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EFFECT OF TIGER NUT TUBERS (*CYPERUS ESCULENTUS*) SUPPLEMENTATIONS ON BLOOD PARAMETERS OF ALBINO RATS

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ABSTRACT

Previous studies showed that tiger nut tubers (*Cyperus esculentus* L.) have been implicated for several therapeutic potentials. In this study, we investigated which concentration of tiger nut tubers added to diet has safe effect in rats. Phytochemical composition of the tiger nut tuber has been screened. The effect of different doses of tiger nut tubers Supplementations on body weight and internal organs of rats was evaluated. Hematological, biochemical and histo-pathological studies were examined. Twenty four female rats were randomly divided into four groups (6 each). Group1 (Control) was fed on standard laboratory rat diet while Groups 2, 3 and 4 were fed on three doses of tiger nut tubers Supplementations (5% , 10% and 20% respectively) for six weeks. Phytochemical screening of the tubers revealed the presence of steroids, carbohydrates, fatty acids, tannins, cumarins and alkaloids. No significant differences were observed in all hematological and biochemical parameters studied for all treated groups when compared to those of the control group ($P < 0.05$). However a significant increase in urea level was noticed in rats treated with 20 % of tiger nut tubers and histopathological examination confirmed this effect. The results in this study suggested that the addition of tiger nut tubers at concentration dose of 5% or 10% to rat diet was safe and edible.

Keywords: Experimental rats; Tiger nut (*Cyperus esculentus*); Hematological; Biochemical parameters.

INTRODUCTION

Cyperus esculentus L. (Tiger nut), a member of the family of Cyperaceae has been considered a foodstuff since ancient times (Pascual *et. al.*, 2000). In Egypt, it was used as an important source of food (Negbi, 1992), medicine and perfumes (De-Vries, 1991). Tiger nut is a crop of early domestication and was added to other crops of the Nile Valley; its dry tubers have been found in tombs from predynastic times about 6000 years ago (Zohary, 1986). Tiger nut has many useful benefits. It can be eaten raw, roasted, grated ،baked or used for ice cream and beverage (Rita, 2009 and Belewu and Abodunrin, 2006). Also, used as sweet meal. Tiger nut flour has been demonstrated to be a rich source of quality oil contains moderate amount of protein. It is also an excellent source of vitamin B1 (David, 2005 and Tiger nut Traders, 2009) and some useful minerals such as iron and calcium which are essential for body growth and development (Oladele and Aina, 2007). Tiger nut was found to be ideal for children, elder people and sportsmen (Martinez, 2003). Various reports had demonstrated that Tiger nut has hypoglycaemic and hypolipidmic properties in humans and experimental animals ((Raut and Gaikwad, 2006; Hassan, 2007; Mahmoud and Aly, 2013). Also, they found that its tubers have aphrodisiac, carminative, diuretic, emmanogogue, stimulant and tonic effect. Furthermore, it had been reported that tiger nut can be used in the treatment of flatulence, indigestion, diarrhea, dysentery and excessive thirst . In addition, tiger nut has been demonstrated to contain more essential amino acids than those proposed in the protein standard for satisfying adult needs (Adejuyitan, 2011). The chemical constituents of *C. esculentus* are flavonoids sterol, resins and alkaloids (Ekeanyanwu *et al.*, 2010). Alkaloids in plants have been reported to cause an increase in the serum level of follicle stimulating hormone in male rats (Udoh *et al.*, 2009). Methanolic extract of *C. esculentus* improved reproductive functions in adult male albino rats by altering the plasma levels of gonadotropins, testosterone and sperm functions in a dose-dependent manner (Agbai and Nwanegwo, 2013). Considering the nutritive and health benefits of the underutilized tiger nuts, there is a need for more utilization and awareness of its health benefits. (Imam, et al . 2013).

The aim of this research is to study the effect of tiger nut tubers supplementation on some blood parameters of albino rats.

MATERIALS AND METHODS

1. Samples

Tiger nut tubers were obtained from the local market at Cairo. Tubers were thoroughly screened to remove the bad ones and stones, cleaned, washed and dried in shade at room temperature for 3 h. The dried tubers were grounded into fine powder and it was kept in an airtight container at 4^{o}C.

2. Chemicals:

Kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Total protein, albumin, blood urea nitrogen (BUN) and creatinine were purchased from Biodiagnostic Company Cairo (Egypt). All other chemicals were considered among the best available commercial grades.

