British Journal of Pharmaceutical Research

16(3): 1-14, 2017; Article no.BJPR.33517
ISSN: 2231-2919, NLM ID: 101631759

Assessment of Haematological and Biochemical Effects of Kolaviron in Male Wistar Rats

Quadri K. Alabi[1,2*], Rufus O. Akomolafe[2], Olaoluwa S. Olukiran[2], Aliyat O. Nafiu[2], Joseph G. Omole[2], Adebukola M. Adefisayo[3] and Ayowole A. Oladele[4]

[1]Department of Haematology and Blood Transfusion, Faculty of Basic Medical Sciences, College of Medicine, Afe Babalola University, Ado Ekiti, Ekiti, State, Nigeria.
[2]Department of Physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[3]Department of Physiology, Faculty of Basic Medical Sciences, University of Medical Sciences, Ondo State, Nigeria.
[4]Department of Medical Laboratory Sciences, College of Medicine, Afe Babalola University, Ado Ekiti, Ekiti, State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors QKA and ROA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OSO and JGO managed the analyses of the study. Authors AMA and AAO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2017/33517
Editor(s):
(1) Partha Krishnamurthy, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, USA.
Reviewers:
(1) Viduranga Waisundara, Rajarata University of Sri Lanka, Mihintale, Sri Lanka.
(2) Eliton da Silva Vasconcelos, Federal University of São Carlos, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/18946

Original Research Article

Received 18th April 2017
Accepted 28th April 2017
Published 6th May 2017

ABSTRACT

This study determined the effects of kolaviron on the hematological and biochemical parameters of rats. The aim was to ascertain if its consumption has deleterious effects on these parameters. Forty adult male Wistar rats divided into four groups of ten animals each were used. The control group received 2 ml/kg propylene glycol only. Kolaviron (KV) was administered at 100, 200 and 400 mg/kg body weight respectively to the experimental groups via oral route for 28 days. At the end of the study period, five rats were sacrificed under ketamine hydrochloride and the other 5 rats

**Corresponding author: E-mail: alabikunlequadri@gmail.com;*

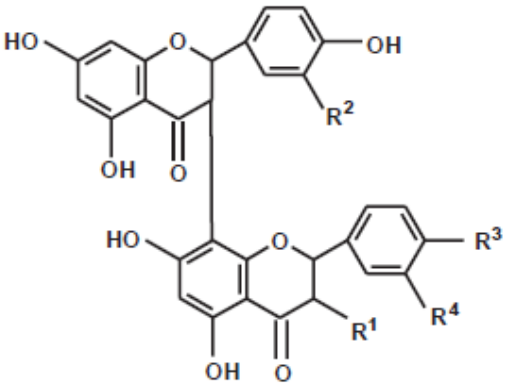
were allowed to recover for 2 weeks. Hematological analysis was carried out, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin and glucose were assayed from the plasma while the liver tissue was used for histopathological examination. Compared with the control group, white blood cell (WBC) and lymphocyte, red blood cells (RBCs) counts, hematocrit (HCT) and hemoglobin (Hb) concentrations were significantly higher in groups treated with 100 and 200 mg/kg b.w of KV. However, plasma AST, ALT, ALP activities and bilirubin in 100 and 200 mg/kg KV were not significant different from that of the control. There was a significantly lower plasma glucose level in all KV treated groups when compared with the control. However, KV at 400 mg/kg had a significantly higher RBCs count with a significantly lower Hb, mean corpuscular hemoglobin concentration (MCHC) and platelet count. The plasma AST, ALT activities and bilirubin level were also higher in 400 mg/kg b.w KV when compared with the control, but plasma ALP remain unchanged. At 400 mg/kg b.w KV, histological examination of the liver tissue showed sign of portal cellular infiltration, periportal congestion and hydropic degeneration of hepatocytes in the liver, but restored toward normal after 2 weeks recovery period. This study confirmed that KV at 100 and 200 mg/kg b.w improve hematological indices with hypoglycemic and immune boosting effects in rats. However, KV at higher dosage (400 mg/kg b.w) has tendency to have deleterious effects on the liver and blood.

Keywords: Kolaviron; hematological; biochemical; liver; rat.

1. INTRODUCTION

Garcinia kola (Heckel) belongs to the family of Guttiferae and is a medium sized tree easily recognized by its hairy flowers and large fruits. Its plant grows in the tropical region of sub-Saharan Africa, naturally in Sierra Leone, Nigeria and Angola [1,2]. The seeds have a bitter astringent taste; hence, it is called “bitter kola” in Nigeria. *Garcinia kola* seed is also referred to as *Orogbo* (*Yoruba*-Western Nigeria), *Namijin goro* (*Hausa*-Northern Nigeria), *Akuilu or Ugugolu* (*Igbo*-Eastern Nigeria), *Efiari (Efik)*, and *Igoligo* in *Idoma-Middle Belt* [3]. The plant has been referred to as a ‘wonder plant’ because almost every part of it has been found to be of medicinal importance [4]. The seed is used in traditional hospitality, cultural and social ceremonies. Its leaves, seeds and barks have also been used in folklore medicine for the treatment of ailments such as liver disorders, urinary tract infections, hepatitis, diarrhea, laryngitis and bronchitis [5-8]. The seeds are used to prevent or relieve colic, cure head or chest colds and relieve cough [6]. They have also been shown to enhance the immune system [9-11]. The seed also has anti-inflammatory, anti-microbial, anti-diabetic, antiviral and anti-ulcer properties [12,13]. The split stems and twigs of the plant are used as chewing sticks in many parts of Africa because they offer natural dental cares [14]. Thus, the seeds have been shown to contain a complex mixture of polyphenolic compounds, bioflavonoids, prenylated benzophenones and xanthones which account for the majority of its effects [15,16].

