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The Hypolipidemic Effect of Aqueous Extract of *Crepis rueppellii* and *Rhamnus staddo* on Acetaminophen-induced Hepatotoxicity of Guinea Pigs

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Abstract

Paracetamol (PCM) overdose/abuse because of self-medication is a common occurrence amongst people living in low/middle income countries. The present study was designed to investigate the hypolipidemic of *Crepis rueppellii* (CR) and *Rhamnus staddo* (RS) aqueous extracts in acetaminophen (paracetamol)-treated guinea pigs. Forty four male guinea pigs (350-650g) were randomly assigned into eleven groups of four guinea pigs each. Group I served as the control group. Groups II received 2g PCM/kg body weight (BW) alone, Groups III received 2g PCM/kg BW and 100 mg silymarin /kg BW, groups IV received 100 mg CR leave extract /kg BW alone while group V received 200 mg CR leave extract / kg BW alone. In-group VI, were administered with 2g PCM/kg BW and 100 mg CR leave extract /kg BW. Meanwhile, group VII were administered with 2g PCM /kg BW and 200 mg CR leave extract /kg BW. Groups VIII received 100 mg RS extract /kg BW alone while group IX received 200 mg RS extract alone /kg BW. In group X, guinea pigs were treated with 2 g PCM /kg BW and 100 mg RS extract /kg BW. Meanwhile, guinea pigs in group XI were treated with 2g PCM /kg BW and 200 mg RS extract /kg BW. The treatment lasted for seven days after which sera were harvested and assayed for serum lipid indices using standard methods. The obtained results showed that, the aqueous extract of *C. rueppellii and R. staddo* significant ($P<0.05$) reduced levels of serum total cholesterol, triglyceride, LDL and glucose in the animal model. However, it was unable to produce significant effect on HDL concentration compare between PCM alone. Data from our study suggest that aqueous leave extracts of CR *and* RS possesses probable hypolipidemic effects.

Keywords: Acetaminophen, *Crepis rueppellii*, *Rhamnus staddo*, Serum lipid indices; Hypolipidemic activity.

Introduction

Acetaminophen, known as paracetamol (PCM), is most often classified as a mild, over-the-counter analgesic used in the treatment of pains/headaches. It is mostly intentionally abused. The drug is generally safe when taken in recommended doses, though even a very small overdose could be deleterious. In the United States of America, PCM overdose has been reported to account for more calls to the poison control center than an overdose of any other pharmacological substance **(Lee, 2004).** It is the number one drug of choice in managing pains globally. However, its mechanism of action in relieving pain is yet to be fully elucidated but suggested to be implicated in a number of pain pathways **(Sharma and Mehta, 2014)**

PCM metabolism in the liver could result in the formation of a highly toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI) by the cytochrome P450 enzyme system **(Kaplowitz, 2004)**. Further detoxification to eliminate the metabolite is accomplished by its conjugation with glutathione, but in cases of overdose or abuse, glutathione stores are depleted resulting in the accumulation of the metabolite and eventual toxicity **(Kaplowitz, 2004)**. Abuse of the drug may lead to toxicity, which could result in hepatocellular necrosis and kidney damage **(Ramadan and Schaalan,2011**). Oxidative stress mediated action of NAPQI accumulation has been implicated in the pathogenesis of PCM -induced liver and renal damage in experimental animals **(Ramadan and Schaalan, 2011**). PCM in third world countries has been on the increase since a greater proportion of the population tends to resort to self-medication. The non-availability of standard health facilities poses another challenge of the arbitrary use of ethno botanicals instead of synthetic drugs to treat complications of PCM abuse.

Medicinal plants have played an important role in the abatement of toxic substances in the human