3. Phytochemical screening:

Tiger nut tubers were subjected to preliminary phytochemical screening and the following tests were performed to check the presence of the following phytoconstituents: alkaloids, phenols, steroids (Gibbs, 1974), terpenoids (Ayoola *et al.*, 2008), tannins (Treare and Evans, 1985), saponins (Kumar *et al.*, 2009), Anthocyanins (Farnsworth, 1966)), emodins (Rizk, 1982), glycosides(Khandewal, 2008) and flavonoids (Alupuli *et al.*, 2009).

4. Animals and experimental design:

Twenty-four female Sprague-Dawley rats (95-115 gm) were housed in the biological laboratory of Chemistry Department, Faculty of Agriculture, Minia University. Rats were acclimatized for at least 1 week prior to any experiment. They were housed in special healthy plastic cages and maintained on *ad libitum* for water and standard diet. The experiments were conducted according to the Institutional Animal Ethics Committee guidelines for animal care and use, Minia University, Egypt.

Rats were randomly assigned into four experimental groups of six animals each. First group was fed on standard chow diet and served as control group. Animals in the second, third and fourth groups were fed on the same diet supplemented with 5% (T1), 10% (T2) and 20% (T3) Tiger nut tubers, respectively.

Body weight was recorded at first and throughout the experimental period. At the end of the experiment (6 weeks). Blood samples were taken from the retro-orbital plexus of all rats of each group after being anesthetized. All blood samples were centrifuged at 3000 rpm for 15 min. The obtained serum samples were kept at -20°C until used for biochemical analysis. Animals were dissected as quickly as possible and liver, kidney, spleen, heart and brain were removed, washed in ice-cold saline, wiped with filter paper and weighted.

5.Hematological and Immunological parameters:

The blood was collected in tubes containing 10% EDTA and then appropriately labeled to reflect the treatment groups as well as the intervals of the study. Red blood cells (RBCs) and white blood cells (WBCs) were counted as described by Dacie and Lewis (1984), while hemoglobin concentration (Hb) was assessed according to VanKampen and Zijlstra (1961). Hematocrit value (HCT %) or packed cell volume (PCV) was determined by centrifuging blood in heparinized microhematocrit tube (capillary tubes of 1mm internal

diameter and 7.5 cm length) for 5 minutes at 15,000 r.p.m (Dacie and Lewis, 1991). The percentage of each type of the total leucocyte population in relation to the total count of WBCs was determined according to Schalm et al. (1975).

6. Biochemical analysis:

Liver functions as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with colorimetric method (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) was measured colorimetrically (Belfield and Golberg, 1971). Total protein, albumin, blood urea nitrogen (BUN) and creatinine as renal functions were determined according to the methods of Gornall *et al.,* (1949), Doumas *et al.,* (1971), Fawectt& Soctt, (1960), and Larsen (1972), respectively. Serum globulin was calculated by the difference between total serum proteins and serum albumin.

7. Histopathological studies

Small pieces of kidney, liver, spleen, heart and brain of each animal of control and treated groups were fixed in 10% formal saline solution for twenty four hours. Then, washed with water and serial dilutions of absolute ethyl alcohol (for dehydration). After routine processing, paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains for histopathological examination through the light microscope. (Banchroft *et al.,* 1996).

8. Statistical analysis:

Results obtained were evaluated by One Way ANOVA (analysis of variance) test. And expressed as mean ± standard deviation (SD) and values of $P< 0.05$ were considered statistically significant (SAS, 1996)

RESULTS AND DISCUSSION

1. Preliminary Qualitative Phytochemical Screening:

The medicinal values of any plant are affected by bioactive phytochemical constituents that cause definite physiological actions on the human body (Krishnaiah *et al.,* 2009). From the phytochemical screening (Table1), the obtained results showed the presence of steroids, carbohydrates, fatty acids tannins, cumarins and alkaloids. Also, it showed the absence of terpenoids, saponins, anthocyanins and emodins.

The absence of saponin and anthocyanins in tiger nut in our study is in contrast with the results of Chukwuma *et al.,* (2010). They reported that saponin and anthocyanins are one of the active constituent. Meanwhile, the same results were obtained regarding to steroid, tannins and alkaloids. The absence may be due to the differences in soil composition or amount of rainfall, leaching effect , climate and type of manure used (Imam, *et al.*, 2013) .