Kolaviron (KV) is a fraction of the defatted ethanol extract of *Garcinia kola* seeds, containing Garcinia biflavonoids GB1, GB2, and kolaflavone as its major components (Fig. 1) [17,18]. The extract is one of the numerous plant product and nutritional supplements that have been found to have a wide range of medicinal values. It is a drug of plant origin which has numerous biochemical importance in the human body system [17]. Kolaviron has shown ample beneficial health effects in animal models of diseases and also in the prevention of hepatotoxicity induced by several toxins such as thioacetamide, paracetamol, carbon tetrachloride and amanita toxins [19,20]. The protective effects of kolaviron against insults from various xenobiotics have been attributed to its antioxidant properties [21]. It has a profound action on the production of erythropoietin in the kidney [22,23]. It protects against oxidative stress induced by toxins in experimental animal models [5,24]. Despite the vast use of kolaviron, there is a paucity of literature on its effects on hematological indices and biochemical parameters in experimental animals. Therefore, this study was designed to investigate the haematological and biochemical effects of graded doses of kolaviron with a view to ascertaining its safety or otherwise of orally ingested dosages of the extract on these parameters.



	R1	R2	R3	R4
GB1	OH	H	OH	H
GB2	OH	H	OH	OH
Kolaflavanone	OH	H	OCH_3	OH

Fig. 1. Chemical structures of the bioactive compounds present in Kolaviron

1.

2.

Images depicting 1. Garcinia kola plant and 2. Seeds

2. MATERIALS AND METHODS

2.1 Extraction of Kolaviron

Kolaviron was isolated according to the method of Iwu et al. [25]. Fresh seeds of *Garcina kola* were peeled, air dried and crushed into powder using an electric pulverizer (DIK-2910, Daiki Rika Kogyo Co. Ltd, Tokyo-Japan). 2.1 kg powdered seeds of *Garcina kola* were defatted with 3.5 liters of petroleum ether (b.p 40°C - 60℃) in a Soxhlet extractor for 24 hours. The defatted dry product was further extracted with 3.5 liters of 80 % acetone (1:2 w/v) in a Soxhlet extractor for 24 hours. The extract was concentrated at 40℃ using a Rotary Evaporator, diluted to twice its volume with distilled water and partitioned with 2 litres of ethyl acetate. The concentrated ethyl acetate fraction yielded kolaviron, a golden brown and was freeze-dried in a Lyophilizer (Ilshin Lab. Co. Ltd, Seoul, Republic of Korea) to a solid form. The sample obtained as a product of freeze drying was weighed to calculate for the percentage yield of the plant extract.

$$\text{Extraction yield in \%} = \frac{\text{Weight of extract of } \textit{Garcina kola}}{\text{Weight of powdered } \textit{Garcina kola}} \times 100$$

$$= \frac{120.39\ \text{g}}{2100} \times 100$$

$$= 5.73\ \%.$$

2.2 Stock Solutions of Kolaviron

The extract's stock solution for 100 mg/kg was prepared by dissolving 1 g kolaviron in 20 mL of propylene glycol. Consequently, every 100 g rat received 0.2 mL of kolaviron to prevent the deleterious effects of extract overload. Accordingly, stock solutions for 200 mg/kg and 400 mg/kg of kolaviron were prepared by dissolving 2 g and 4 g of the extract, respectively, each in 20 mL of propylene glycol.

2.3 Animal Care and Management

Forty adult male Wistar rats (110-150 g) were used for this study; they were obtained from the Animal House of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun State. The animals were kept inside plastic cages under normal environmental conditions of light/dark cycle and had free access to standard rodent pellet diet (Ace Feed PLC Ibadan, Nigeria) and

water *ad libitum*. They were allowed to acclimatize in the laboratory for two weeks before the commencement of the study. The experimental protocols were in strict compliance with the guidelines for animal research, as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals [26] and approved by Institutional Research Committee.

2.4 Experimental Design

The rats were divided into 4 groups (n = 10 per group) as follows; Group I (control) received propylene glycol at 2 ml/kg via oral route for 28 days. Groups II, III and IV received graded doses of KV at 100, 200 and 400 mg/kg/day via oral route for 28 days. At the end of the study period, five rats were sacrificed under ketamine hydrochloride (10 mg/kg/b.w via intramuscular route) and the remaining 5 rats were left untreated for 2 weeks before sacrifice. Blood samples from all the rats were drawn via cardiac puncture and collected into separate EDTA and heparinize bottles. Blood dispensed into potassium (EDTA) bottles was used for hematological analysis while that collected into heparinize bottles was centrifuged at 4000 revolutions per minute for 15 minutes at – 4℃ using a cold centrifuge (Centurium Scientific, Model 8881) to obtain the plasma which was used for the assessment of biochemical parameters.

2.5 Measurement of Food Consumption, Body and Organ Weight

Assessment of weekly weight change was carried out using Hanson digital weighing balance (Hanson, China). However, organ weights were determined using Camry sensitive weighing balance (Camry, China). The percentage weight change was calculated using the formulae below;

Percentage Weight Change (PWC)% =

$$\frac{(\text{Final body weight} - \text{Initial body weight})\ g}{\text{Initial body weight (g)}} \times 100$$

With the aid of the metabolic cage, the food consumption for each rat in the groups were carried out. Food consumption was measured with the aid of a digital weighing balance (Hanson, China).

2.6 Biochemical Analyses

Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and glucose were assayed using biochemical kits purchased from Randox Laboratories (Crumlin, Co. Antrim UK).

2.7 Hematological Indices

Red blood cell (RBC), haematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin Concentration (MCHC), white blood cell (WBC), granulocyte, monocytes, lymphocytes and platelet counts were determined using an auto-analyzer machine (SFRI blood cell Counter, H18 light, France).