body. They also function as vital hypolipidemic agents **(Luo *et al.*, 2004).** The *Crepis rueppellii* belongs to the Asteraceae family. *C. rueppellii* is a narrow endemic species of southern Greece. *C. rueppellii* is well known for its abundance in sesquiterpene lactones. In total, from its aerial parts nine compounds were isolated for the first time, i.e. three guaianolides, grosheimin, crepiside E, crepiside D; two germacranolides, taraxinic acid and its 1′-O-β-d-glucopyranosyl ester, the nor-isoprenoid (3S, 5R)-loliolide and three flavonoids, luteolin, luteolin 7-O-β-d-glucopyranoside and quercetin 3-O-β-d-glucopyranoside. Furthermore, the root extract afforded two triterpenes, oleanolic acid and lupeol. The structures of the isolated compounds were elucidated by high-field NMR spectroscopy **(Christina *et al.*, 2018).** *Rhamnus staddo* (Rhamnaceae) has been traditionally used in East Africa to treat malaria and venereal. Methanol extract of the plant has been reported to show significant antiplasmodial activity and its *in vivo* antimalarial activity in mice against a chloroquine (CQ)-tolerant plasmodium berghei NK65, was recently reported. Methanolic extract of *R. staddo* had statistically significant parasitaemia suppressions of 31.7–59.3% and in combination with CQ; it gave statistically significant and improved suppressions ranging from 45.5 to 85.1%. In a search for potential antimalarial candidates from the plants traditionally used to cure malaria in Africa, a new bianthraquinone,1,1',8,8'-tetrahydroxy-6-methoxy-3,3'-dimethyl-[2,2'bianthracene]9,9',10,10'-tetraone together with known anthraquinone, flavonoids and benzofuranone were isolated from *R. staddo* and characterized using spectroscopic techniques **(Rainer and Narel, 2020)** However, there is scanty information on the probable lipid lowering potentials of *C. rueppellii and R. staddo* extracts in acetaminophen-treated guinea pigs. This study was therefore designed to investigate this information gap and recommend its use as alternative therapy in acetaminophen toxicities.

Materials and Methods

2.1 Collection of plants and identification

Green leaves of *C. rueppellii* were collected from filed of Ibb University filled; Ibb, Yemen, and green leaves of *R. staddo* were collected from Alross filed, Oden Village, Ibb, Yemen. The identification and authentication of plant specimen was done by Dr. Esam Aqlan, Assistant Professor of Plant Taxonomy and Flora, Department of Biology, Faculty of Sciences, Ibb University. A voucher specimen was deposited at the Biology Herbarium, Faculty of Sciences, Ibb Universirt under the code CM202115.

2.2 Plant extraction

The collected fresh plants material were washed thoroughly with tap water to get rid of filth and dried in the oven at a temperature of (40 °C) until the leaves became brittle. Once dried, the leaves were crushed coarsely and powdered using a blender. 8.6 grams of leave powder and 1000 mL of distilled water were taken. The extraction was repeated 3 times, then the glass beaker was placed from the electric shaker for 24 hours, then the extract was filtered and the water was removed by evaporating the extract in an electric oven at a temperature of 40 °C ° to obtain the extract powder.

2.3 Experimental protocol

All procedures involving the use of laboratory animals were reviewed and approved by Institutional Animal Ethics Committee of Ibb University-Yemen. Forty four male guinea pigs (350-650g) were obtained from the animal house of Biology Department, Ibb University-Yemen. The animals were housed in a controlled environment with room temperature and a 12-h light-dark cycle to accommodate with free access to feed and water *ad libitum*. Guinea pigs were randomly divided into eleven groups containing 4 animals of each and all treatments were given daily for seven days. PCM at level 2g/kg BW and plants extract at 100 and 200 mg /kg of BW were administered orally. Guinea pigs in group I served as the control group and were administered distilled water only. Groups II received 2g/kg BW PCM alone, Groups III received 2g PCM /kg BW and 100 mg/kg BW silymarin, Groups IV received 100 mg/kg *C. rueppellii* aqueous leave extract alone while Group V received 200 mg/kg *C. rueppellii* aqueous leave extract alone. In Group VI, were administered with 2 g PCM /kg BW and 100 mg/kg *C. rueppellii* aqueous leave extract. Meanwhile, in Group VII pigs were administered with 2g PCM /kg BW and 200 mg/kg *C. rueppellii* aqueous leave extract. Groups VIII received 100 mg/kg *R. staddo* extract alone, while group IX received 200 mg/kg *R. staddo* extract alone. In group X, Guinea pigs were treated with 2 g PCM /kg BW and 100 mg/kg *R. staddo* extract. Meanwhile, pigs in group XI were treated with 2g PCM /kg BW and 200 mg/kg *R. staddo* extract. On day 8, all animals were anaesthetized with chloroform and blood was collected.