Table(1):Qualitative analysis of the Phytochemical of tiger nut tubers

Constituents	Bioassay
Steroid	(+)
Carbohydrates	(+)
Terpenoids	(-)
Fatty acid	(+)
Tannins	(+)
Saponins	(-)
Anthocyanins	(-)
Coumarins	(+)
Emodins	(-)
Alkaloids	(+)

(+) presence of constituent (-) absence of constituent

2. Changes in body weight and food consumption:

All animals did not show abnormal physical, behavioral changes or unexpected deaths throughout the experiment. Data in Table (2) showed a significant reduction in body weight gain about 12% especially in rats that supplemented with 20 % of tiger nut tubers. Although there was insignificant decrease in body weight gain of rats supplemented with 5% and 10 % tiger nut. On other hand the daily feed intake significantly decreased in all groups when compared with the control group.

Table (2): Effect of 5, 10 and 20 % tiger nut supplementation on body weight (g) and feed intake of female rats

Groups	Initial weight	Final weight	Body weight gain	Daily body weight gain	Daily feed intake	FER* %
Control	100.0 ± 5.66	206.85±6.39	106.85±7.02	2.55±0.19	17.5	14.6±1.08
T1 5%	102.75 ± 3.68	199.0±6.85	96.23±8.14	2.30±0.16	16.8[a]	13.62±1.11
T2 10%	102.25± 4.72	202.3 ±1.75	99.65 ± 5.66	2.38±0.15	16.6 [ab]	14.33 ±0.94
T3 20%	105.0 ± 4.24	199.5 ± 5.74	94.05 ±7.14 [a]	2.22±0.17 [a]	16.4[ab]	13.60±0.94

Data represent the mean ±S.D. of observations from six rats. [a] Significantly different from control group at $P < 0.05$. [b] Significantly different from T1 group at $P < 0.05$

*FER= Feed efficiency Ratio

C. esculentus tubers have high dietary fiber content (Umerie and Enebeli, 1997), so they may play a major role in lowering blood glucose level. This observation supports an earlier hypothesis that the tuber may be important for diabetics and those seeking to reduce weight (Kordyias, 1990). Meanwhile, tubers contain important nutrients and some essential macro and micro nutrients necessary for human and animal good health. (Chukwuma, *et al.*, 2010).

3. Changes in relative weight of some internal organs:

Table (3) indicated that adding 5, 10 and 20 % of tiger nut did not cause significant changes in liver, kidney, spleen heart and brain relative weights to the total weight of the treated female albino rats when compared to the control group. As exception, results from rats received 20% of tiger nut demonstrated an increase in relative weight of liver and a decrease in relative weight of brain after 6 weeks of treatment.

Table(3): Effect of 5, 10 and 20 % tiger nut supplementations on internal organs of female rats

Body weight (g) (BW)	Control	T1 (5%)	T2 (10%)	T3 (20%)
Initial Final	100.0 ± 5.66 206.85±6.39	102.75 ± 3.68 199.0±6.85	102.25 ± 4.72 202.3 ±1.74	105.0 ± 4.24 199.5 ± 5.74
Organ weight (g)				
Liver weight % of BW	7.48 ±0 .70 3.52 ± 0.30	7.22 ± 0.63 3.53 ± 0. 17	7.8 ± 1.16 3.35 ± 0.30	7.77 ± 0.62 3.87 ± 0.25
Kidney weight % of BW	1.37 ± 0.17 0.65±0.04	1.35 ± 0.10 0.66± 0.5	1.31 ± 0 .09 0.67± 0.01	1.32 ± 0.08 0.66± 0.03
Spleen weight % of BW	0.78 ± 0.04 0.37±0.03	0.70 ± 0.06 0.34±0.02	0.89 ± 0.21 0.43±0.08	0.75 ± 0.05 0.38±0.03
Heart weight % of BW	0.79 ± 0.09 0.37±0.03	0.77 ± 0.07 0.38±0.02	0.8 3 ± 0.11 0.39±0.03	0.79 ± 0.09 0.39±0.03
Brain weight % of BW	1.69 ±.07 0.79±0.02	1.57 ±.05 0.78±0.06	1.65 ±.09 0.79±0.06	1.59 ±.06 0.80±0.02

Data represent the mean ±S.D. of observations from six rats.