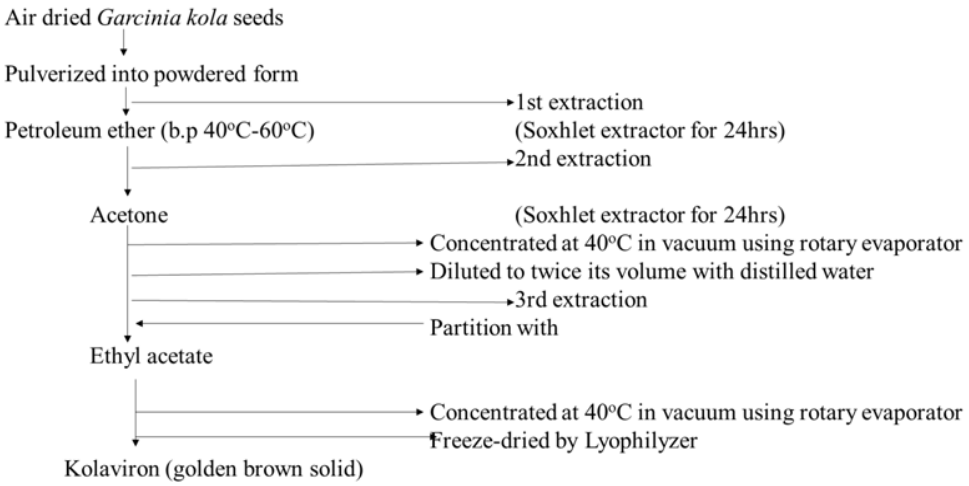


Fig. 2. Scheme illustration of extraction process of kolaviron

2.8 Histological Processes

The fixed liver samples of the rats were dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax. The tissues were then cut into 2-3 µm thick sections by a microtome, fixed on the slides and stained with haematoxylin-eosin. The slides were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan) and photomicrographs were taken with a Leica DM 750 Camera at x 100 and 400 magnifications.

2.9 Statistical Analysis

The results obtained were expressed as mean ± SEM. Data were analyzed using One-way analysis of variance (ANOVA) followed by post-hoc test using Student-Newman-Keuls and P value less than 0.05 was considered statistically significant. The statistical analysis was performed with the aid of GraphPad Prism 5.03 (GraphPad Software Inc., CA, USA) and Microsoft Office Excel, 2016 package.

3. RESULTS

3.1 Relative Liver Weight, Percentage Body Weight Change and Food Consumption

3.1.1 4 Weeks treatment

The relative liver and absolute liver weight of all kolaviron treated groups (100, 200 and 400 mg/kg) showed no significant ($p > 0.05$) different when compared with the control (Table 1). However, there was a significantly ($p < 0.05$) increased in body weight change and food consumption in all kolaviron treated groups (100, 200 and 400 mg/kg) when compared with the control (Table 1).

3.1.2 2 Weeks recovery

After 2 weeks recovery period, the relative liver and absolute liver weight of all kolaviron treated groups (100, 200 and 400 mg/kg) showed no significant ($p > 0.05$) different when compared with the control (Table 1). The body weight change and food consumption in all kolaviron treated groups (100, 200 and 400 mg/kg) were significantly increased after recovery period from kolaviron treatment (Table 1).

3.2 Hematological Parameters

3.2.1 4 Weeks treatment

The mean values of hematological parameters in rats treated with graded doses of kolaviron are presented in Tables 2 and 3. Rats administered with 100 and 200 had a significantly ($p < 0.05$) increased white blood cells (WBC) and lymphocyte values (Table 2). The red blood cell, hemoglobin and hematocrit values were significantly increased at the doses of 100 and 200 mg/kg. The RBC and mean corpuscular volume (MCV) values were significantly ($p < 0.05$) increased in the group that received 400 mg/kg with a significantly ($p < 0.05$) reduction in hemoglobin and mean corpuscular hemoglobin concentration(MCHC) when compared with the control (Table 3). Also, there was a significantly ($p < 0.05$) decreased in platelets count at the dose of 400 mg/kg when compared with the control (Table 2).

Table 1. Effect of kolaviron on body weight change, absolute and relative liver weight and food consumption of rats

Groups	Body weight change (%)	Absolute liver weight (g)	Relative liver weight (%)	Food consumption (g)
4 Weeks treatment				
I (Control)	38.54±3.50	4.52±0.28	2.99±0.03	14.20 ± 0.58
II (100 mg/kg KV)	50.25 ± 8.27*	4.16±0.76	2.67±0.76	17.80 ± 0.49*
III (200 mg/kg KV)	57.65 ± 5.99*	4.19±0.57	2.58±0.68	19.00 ± 0.63*
IV (400 mg/kg KV)	49.47 ± 1.02*	5.09±0.49	3.12±0.88	16.80 ± 0.37*
2 Weeks recovery				
I (Control)	38.54±3.50	4.52±0.28	2.99±0.03	18.60±0.40
II (100 mg/kg KV)	70.48±10.03*	4.10±0.16	2.14±0.15	23.20±0.37*
III (200 mg/kg KV)	74.68±8.61*	4.05±0.28	2.11±0.32	25.20±0.49*
IV (400 mg/kg KV)	69.54±2.47*	5.00±0.26	3.00±0.65	22.00±0.55*

*Values are given as mean ± SEM (n=5). *p < .05 compared to control*

Table 2. Hematological parameters of rats after 4 weeks' treatment with kolaviron

Parameters	I (control)	II (100 mg/kg)	III (200 mg/kg)	IV (400 mg/kg)
WBC ($x10^3$/µL)	7.08±0.62	9.86±0.90*	9.96±0.06*	8.00±0.82
LYM %	82.04±0.79	94.40±4.75*	95.34±5.64*	81.88±1.76
MON %	7.50±0.36	8.28±1.05	9.94±1.40	7.90±0.84
GRAN %	16.42±1.09	17.32±8.42	19.72±9.72	16.22±3.05
LYM ($x10^3$/µL)	6.52±0.52	9.18±0.88*	9.08±1.11*	5.20±0.96
MON ($x10^3$/µL)	0.54±0.04	0.58±0.09	0.68±0.08	0.48±0.05
GRAN ($x10^3$/µL)	0.94±0.10	1.10±0.25	1.20±0.16	0.90±0.09
RBC ($x10^6$/µL)	6.66±0.24	8.29±0.26*	8.34±0.95*	9.03±0.17*
HGB (g/dl)	14.92±0.61	16.98±0.53*	16.86±0.32*	12.76±0.41*
HCT (%)	47.76±2.48	59.30±1.44*	59.26±5.71*	37.64±1. 37*
MCV (fl)	60.02±0.99	60.56±0.47	59.92±10.92	87.98±0. 40*
MCH (pg)	19.54±0.19	19.60±0.084	20.22±5.12	19.96±0. 34
MCHC (g/dl)	32.30±0.31	32.46±0.18	32.48±8.43	27.68±0.27*
PLT ($x10^3$/µL)	637.40±58.63	674.00±34.06	598.60±76.82	342.80±60.58*
MPV (fl)	7.08±0.08	7.16±0.08	6.90±0.18	5.30±0.07
PCT (%)	0.49±0.04	0.46±7.33	0.49±0.06	0.43±0.04

*Values are given as mean ± SEM (n=5). *p < .05 compared to control. WBC = White blood cell, LYM= lymphocyte, MON= Monocyte, GRAN= Granulocyte, HGB= Haemoglobin, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, HCT= Hematocrit, MCV= Mean Corpuscular Volume, PLT= Platelet Count, Platelet crit*

3.2.2 2 Weeks recovery

After 2 weeks recovery period, there was a significantly ($p < 0.05$) increased in WBC and lymphocytes value in all kolaviron treated groups when compared with the control (Table 3). But other hematological parameters showed no significant ($p > 0.05$) different when compared with the control ($p > 0.05$) (Table 3).

