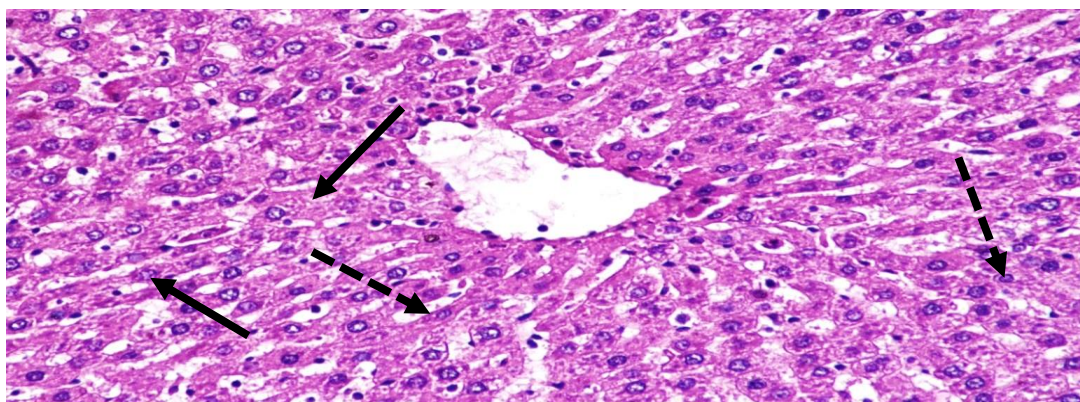
**Photo (7):** Liver of control positive rat which treated with mix diets showing mild vacuolar degeneration (arrow) and necrosis (dashed arrow) of the hepatic cells. (H&E, X200).



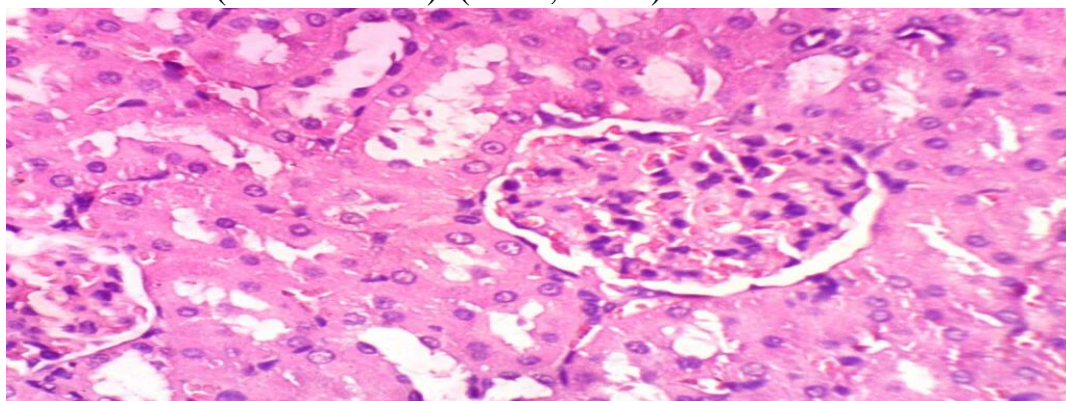
**Photo (8):** Liver of control positive rat which treated with mix diets showing mild hepatocellular necrobiotic changes (dashed arrow) and slight activation of Kupffer cells (arrow). (H&E, X400).