These results are confirmed with the histopathological examination which revealed no histopathological changes in rats received 10% tiger nut while a few leucocytes were noticed in hepatic sinusoids and neuronophagia of necrotic neurons in animals received 20% tiger nut.

4. Changes in hematological parameters:

The changes in RBCs, WBCs, HB and PCV were presented in Table (4) , which indicated narrow ranges in all groups supplemented with 5, 10 and 20 % of tiger nut compared with those values in control animals. Statistical analysis revealed no significant differences between all groups. While, HB concentration and RBCs counts were increased in all treated groups.

Table (4): Effect of 5, 10 and 20 % tiger nut supplementations on some blood parameters of female experimental rat groups

Groups	RBCs (M/mm^3)	WBCs (m/mm^3)	HB (g/dI)	PCV (%)
Control	4.27±0.40	3.63 ±0.35	12.97±0.97	36.14± 2.17
T1 5%	5.11±1.64	2.41±0.82	14.45 ±0.85	36.50±9.84
T2 10%	4.93±0.56	2.29±0.85	14.38±1.61	32. 39±4.44
T3 20%	4.55±0.90	2.90±0.95	14.15 ±1.22	39.25±9.74

Data represent the mean ±S.D. of observations from six rats.

The influence of food components on some haematological traits such as PCV and Hb concentration are very strong indicator of nutritional value of Tiger nuts which affect the nutritional status of animals (Hackbath *et al.*, 1983) . Also, iron(F) content is highly important because of its requirement in blood formation ,almost two – third of iron in the body is found in hemoglobin which helps in carrying oxygen to body tissues (National Institute of Health, 2013).

5. Changes in Biochemical parameters:

Serum biochemical parameters such as AST (GOT), ALT (GPT) and ALP were not significantly changed in rats supplemented with 5, 10 and 20 % of tiger nut. However a decrease in AST and ALP were noticed in rats treated with 10 and 20 % of tiger nut when compared with the control group.

Table (5): Effect of 5, 10 and 20 % tiger nut supplementations on enzyme activities of serum (AST), (ALT), (ALP), protein , albumin and globulin of rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Protein (g/dI)	Albumin (g/dI)	Globulin (g/dI)
Control	43.26 ± 2.07	12.58 ± .77	93.44 ± 5.78	7.90 ±.51	4.15±.46	3.75 ±.18
T1 5%	42.94 ± 1.51	12.52 ±1.2	93.35±7.84	8.48 ±.62	3.95 ±.54	4.52 ±.15
T2 10%	39.82 ± 1.99	12.32 ±.45	83.77±9.17	8.03±.96	3.59 ±.30	4.44 ±.77
T3 20%	39.32 ± 1.24	12.30 ±.34	88.70±13.61	8.14 ± .55	3.74 ±.11	4.39 ±.65

Data represent the mean ±S.D. of observations from six rats.

As shown in Table (5) the data indicate that tiger nut at different doses did not cause any significant difference in total protein and albumin contents throughout the experimental course when compared with the control rats.

6. Changes in renal functions:

Serum creatinines as well as urea were assessed as markers of renal functions. It is well known that

oxalates can remove calcium(ca) in the form of calcium oxalate (Savage, 1993) in the blood, which result in kidney damage. Calcium is always found in the body with phosphorus (P)contributing to the blood . Low Ca/P ratio facilitates calcification in the small intestins(Nieman *et al.*, 1992). Phosphorus was found to be one of the highest minerals (219mg/g) in *C. esculentus* tubers and more than calcium (100mg/g) (Ekeanyanwu and Ononogbu, 2010).