3.3 Biochemical Parameters

3.3.1 4 Weeks treatment

The result of the effects kolaviron on plasma biochemical parameters is presented in Table 4. There was significant ($P<0.05$) increase in the levels of AST, ALT and total bilirubin at the dose of 400 mg/kg. There were however no significant ($P > 0.05$) different in the levels of AST, ALT and total bilirubin at the doses of 100 and 200 mg/kg. Plasma ALP was not significant ($P > 0.05$) different in all the treated doses of the kolaviron when compared with the control. Also, there was significant ($P<0.05$) decrease in the level of plasma glucose at the doses of 100 and 200 mg/kg, but 400 mg/kg had no significant ($P>0.05$) different in plasma glucose when compared with control.

3.3.2 2 Weeks recovery

After recovery period for 2 weeks, the plasma levels of AST, ALT, ALP and bilirubin showed no significant ($p > 0.05$) different in all kolaviron treated groups when compared with the control (Table 4). However, there was a significantly ($p < 0.05$) decreased in plasma glucose levels in all kolaviron treated groups after recovery from kolaviron when compared with the control (Table 4).

3.4 Histopathological Results

The result of the histological rat liver treated with kolaviron is presented in Figs 3 and 4.

3.4.1 4 Weeks treatment

No visible lesion was observed in the liver of the control group and doses at 100 and 200 mg/kg. However, severe periportal congestion, portal cellular infiltration and diffuse hydropic degeneration of hepatocytes was seen at 400 mg/kg.

3.4.2 2 Weeks recovery

Control liver showed normal histoarchitecture with no visible lesion; 100 mg/kg KV recovery showed normal histoarchitecture as the control; 200 mg/kg KV recovery showed normal histoarchitecture of the liver as the control and 400 mg/kg KV recovery liver showed sign of recovery with mild degeneration of hepatocytes.

Table 3. Hematological parameters of rats after 2 weeks' recovery from kolaviron treatment

Parameters	I (control)	II (100 mg/kg)	III (200 mg/kg)	IV (400 mg/kg)
WBC (x10^3/µL)	7.08±0.62	9.54±1.50*	10.80±0.33*	10.22±0.85*
LYM %	82.04±0.79	99.32±4.38*	99.94±3.95*	95.96±6.01*
MON %	7.50±0.36	9.12±0.75	10.96±1.39	9.76±0.67
GRAN %	16.42±1.09	19.56±2.61	19.10±1.20	18.28±2.20
LYM (x10^3/µL)	6.52±0.52	9.56±1.08*	9.04±0.17*	8.84±0.75*
MON (x10^3/µL)	0.54±0.04	0.60±0.14	0.64±0.12	0.68±0.06
GRAN (x10^3/µL)	0.94±0.10	1.38±0.33	1.12±0.10	1.70±0.12
RBC (x10^6/µL)	6.66±0.24	7.81±0.17	7.25±0.39	7.89±0.07
HGB (g/dl)	14.92±0.61	15.94±0.42	15.10±1.83	15.58±0.54
HCT (%)	47.76±2.48	46.24±1.01	41.50±2.32	45.70±0.46
MCV (fl)	60.02±0.99	61.28±1.88	57.30±0.22	57.98±0.40
MCH (pg)	19.54±0.19	20.40±0.43	19.36±0.11	19.70±0.22
MCHC (g/dl)	32.30±0.31	34.48±0.67	33.92±0.09	34.04±0.19
PLT (x10^3/µL)	637.40±58.63	525.40±75.04	634.60±42.23	645.80±19.28
MPV (fl)	7.08±0.08	7.78±0.66	7.04±0.11	7.24±0.10
PCT (%)	0.49±0.04	0.31±0.04	0.29±0.04	0.35±0.04

*Values are given as mean ± SEM (n=5). *p < .05 compared to control. WBC = White blood cell, LYM= lymphocyte, MON= Monocyte, GRAN= Granulocyte, HGB= Haemoglobin, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, HCT= Hematocrit, MCV= Mean Corpuscular Volume, PLT= Platelet Count, Platelet crit*