2.4 Sample preparation

The blood sample putted in tubes does not contain heparin to allow the blood clot at the room temperature for thirty minutes. Then to get the serum the blood was centrifuge at 3000 rpm for 15 minutes. The serum samples were collected and saved them at -20 ^{o}C to biochemical analysis. The liver was obtained after the guinea pig was sacrificed, washed in normal saline for histological studies, the liver was preserved in 10% formalin solution.

2.5 Lipid profile biochemical parameters

Triglycerides, cholesterol were determined by using Roche diagnostic Kits (Germany) according to

Tietz (1995) and HDL-cholesterol, LDL-cholesterol **(Cohn *et al*., 1988).**

2.6 Determination of Glucose

Standard methods were used to estimate glucose **(Tietz, 2006).**

2.7 Histological analysis

Examination of liver histology was performed according to routine histology techniques. Briefly, after the animal was sacrificed, the liver was harvested, rinsed in normal saline, and sectioned into small pieces. The sectioned tissue was then fixed in 10% formalin, dehydrated in stepwise with increasing concentration of ethanol solution (50% to 100%), and embedded in paraffin. Using a microtome, tissue sections of 4-µm thickness were produced, fixed overnight on the slide, subsequently stained with hematoxylin and eosin (H&E), then examined under a light microscope (Olympus BX41, Japan).

2.8 Statistical analysis

Data were expressed as the mean values ± standard deviation (S.D.) for each measurement. The data were also analyzed by two-way analysis of variance (two-way ANOVA) using SPSS (version 20), the test is significant at 5%.

3.1 Effects of *C. rueppellii and R. staddo* extracts on lipid profile of paracetamol - induced hepatotoxicity of Guinea pigs

Compared with the control group, Guinea pigs treated with PCM (PCM group) had significantly increased ($P<0.05$) levels of serum total cholesterol, triglyceride, LDL, while significantly decreased ($P<0.05$) of HDL level (Table 1). Silymarin drug group showed significantly decreased ($P<0.05$) of total cholesterol, triglyceride, LDL, while the level of HDL was insignificantly decreased. Treatment with *C. rueppellii* and *R. staddo* extracts 100 mg/kg and 200 mg/kg BW showed significantly decreased ($P<0.05$) of total cholesterol, triglyceride, LDL, however significantly decreased ($P<0.05$) HDL level compared with the PCM alone group.

3.2 Effects of *C. rueppellii and R. staddo* extracts on glucose level in paracetamol-induced hepatotoxicity of Guinea pigs

The overdose of PCM raised significantly ($P<0.05$) the concentrations of glucose. Treatment with *C. rueppellii* and *R. staddo* extracts 100 mg/kg and 200 mg/kg and silymarin reversed these alterations, significantly decreasing the toxicity of PCM, which noted a significant decrease ($P<0.05$) in glucose level compared with the PCM alone group (Table 1).

Results

Table 1. Effect of *C. rueppellii and R. staddo* extracts on lipid profile and glucose levels in paracetamol induced hepatotoxicity of Guinea pigs

Parameters	Total cholesterol (mg/dL)	Triglycerides (mg/ dL)	HDL (mg/ dL)	LDL (mg/ dL)	Glucose (mg/ dL)
Control	120.25 ± 0.50	127.33 ± 0.44	39.67 ± 0.68	63.00 ± 0.43	109.00 ± 0.81
PCM(2g/kg BW) alone	175.50 ± 0.37$^{\#}$	153.92 ± 0.45$^{\#}$	33.50 ± 0.41 $^{\#}$	105.50±0.88$^{\#}$	153.33 ± 0.71$^{\#}$
PCM(2g/kg BW) + Silymarin(100mg/kg)	141.10 ± 0.55$^{\#*}$	131.33 ± 0.18*	37.10 ± 0.55	77.13±0.73*	116.05 ± 0.66*
CR (100mg/kg)	125.50 ± 0.35*	126.00 ± 0.63*	39.50 ± 0.84*	65.50±0.21*	110.00 ± 0.82*
CR (200mg/kg)	126.00 ± 0.23*	127.48 ± 0.75*	39.78 ± 0.51*	63.00±0.59*	100.75 ± 0.25*
PCM(2g/kg BW) +*CR* (100mg/kg)	152.00 ± 0.81$^{\#*}$	128.67 ± 0.58*	35.67 ± 0.85	86.30±0.59$^{\#*}$	118.00 ± 0.58*
PCM(2g/kg BW) +*CR* (200mg/kg)	158.75 ± 0.50$^{\#*}$	140.17 ± 0.57$^{\#*}$	37.33 ± 0.12	93.00±0.83$^{\#*}$	112.00 ± 0.85*
RS (100mg/kg)	125.25 ± 0.61*	127.90 ± 0.41*	40.10 ± 0.81*	63.00±0.44*	109.00 ± 0.81*
RS (200mg/kg)	124.90 ± 0.64*	127.78 ± 0.87*	39.67 ± 0.65*	63.25±0.32*	108.00 ± 0.63*
PCM(2g/kg BW) +*RS*(100mg/kg)	145.50 ± 0.20$^{\#*}$	143.70 ± 0.43$^{\#}$	37.42 ± 0.92	90.25±0.13$^{\#*}$	116.66 ± 0.89*
PCM(2g/kg BW) +*RS*(200mg/kg)	140.50 ± 0.35$^{\#*}$	130.00 ± 0.81*	36.00 ± 0.64	86.00±0.35$^{\#*}$	120.25 ± 0.91*