Table (6): Effect of 5, 10 and 20 % tiger nut supplementations on renal function tests(serum BUN and Creatinine) of rats

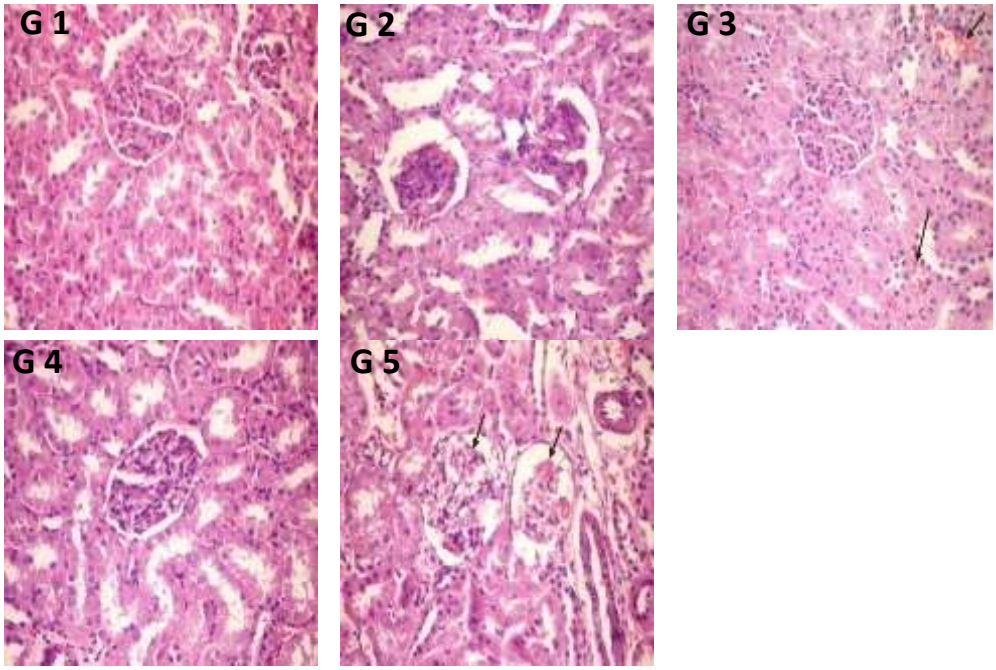
Groups	BUN (mg/dl)	Creatinine (mg/dl)
Control	63.72±10.32	0.68 ±.02
T1 5%	71.15±9.23	0.55 ±.19
T2 10%	71.43±7.60	0.65 ±.15
T3 20%	87.78±4.90[a]	0.59 ±.12

Each value represents mean of 6 replicates ±S.D [a] Significantly different from control group at $P < 0.05$.

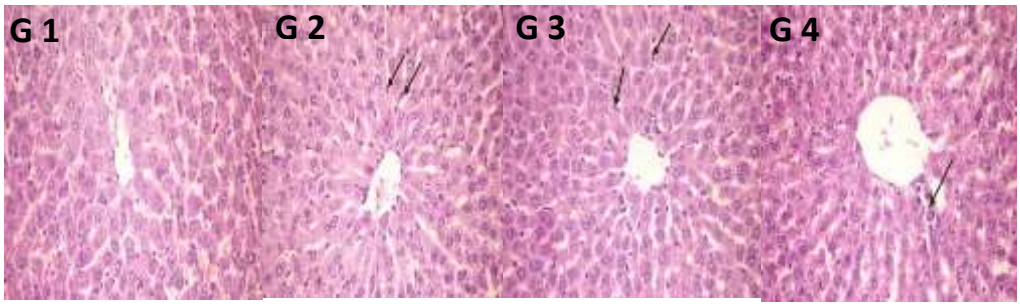
Serum creatinine was not significantly changed in rats with 5, 10 and 20 % of tiger nut (Table 6). However a significant increase in urea level was noticed in rats treated with 20 % of tiger nut when compared with the control group. On the other hand, there was insignificant increase with the other two doses (5% and 10%) throughout the experimental course.