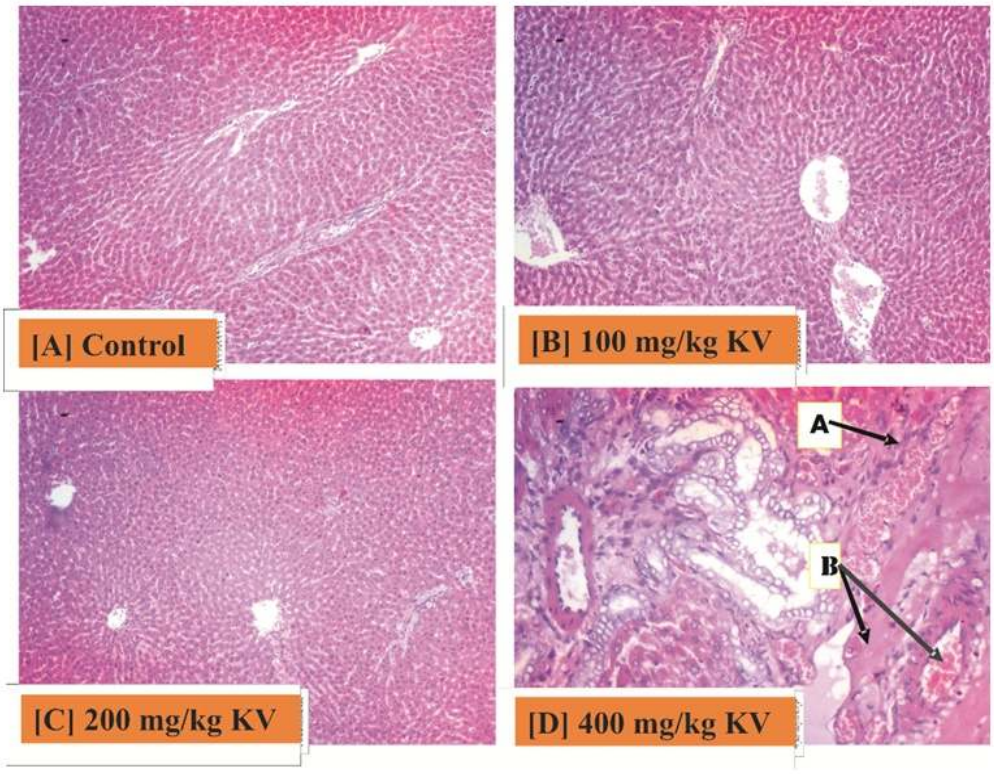


Fig. 3. Microscopic views of H&E - stained rat after 4 weeks' treatment of kolaviron. [A] = control; Liver from the control showed normal histoarchitecture with no visible lesion, [B] 100 mg/kg KV; showed normal histoarchitecture of the liver with well defined nucleus, [C] 200 mg/kg KV; showed normal histoarchitecture of the liver with well defined nuclei, [D] 400 mg/kg KV; showed severe periportal congestion; A, portal cellular infiltration; B diffused hydropic degeneration of hepatocytes (Magnification: × 100)

Table 4. Effect of Kolaviron on the activities of some plasma enzymes and total bilirubin and glucose of rats

	4-Week treatment					2 Weeks recovery				
Parameter	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Total Bilirubin (µmol/l)**	**Plasma glucose (mg/dl)**	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Total Bilirubin (µmol/l)**	**Plasma glucose (mg/dl)**
I (Control)	50.66±3.28	24.13±4.70	101.9±5.65	4.31±0 .85	92.06±11.24	50.66±3.28	24.13±4.70	101.9±5.65	4. 31±0.85	92.06±11.24
II (100 mg/kg KV)	54.68±2.04	29.18±2.63	106.7±6.45	3.95±0.56	70.79±5.07*	48.85±3.68	22.21±1.66	98.21±2.07	2.89±0.38	43.74±3 .89*
III (200 mg/kg KV)	54.42±1.21	28.41±2.14	109.3±4.40	3.75±0.84	62.20±4.53*	48.12±4.45	20.86±1.14	97.71±2.38	2.99±0.42	50.98±5 .92*
IV (400 mg/kg KV)	65.33±4.13*	38.50±2.66*	110.1±3.99	6.78±0.52*	97.32±3.81	49.55±4.78	21.69±1.42	98.30±4.26	3.27±0 .53	65.82±6.89*

*Values are given as mean ± SEM (n=5). *p < .05 compared to control*

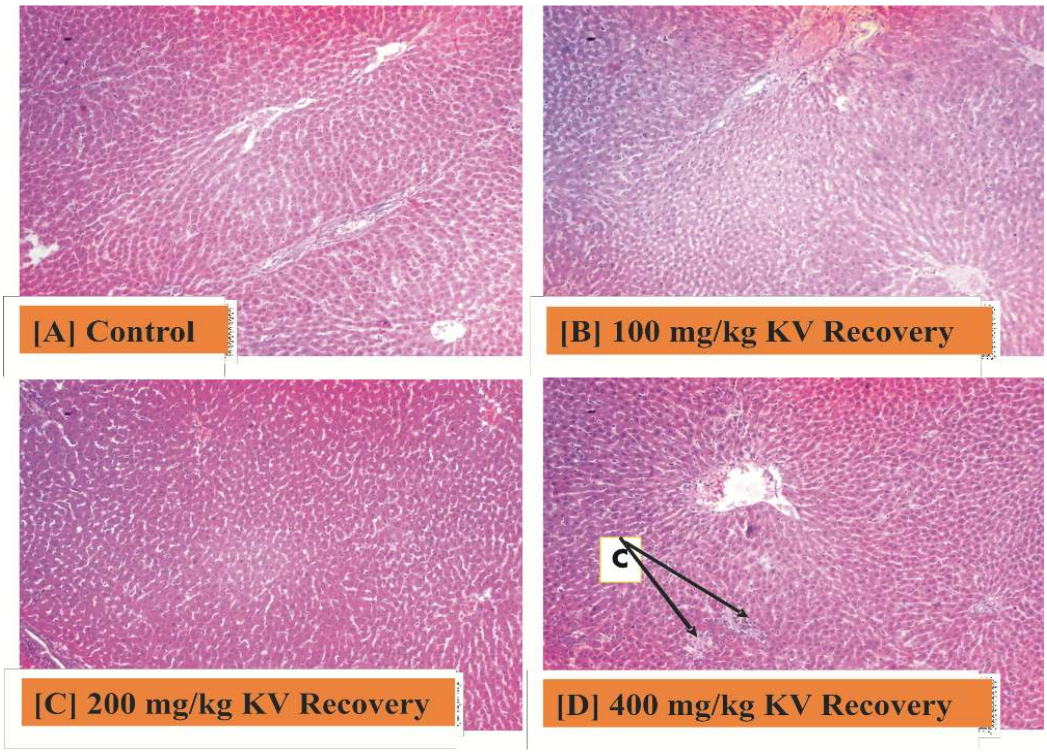


Fig. 4. Microscopic views of H&E - stained of rat after 2 weeks' recovery from kolaviron treatment. [A] = control; showed normal liver histoarchitecture with no visible lesion, [B] 100 mg/kg KV Recovery; showed normal histoarchitecture as the control, [C] 200 mg/kg KV Recovery; showed normal histoarchitecture of the liver as the control, [D] 400 mg/kg KV Recovery; Liver showed sign of recovery; C, with mild degeneration of hepatocytes, (Magnification: × 100)

4. DISCUSSION

The present study showed that kolaviron did not altered the feeding pattern of rats. The significant increase in food intake in all kolaviron treated groups that was observed in this study when compared with the control may suggests that kolaviron does not have any deleterious effects on the gastrointestinal (GIT) or appetite center in the hypothalamus. This observation is not in agreement with the report of Braide [27]; Obi and Nwoha [28] who reported that kolaviron caused an intestinal mal-absorption; general reduction in body weight and retarded growth rate. The lack of consistency is likely attributable to the duration and dose used in their study.