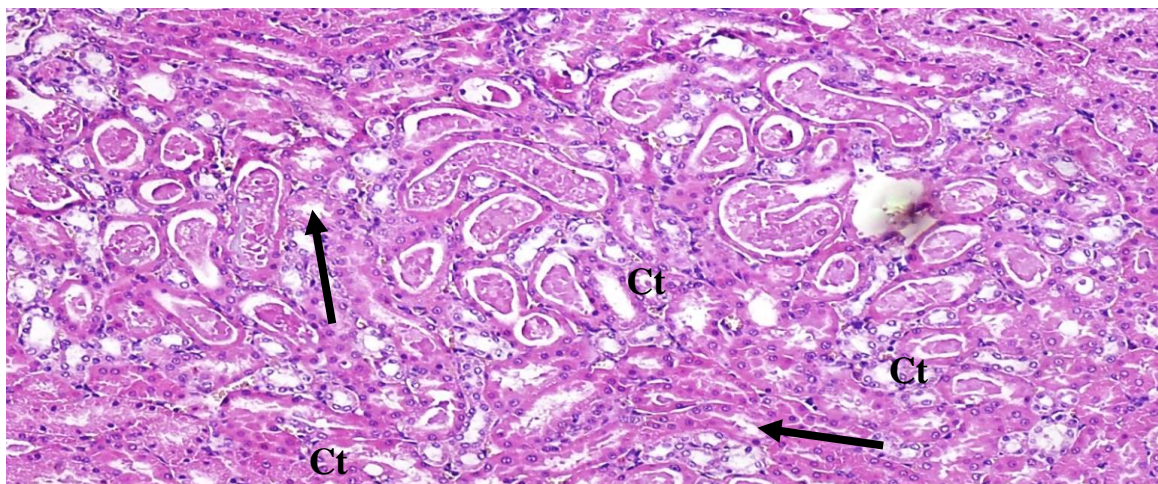
**Photo (9):** Liver of control positive rat which treated with phyllanthus diets showing good restoration of the hepatic parenchyma with scattered necrotic cells (arrow). (H&E, X200).



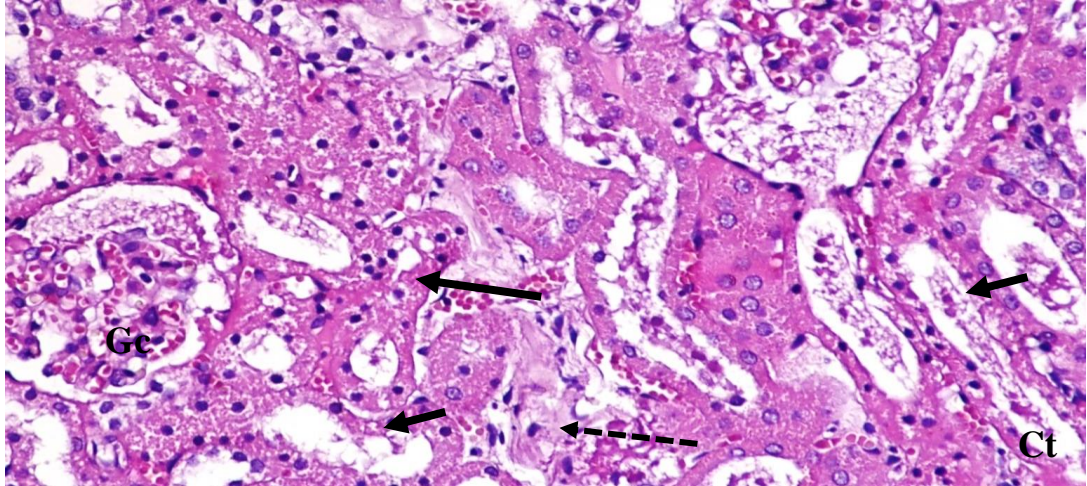
**Photo (10):** Liver of control positive rat which treated with phyllanthus showing vacuolation (arrow) of scattered hepatic cells and some necrotic ones (dashed arrow). (H&E, X400).