7. Histopathological results:

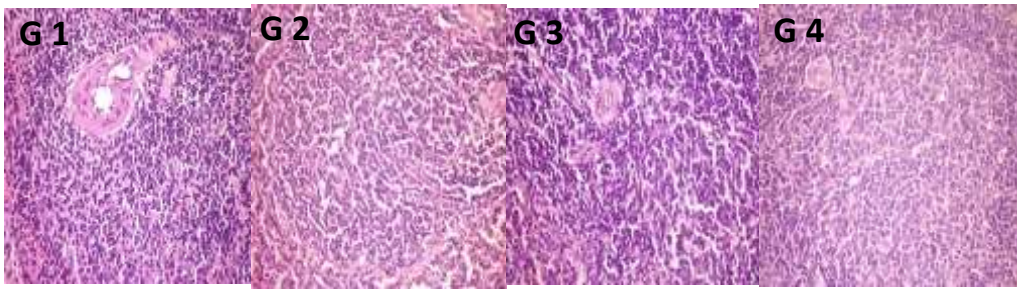
The effects of the three doses of tiger nut supplemented to rat diets for 6 weeks were evaluated by histopathological examination of kidney, liver, heart, spleen and brain tissues. Results showed significant correlation with the biochemical results. The microscopic examination of the tissue of these organs in control and rats received 5% tiger nut showed normal structure of the all organs cells (Fig. 1). Also, rats received 10% tiger nut showed normal appearance for kidney, liver, heart, spleen and brain in some sections while in other sections showed congestion of renal intertubular blood vessels and neuronophagia of necrotic neurons. Meanwhile, rats received 20% tiger nut had congestion and vacuolations of renal glomerular tuft, few leucocytes in hepatic sinusoids, necrosis of sporadic cardiac myocytes and showed neuronophagia of necrotic neurons in some sections.



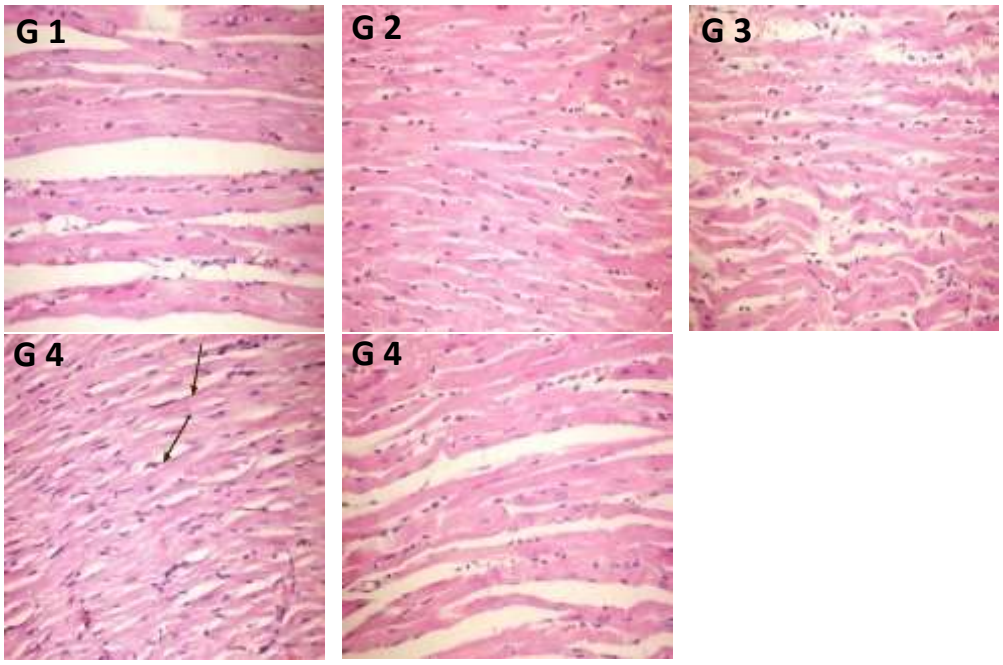
Histological examinations of kidney tissue of control rats (G 1) showing the normal histological structure of renal parenchyma .(G2) rats treated with 5% tiger nut showing no histopathological changes., (G3) 10% tiger nut showing congestion of intertubular blood and other section showing no histopathological changes and(G 4) 20% tiger nut showing congestion and vacuolations of glomerular tuft (H & E X 400)