The significant increase in body weight change that was seen in the experimental groups treated with kolaviron is an indication of increase in food intake that was observed in this study. This may be that kolaviron, might probably favor rapid absorption of nutrient from the gastro-intestinal tract. However, this is not in agreement with the report of Obi and Nwoha [28], who reported that kolaviron administered at 400 mg/kg caused a significant decrease in the body weight. This disparity may have resulted from the differences in the duration of the study. Another possible explanation for the significant increase in food consumption and body weight that was observed in the groups treated with kolaviron could be due to hypoglycemic property attributed to flavonoids and other phytochemical constituents [29]. Kolaviron is a flavonoid complex that has been reported to cause a significant reduction in plasma glucose level in streptozotocin - induced diabetic rats [30]. Decrease in the plasma glucose level is said to inhibit the satiety center, thus activating the feeding center to increase appetite leading to increase in food intake [31]. Hence, the increase in food consumption and body weight of rats treated with kolaviron is an indicative of pharmacological function of KV in rats.

Hematological indices are diagnostic tools for routine clinical evaluation of the state of health. The present study showed that treatment with kolaviron at 100 and 200 mg/kg for 4 weeks significantly increased WBC and lymphocyte counts when compared with the control, whereas monocyte and granulocyte counts were not significant different from the control rats after treatment with kolaviron for 4 weeks (Table 2). After 2 weeks' withdrawal of kolaviron treatment, there was a significantly increased in WBC and lymphocyte counts in all kolaviron treated groups compared with the control (Table 3). The observed increase in WBC and lymphocyte counts following kolaviron treatment revealed that it may have beneficial effects on the immune system. WBC and lymphocytes are synthesized in the bone marrow along with other types of blood cells. They protect the body from infection. The high numbers of WBC and lymphocytes observed in kolaviron-treated rats indicate low risk of infection. This finding is in accordance with the study of Uko et al. [32] and Dada and Ikuerowo [33], who reported a proliferation in total leucocyte counts but different from the study of Ahumibe and Braide [34], who observed no significant difference in white blood cell (WBC) counts after treatment with *Garcinia kola*. The observed effect of kolaviron on WBC explains the antimicrobial potential of the plant extract in view of the major role that WBC assume in the immunity defense mechanism of the body in both man and animal as reported by Dada and Ikuerowo [33]. The significantly increased lymphocytes signified an improvement in immune system. This supports the findings of Okoko and Oruambo [35] and Dada and Ikuerowo [33].

Decrease in red blood cell counts (RBC) could revealed an imbalance between its production and loss [36]. The results obtained in this study showed that kolaviron at 100 and 200 mg/kg b.w treatment for 4 weeks caused significant increase in red blood cell (RBC) counts with subsequent increase in hemoglobin (Hb) and hematocrit (HCT) concentrations when compared with the control. These results are in line with the report of Ahumibe and Braide [37], who observed increase in RBC count, HCT and Hb concentrations after administration of kolaviron in humans and rats' model. The life span of RBCs is also related to the blood antioxidant capacity [38]. Hence, the increase in RBC count that was observed in the 100 and 200 mg/kg kolaviron treated rats may have resulted from the antioxidant capacities of kolaviron [39-42]. However, kolaviron treated group at 400 mg/kg b.w had increase the number of RBCs, mean corpuscular volume (MCV) and this is accompanied by a decreased in hemoglobin (Hb) concentrations and mean corpuscular hemoglobin concentration (MCHC) and platelet count. This seems to appear that kolaviron at 400 mg/kg b.w might have caused a decrease in tissue iron concentration and/or interferes with hemoglobin (Hb) biosynthesis. The observed significant increase in RBCs count and reduction in Hb concentration and MCHC at high dose (400 mg/kg of kolaviron) is an indication of macrocytic hypochromic anemia. This finding is in accordance with the study of Uko et al. [32], who reported a reduction in Hb concentration and hematocrit following treatment with *Garcinia kola*. The reduction observed in the platelet counts in the group that received 400 mg/kg b.w of kolaviron may suggests destruction of megakaryocytes which are responsible for platelets formation [43]. Therefore, continued administration of the extract at high doses (400 mg/kg KV) may result in widespread hemorrhages as seen in the histology (Fig. 3), due to coagulation deficiency because platelets play a crucial role in reducing blood loss and repairing vascular injury [44,45]. However, after 2 weeks' withdrawal of the extract, there was no significant different in hematological indices of the rats treated with kolaviron when compared with the control (Table 3).

Plasma glucose of the rats that were administered with 100 and 200 mg/kg kolaviron was significantly lower than that of the control, of which rats that were administered with 400 mg/kg of kolaviron showed no significant difference in plasma glucose when compared with the control (Table 4). After 2 weeks' withdrawal of kolaviron treatment, the plasma glucose in all kolaviron treated groups was significantly decreased when compared with the control (Table 4). This report showed that kolaviron has the ability to lower plasma glucose. The mechanisms of hypoglycemic effect of kolaviron might be due to the combination of its stimulating action on the pancreatic β cells to release insulin and also an insulin independent effect and extrapancreatic action which may involve glucose utilization in extrahepatic tissues [46,47]. Furthermore, the hypoglycemic effect of flavonoids can be mediated through an increase in hepatic glucose storage by stimulating the action of glycolytic and glycogenic enzymes or by inhibiting glucose-6-phosphatase. This consequently results in the uptake of glucose into cells and the reduction in the blood glucose level

through the upregulation of glycogen formation, downregulation of the rate of glycogen breakdown and glucose synthesis [48-49].

Hepatoprotective activity of kolaviron has been reported by various studies [17,5,50,51], but may be hepatotoxic at very high doses. It was observed in this study that administration of kolaviron at 400 mg/kg body weight significantly increased the level of plasma AST, ALT and bilirubin when compared with the control (Table 4). The increase in the level of AST and ALT may be due to the leakage of the enzymes from damaged hepatocyte into the systemic circulation. This may have accounted for the diffuse hepatic degeneration that was observed in the photomicrograph of rats treated with 400 mg/kg body weight (Fig. 3). Plasma ALT is also known to increase in liver disease and it has been used as a tool for measuring hepatic necrosis [52].