PCM=paracetamol, *CR*= *C.rueppellii*, *RS*= *R. staddo*, All value represents mean± SD of six animals. # $P < 0.05$ compared with normal control value. * $P < 0.05$ compared with non-treated control values.

3.3 Effect of *C. rueppellii and R. staddo* extracts on liver histopathology of paracetamol- induced hepatotoxicity of Guinea pigs

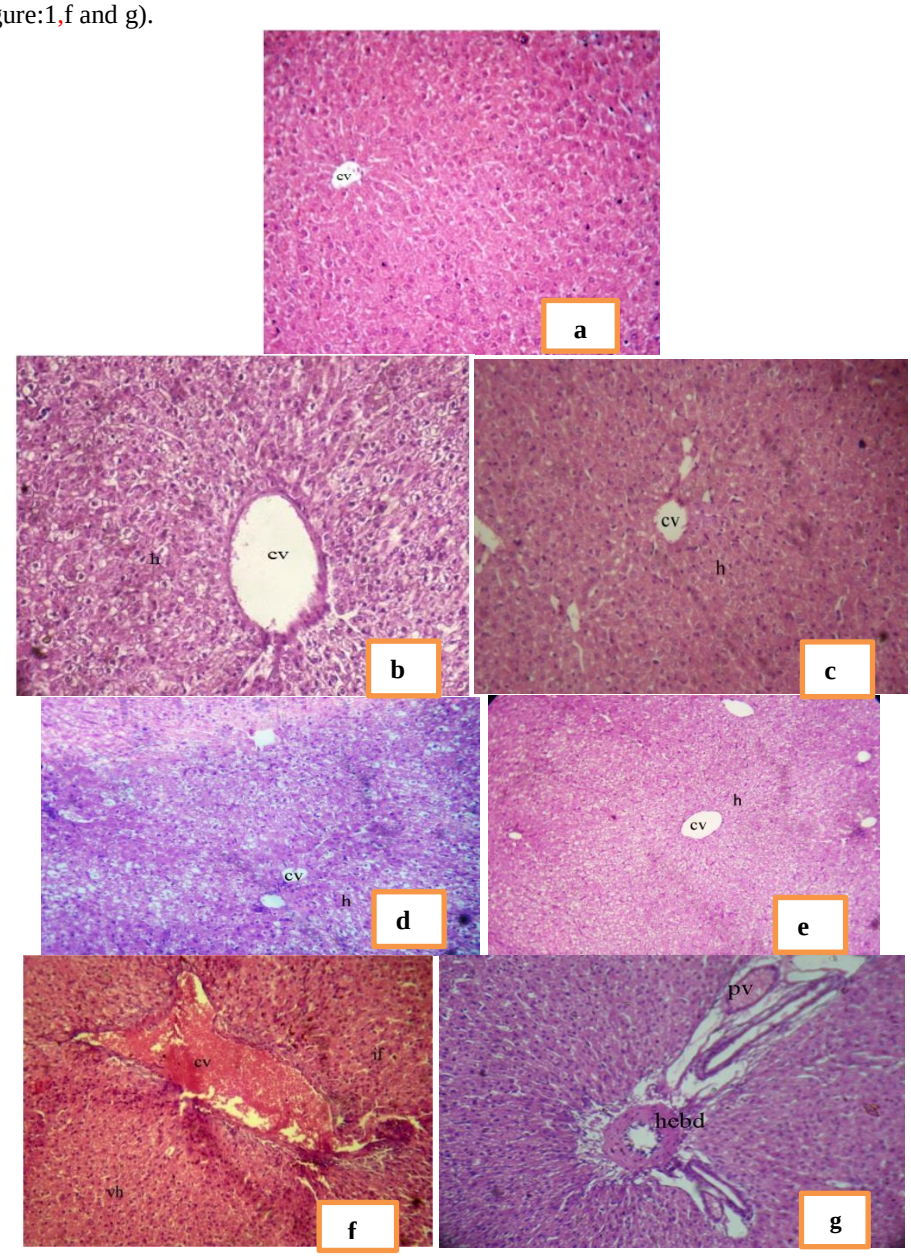
The microscopic examination of the liver of Guinea pigs in the control group showed no microscopic changes. The hepatocytes appeared as polygonal cells with rounded nuclei and there is a few spaced hepatic sinusoids with fine arrangement of Kupffer cells. In addition, it appear normal lobular architect with hepatocyte arranged in cords encircling the central vein (Figure1, a).