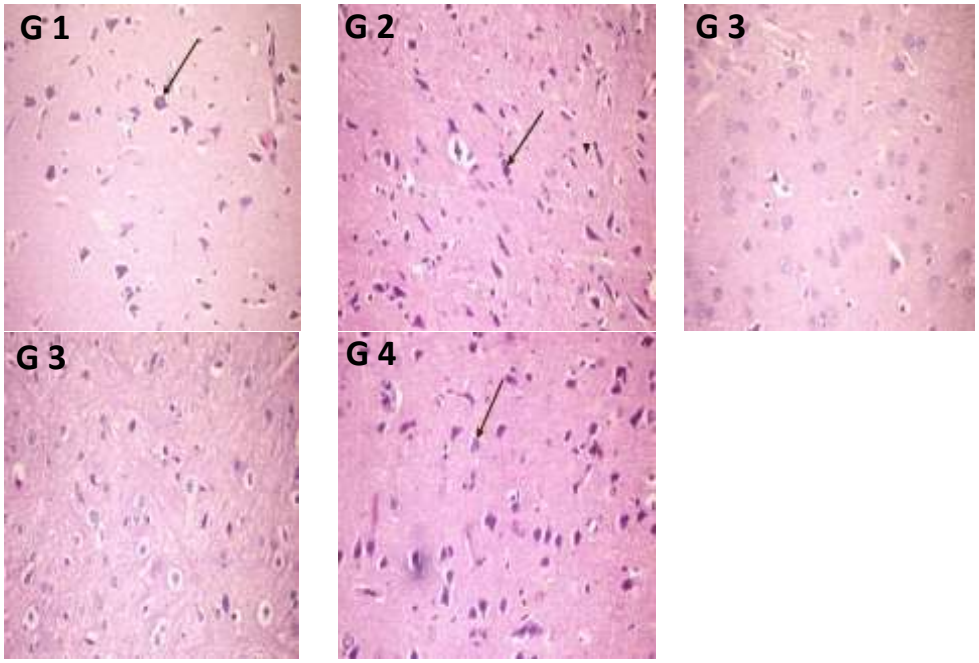
Histological examinations of liver tissue of normal control rats (G 1) showing normal histological structure of hepatic lobule, (G2) rats treated with 5% tiger nut and(G3) 10% tiger nut showing Kupffer cells activation and(G 4) 20% tiger nut showing few leucocytes in hepatic sinusoids (H & E X 400).



Histological examinations of Spleen tissue of rats control (G 1), rats treated with 5% tiger nut (G2), 10% tiger nut (G3) and 20% tiger nut (G 4) showing the normal histological structure of hepatic lobule (H & E X 400).



Histological examinations of heart tissue of control rats (G 1), rats treated with 5% tiger nut (G2), 10% tiger nut (G3)) showing normal cardiac myocytes and(G 4) 20% tiger nut showing necrosis of sporadic cardiac myocytes and other section showing no histopathological changes (H & E X 400



Histological examinations of brain tissue of control rats (G1) showing no histopathological changes.(G2)rats treated with 5% tiger nut showing neuronophagia of necrotic neurons, (G3)10% tiger nut showing neuronophagia of necrotic neurons and other section showing no histopathological changes and(G 4) 20% tiger nut showing neuronophagia of necrotic neurons (H & E X 400).

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تأثير التدعيم الغذائي لدرنات حب العزيز على مكونات الدم في جرذان الألبينو

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أظهرت الدراسات السابقة أن درنات حب العزيز قد تم استخدامها في العديد من المحاولات العلاجية. وفي هذه الدراسة تم التحقق من التركيزات الآمنة لحب العزيز المضافة لغذاء الجرذان. أولا تم حصر المكونات الكيميائية النباتية لدرنات حب العزيز ثم تم تقييم تأثير الجرعات المختلفة وهى 5% و10% و 20% من درنات حب العزيز على وزن الجسم ووزن الأعضاء الداخلية في الجرذان. وتم فحص القياسات الهيماتولوجية والبيوكيميائية والهستولوجية. قسم 24 جرذ إلى 4 مجاميع كل مجموعة 6 جرذان. المجموعة الأولى (المجموعة الضابطة) تم تغذيتها على غذاء قياسي بينما المجاميع الثانية والثالثة والرابعة غذيت على حب العزيز بتركيز 5 % و10% و20% بالتتابع لمدة 6 أسابيع.

أظهر حصر المكونات الكيميائية النباتية لدرنات حب العزيز وجود الاستروييد والكربوهيدرات والأحماض الدهنية والتانينات والكيومارين والقلويدات. لم يلاحظ اختلافات معنوية في كل القياسات الهيماتولوجية والبيوكيميائية التي درست في كل المجاميع المعاملة عندما قورنت بالمجموعة الضابطة. ومع ذلك لوحظ زيادة معنوية في مستوى اليوريا في الجرذان التي غذيت على 20% حب العزيز و أكد ذلك نتائج دراسة الأنسجة. اقترحت نتائج هذه الدراسة أن إضافة درنات حب العزيز بتركيزات 5% 10% للغذاء آمن وصالح للأكل.