Plasma ALP activity in this study showed no significant different when compared with the control, but the level of bilirubin increase in rats treated with 400 mg/kg of KV when compared with the control. Elevated serum bilirubin concentration is indicative of bile ducts obstruction. Bilirubin may also be elevated when the secretory function of the liver is impaired. Thus, the increase in plasma levels of AST, ALT and bilirubin that was observed in the higher dosage (400 mg/kg b.w) showed the evident of liver necrosis. However, the administration of kolaviron extract appears to be relatively non-toxic to animals at low and moderate doses (100 and 200 mg/kg b.w). This is because there was no apparent damage to the physiology and biochemistry of the blood of rats in this study. However, at high dose (400 mg/kg b.w), the alterations observed in rat RBCs, Hb, MCHC, platelets, AST, ALT and bilirubin suggest dose selective toxicity of kolaviron extract when repeatedly consumed on a daily basis for a prolonged time. But after the rats were left untreated for 2 weeks, the level of hematological and biochemical parameters restored back to normal.

5. CONCLUSION

In conclusion, the study justified that kolaviron at 100 and 200 mg/kg increased food consumption, body weight and improved hematological indices with hypoglycemic and immunity boosting effects in rats. However, kolaviron at 400 mg/kg b.w has tendency to cause pathological changes in body systems, evidenced by the reduced hemoglobin content, alterations in markers of liver function and histology. Thus, caution must be taken in administering high doses of kolaviron for a prolonged period of time.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “Guidelines for care and use of laboratory animals” [26] were followed, as well as specific national laws where applicable. All experiments have been examined and approval by Animal Ethics Research Committee of the Institute of Public Health, College of Health Sciences, Obafemi Awolowo University, Ile-Ife.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Okwu DE. Phytochemical, vitamins and mineral contents of two Nigerian medicinal plants. International Journal Molecular Medicine and Advance Science. 2005;1(4): 375-381.
2. Adesanya OA, Oluyemi KA, Ofusori DA, Omotuyi IU, Okwuonu CO, Ukwenya VA, Adesanya AR. Micromorphometric and stereological effects of ethanolic extracts of *Garcinia cambogia* seeds on the testes and epididymides of adult wistar rats. International Journal Alternate Medicine. 2007;5(1):1-9.
3. Yakubu, Quadri. Garcinia Kola seeds: Is the aqueous extract a true aphrodisiac in male wistar rats? African Journal of Traditional, Complementary and Alternative Medicine. 2012;9(4):530-535.
4. Hutchinson J, Dalziel JM. Flora of West Tropical Africa. 2nd ed. H.M.S.O; London. 1956;11:295.
5. Akintonwa A, Essien AR. Protective effect of garcinia kola seed extract against parancetamol-induced hepatotoxicity in rats. J. Elhnopharmocol. 1990;29(2):207-211.
6. Iwu MM. Handbook of African Medicinal Plants. CRC Press, London. 1993;183–184.

7. Orie NN, Ekon EU. The bronchodilator effect of Garcinia Kola. East African Medical Journal. 1993;70(3):143.
8. Adesanya OA, Oluyemi KA, Ofusori DA, Omotuyi IU, Okwuonu CO, Ukwenya VA, Adesanya AR. Micromorphometric and stereological effects of ethanolic extracts of *Garcinia cambogia* seeds on the testes and epididymides of Adult Wistar Rats. International Journal Alternate Medicine. 2007;5(1):1-9.
9. Iwu, M. Garcinia kola: A new adaptogen with remarkable immunostimulant, antiinfective and anti-inflammatory properties. A colloquim on Garcinia kola presented in International Conference on Ethnomedicine and Drug Discovery, November 3-5, Maryland, USA, PL-26; 1999.
10. Meserole L. Garcinia kola in clinical therapeutics – present and potential indications as a tonic and therapeutic medicine. A colloquim on Garcinia kola presented in International Conference on Ethnomedicine and Drug Discovery, November 3-5, Maryland, USA. PL 27; 1999.
11. Esimone CO, Nworu CS, Adikwu MU, Odimegwu DC, Ezugwu CO. The effect of a new adaptogen, Garcinia kola seed, on the bioavailability of ofloxacin in humans. Scientific Research Essays. 2007;2(11): 482-485.
12. Iwu M. Biflavanones of garcinia: Pharmacological and biological activities. Progression in Clinical Biological Research. 1986;213:485.
13. Olaleye SB, Ibironke GF, Balogun WO,. Aremu A. Effects of diets containing seeds of garcinia kola (Heckel) on gastric acidity and experimental ulceration in rats. Phytotherapy Research. 1997;11:312–313.
14. Agyili J, Sacande M, Kouame C. Garcinia kola Heckel. Seed leaflet No. 113. Millenium seed bank project, Ghana; 2006.
15. Hussain RA, Owegby AG, Parimoo P, Eatomam PG. Kolavonone, a novel polyisoprenylated benzophenone with antimicrobial properties from fruit of Garcinia kola. J Medi Plan Res. 1982;44: 78-81.
16. Iwu M, Igboko O. Flavonoids of Garcinia kola seeds. J Nat Prod. 1982;45(5):650-651.
17. Iwu MM. Antihepatotoxic constituents of garcinia-kola seeds. Experimentia. 1985;41:699-700.
18. Farombi EO, Akanni OO, Emerole GO. Antioxidant and scavenging activities of flavonoid extract (kolaviron) of garcinia kola seeds. Pharmaceutical Biology. 2002;40(2):107-16.
19. Olaleye SB, Farombi EO, Adewoye EA, Owoyele BV, Onasanwo SA, Elegbe RA. Analgesic and anti-inflammatory effects of kolaviron (A garcinia kola seed extract). African Journal of Biomedical Research. 2000;3:171-174.
20. Farombi EO, Adepoju BF, Ola-Davies OE, Emerole GO. Chemoprevention of aflatoxin B1-induced genotoxicity and hepatic oxidative damage in rats by kolaviron, a natural biflavonoid of garcinia kola seeds. Eur J Cancer Prev. 2005;14(3):207-214.
21. Farombi EO, Møller P, Dragsted LO. Ex-*vivo* and *in vitro* protective effects of kolaviron against oxygen-derived radical-induced DNA damage and oxidative stress in human lymphocytes and rat liver cells. Cell Biology and Toxicology. 2004;20:71-82.
22. Ayensu ES. Medicinal plants of West Africa. Reference Publications, Algonac; 1987.
23. Dhainaut JK, Thiji LG, Park G. Septic shock. Saunders; 2000.
24. Farombi EO. Mechanisms for the hepatoprotective action of kolaviron: Studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride treated rats. Pharmacological Research. 2000;42:75-80.
25. Iwu MM, Igboko OA, Okunji CO and Tempesta MS. Antidiabetic and aldose reductase activities of biflavanones of Garcinia kola. Journal of Pharmacy and Pharmacology. 1990;42(4):290-292.
26. Guide for the care and use of laboratory animals, 2011. 8th ed.
    Available:https://grants.nih.gov/grants/../Guide-for-the-Care-and-use-of-laboratory-animals.pdf
    (Access date: 5.10.15)
27. Braide VB. Pharmacological effects of chronic ingestion of garcinia kola seeds in the rat. Phytotherapy Research. 1990;4, 39-41.
28. Obi AU, Nwoha PU. Effects of kolaviron, the major constituent of garcinia kola, on the histology of the hypothalamus, pituitary, and testes using adult male wistar