After seven days the microscopical findings PCM intoxicated liver tissue showed large area of haemorrhagic necrosis around centrilobular region and inflammatory cell infiltration, dilatation of portal vessel and hyperplasia of the lining epithelium of the bile duct (Figure:1,f and g).

The microscopic examination of the liver in groups treated with silymarin (100 mg/kg) revealed mild congestion of central vein, necrosis some liver cells (Figure 1, h).

The examined liver of Guinea pigs treated with 100 and 200 mg/kg CR treatment on PCM intoxicated liver tissue showing mild sinusoidal congestion of central vein and moderate degree necrosis some liver cells (Figure:1, i and j).

The examined liver of Guinea pigs treated with 100 and 200 mg/kg RS treatment on PCM intoxicated liver tissue showing mild sinusoidal congestion of central vein and moderate degree necrosis some liver cells (Figures:1, k and l).



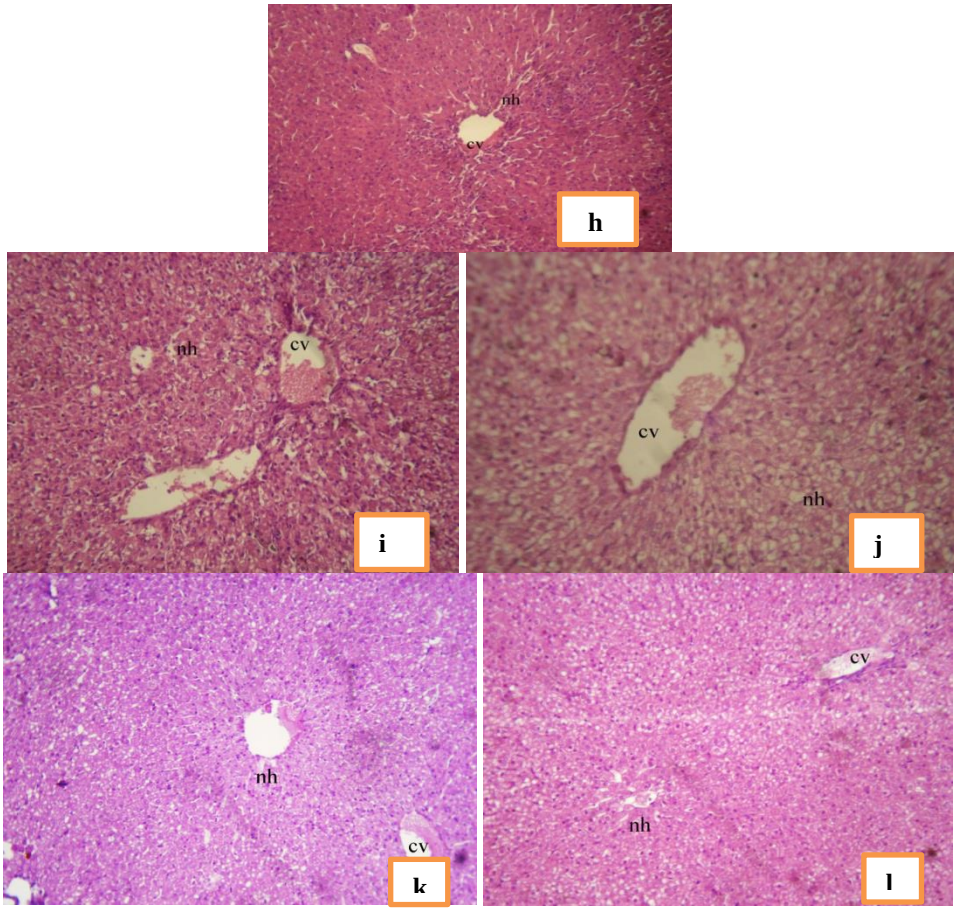


Fig.1: Histopathological study of liver tissue of guinea pigs. **(a)** Control group given (2 mL/kg)distilled water for seven days showed normal liver architecture(H&E stain ×200), **(b, c, d and e)** group given CR (100 and 200 mg/kg) and RS (100 and 200 mg/kg) showed normal liver architecture, the hepatocytes appeared as polygonal cells with rounded nuclei and there is a few spaced hepatic sinusoids with fine arrangement of Kupffer cells(H&E stain ×200), **(f and g)** PCM intoxicated liver tissue showed large area of haemorrhagic necrosis around centrilobular region and inflammatory cell infiltration (**I** and **f**), dilatation of portal vessel (pv) and hyperplasia of the lining epithelium of the bile duct (hepd),**(h)** effect of 100 mg/kg silymarin treatment on PCM intoxicated liver tissue showing mild congestion of central vein(cv), necrosis some liver cells (nh) (H&E stain x 100), **(i)** effect of 100 mg/kg CR treatment on PCM intoxicated liver tissue showing mild sinusoidal congestion of central vein and moderate degree necrosis some liver cells (nh), **(j)** effect of 200 mg/kg CR treatment on PCM intoxicated liver tissue showing mild sinusoidal congestion of central vein and moderate degree necrosis some liver