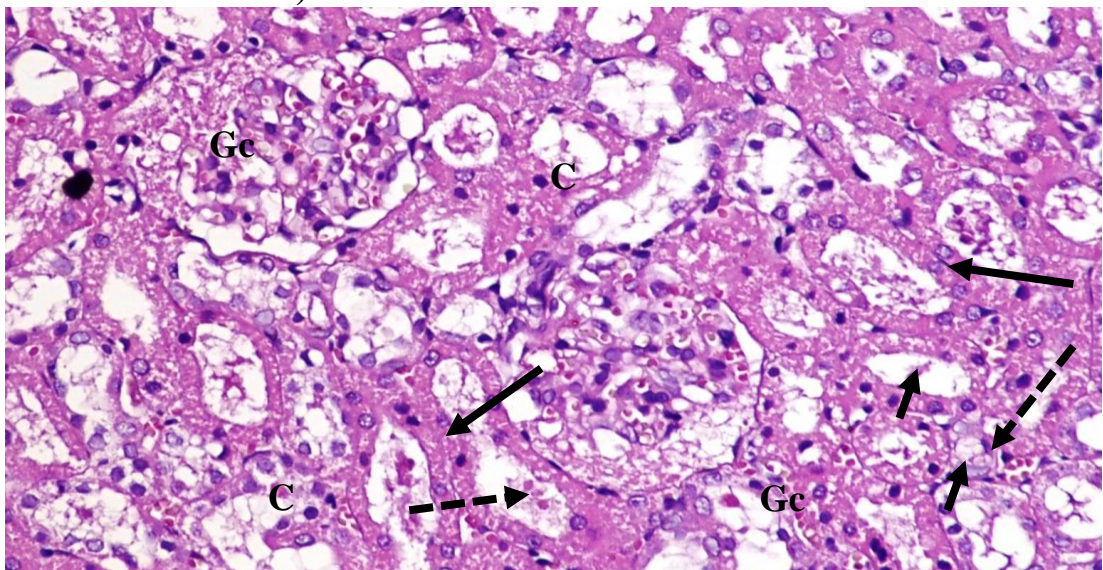
**Photo (11):** Photomicrograph of kidney of rat from group 1 healthy rats showing the normal histological structure of renal parenchyma (H & E X 400).



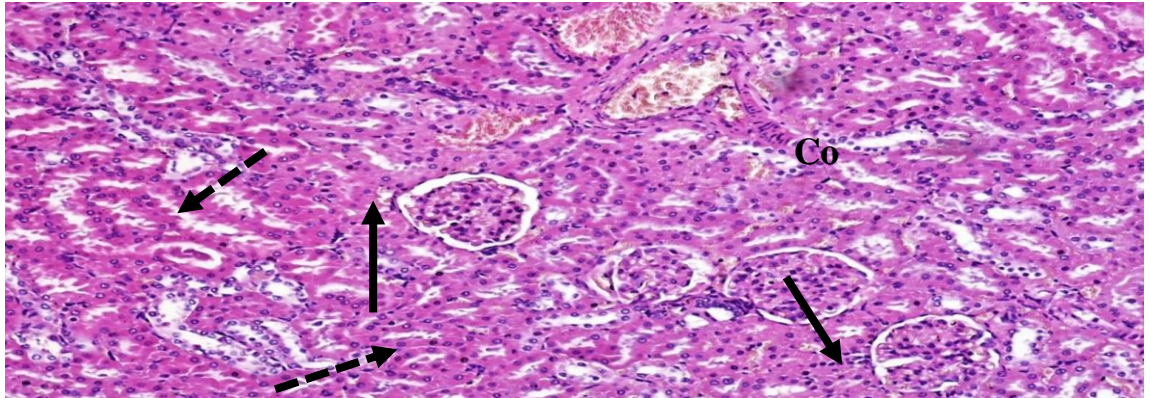
**Photo (12):** Kidney of control positive rat showing severe diffuse degeneration and necrosis (arrow) of the tubular epithelial linings with marked granular to homogenous esinophilic cast formation (Ct) in the lumen of most of the renal tubules. (H&E, X200).