rats as a model organism. Forensic Medicine and Anatomy Research. 2014;2: 80-87.

29. Chen MD, Lin PY, Tsou CT, Wang JJ, Lin WH. Selected metal status in patients with non-insulin-dependent diabetes mellitus. Biol Trace Elem Res. 1995;50:119–24.
30. Ayepola RO, Chegou NN, Brooks NL, Oguntibeju OO. Kolaviron, a garcinia biflavonoid complex ameliorates hyperglycemia-mediated hepatic injury in rats via suppression of inflammatory responses. BMC Complementary and Alternative Medicine. 2013;13:363.
DOI: 10.1186/1472-6882-13-363
31. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998;395:763–70.
32. Uko OJ, Usman A, Ataja AM. Some biological activities of Garcinia kola in growing rats. Vet. Arhiv. 2001;71:287-297.
33. Dada AA, Ikuerowo M. Effects of ethanolic extracts of garcinia kola seeds on growth and haematology of catfish (*Clarias gariepinus)* broodstock. African Journal of Agricultural Research. 2009;4(4):344-347.
34. Ahumibe AA, Braide BV. Effect of gavage treatment with pulverized Garcinia kola seeds on erythrocyte membrane integrity and selected haematological indices in male albino Wistar rats. Nigerian Journal of Physiological Sciences. 2009;24(1):47-52.
35. Okoko T, Oruambo FI. Garcinia kola extract reduced lipopolysaccharide activation of macrophages using U937 cells as a model. African Journal of Biotechnology. 2008;7(6):792-795.
36. Sirag HM. Biochemical and hematological studies for protective effect of oyster mushroom (*Pleurotus ostreatus*) against glycerol induced acute renal failure in rats. J Biol Sci. 2009;9(7):746-752.
37. Ambali FS, Onukak C, Idris BS, Yaqub SL, Aliyu MH, Kawu UM. Vitamin C attenuates short-term hematological and biochemical alterations induced by acute chlorpyrifos exposure in Wistar rats. Journal of Medicine and Medical Sciences. 2010; 1(10):465-477.
38. Robak J, Gryglewsk IR. Flavonoids are scavengers of superoxide anions. Biochem. Pharmacol. 1988;37:837–841.
39. Torrel J, Gilliard J, Gilliard P. Anti-oxidative activity of biflavonoids and reactivity with peroxy radicals. Phyto. Chem. 1986; 25:383–386.
40. Adaramoye OA, Akinoloye O. Possible protective effects of kolaviron on CCl4 – induced erythrocyte damage in rats. Biol; Sci. Reposit. 2000;20(4):259-264.
41. Okoko T. *In vitro* antioxidant and free radical scavenging activities of Garcinia kola seeds. Food and Chemical Toxicology. 2009;47:2620–2623.
42. Guyton AC, Hall JC. Text book of medical physiology. W.B Sanders Company. Philadelphia. 2006;966-971.
43. Adedapo AA, Abatan MO, Olorunsogo OO. Effects of some plants of the spurge family on the haematological and biochemical parameters of rats. Vet. Arhiv. 2007;77: 29-38.
44. Dahlback B. Advances in understanding pathogenic mechanisms of thrombophilic disorders. Blood. 2008;112:19-27.
45. Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA, Shay N. Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese zucker rats and murine RAW 264.7 cells. Journal of Nutrition. 2003;133(5):1238-43.
46. Pinent M, Blay M, Bladé MC, Salvadó MJ, Arola L. Grape seed-derived procyanidins have an antihyperglycemic effect in streptozotocin-induced diabetic rats and insulinomimetic activity in insulin-sensitive cell lines. Endocrinology. 2004;145:4985-4990.
47. Naik SR, Fliho JMB, Dhuley JN, Deshmukh A. Probable mechanism of hypoglycaemic activity of basic acid, a natural product isolated from Bumelia sartorum. Journal of Ethnopharmacol. 1999;33:37–44.
48. Waltner-Law ME, Wang XL, Law BK, Hall RK, Nawano M, Granner DK. Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. The Journal of Biological Chemistry. 2002;277:34933–34940.
49. Sarkhail P, Rahmaipour S, Fadyevatan S, Mohammadirad A, Dehghan G, Amin G. Antidiabetic effect of *Phlomis anisodonta*: Effects on hepatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. Pharmacological Research. 2007;56:261–266.
50. Braide VB. Antihepatotoxic biochemical effects of kolaviron, A biflavonoid of

Garcinia kola seeds. Phytotherapy Research. 1991;5:35–37.

51. Farombi EO, Shrotriya S, Surh YJ. Kolaviron inhibits dimethyl nitrosamineinduced liver injury by suppressing COX-2 and iNOS expression via NF κB and AP-1. Life Sciences. 2009;84:149–155.

52. Bush BM. Plasma biochemistry. In: Interpretation of Laboratory Results for Small Animal Clinicians. Oxford: Blackwell Sci. Publications. 1991;221-347.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18946