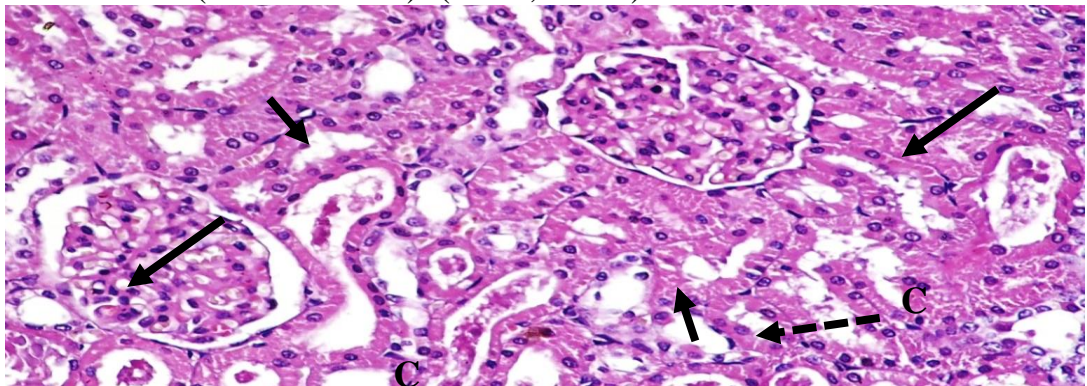
**Photo (13):** Kidney of control positive rat showing diffuse granular and vacuolar (arrow) degeneration, necrosis (dashed arrow), nuclear pyknosis (short arrow) and desquamation with granular cast formation (Ct). Notice the granular cast (Gc) in the Bowmans' space with congested glomerular capillaries and mesangial hyalinization. (H&E, X400).



**Photo (14):** Kidney of control positive rat showing eosinophilic granular cast (Gc) in the Bowmans' space with focal hyalinization of the glomerular tuft, tubular epithelial vacuolar degeneration (arrow), necrosis (dashed arrow), widespread nuclear pyknosis (short arrow) and luminal cast formation (C). (H&E, X400).

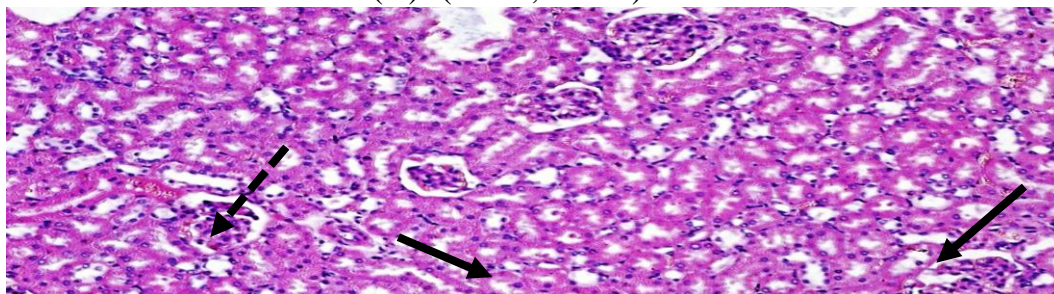


**Photo (15):** Kidney of control positive rat which treated with galangal diet showing good protection of the renal tissue, with some congested interstitial vessels (Co), mild degenerative and necrotic (arrow) changes of the tubular epithelium and some regenerated tubules (dashed arrow). (H&E, X200).

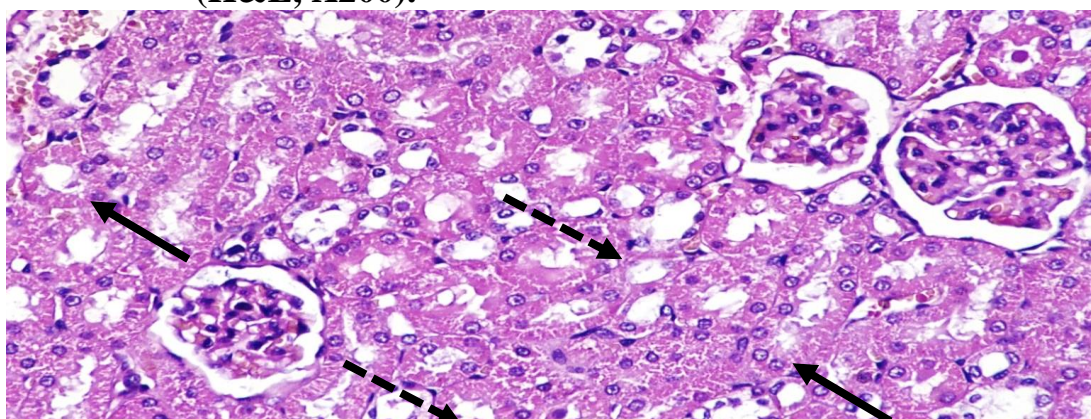


**Photo (16):** Kidney of control positive rat which treated with galangal diet showing near to normal appearance of the renal glomeruli with mild endothelial vacuolation (arrow) and mild to moderate degree of degeneration (dashed arrow) and necrosis (short

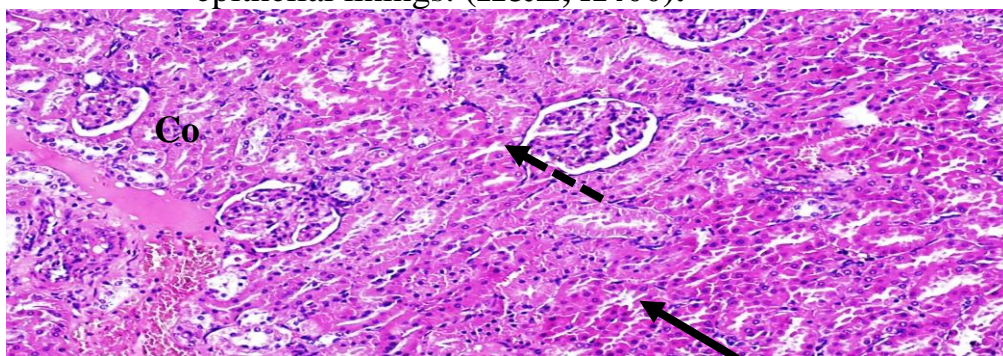
arrow) of the tubular epithelium with some cast formation (C). (H&E, X400).



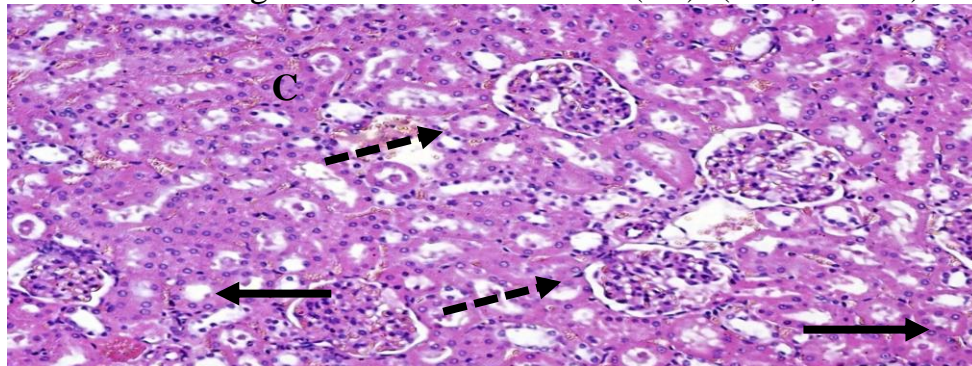
**Photo (17):** Kidney of control positive rat which treated with mix diets showing near to normal appearance of the renal parenchyma with very mild necrobiotic changes (arrow) of the tubular epithelial linings and scattered congested inter-tubular vessels (dashed arrow). (H&E, X200).



**Photo (18):** Kidney of control positive rat which treated with mix diets showing mild granular degeneration (arrow) and scattered necrosis (dashed arrow) of the renal tubular epithelial linings. (H&E, X400).



**Photo (19):** Kidney of control positive rat which treated with phyllanthus diets showing moderate tubular epithelial swelling, degeneration, necrosis (arrow) and some desquamation (dashed arrow), notice the congested interstitial vessels (Co). (H&E, X200).



**Photo (20):** Kidney of control positive rat which treated with phyllanthus diets showing good restoration of the renal tissue with mild necrobiotic changes (arrow) of the renal tubular epithelial linings and few cast formation (dashed arrow) in some tubular lumen. (H&E, X200).

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