cells (nh), **(k)** effect of 100 mg/kg RS treatment on PCM intoxicated liver tissue showing mild sinusoidal congestion of central vein and moderate necrosis some liver cells (nh), **(l)** effect of 200 mg/kg RS treatment on PCM intoxicated liver tissue showing mild sinusoidal congestion of central vein and moderate necrosis in some liver cells (nh).

Discussion

Lipid profile/ lipid panel is a panel of blood tests **(Umoren *et al*., 2020)** that serves as an initial screening tool for abnormalities in lipids **(Fahy *et al*, 2009)** such as cholesterol and triglycerides **(IUPAC, 2019).** The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease **(Go *et al*., 2013)** certain forms of pancreatitis **(Yadav and Lowenfels, 2013)** and other diseases **(Fahy *et al*., 2009).**

The prevalence of drug abuse because of overdose has been increasing, especially in economically deprived communities in the developing countries where there is little or no access to standard health facilities and the majority of the population resort to self-medication **(Bennadi ,2014).** In the present study, the effect of *C. rueppellii* and *R. staddo* leaves extracts on some selected lipid biomarkers in paracetamol-treated Guinea pigs was reported. The extracts of *C. rueppellii* and *R. staddo* show potent lipid lowering activity, which is similar to the pattern in a study by **Iwara *et al.* (2015)**. Acetaminophen poisoning may be due to ingestion of excessive/repeated or too frequent doses **(Kett *et al*., 2011).** *C. rueppellii* and *R. staddo* are safe because its dose at 100 and 200 mg/kg BW in mice showed no toxicity from our studies. The animal model groups treated with 100

mg/kg BW and 200 mg/kg BW of the *C. rueppellii* and *R. staddo* and standard drug (silymarin) showed significantly lower total cholesterol, LDL, triglycerides, as well as increased HDL levels. This finding is in agreement with those reported by **Iwara *et al*. (2015)** where the extract was administered to rats it exhibited a lipid lowering effect. Oxidative stress conditions will often trigger lipid peroxidation as represented by increased levels of malondialdehyde concentrations. The metabolite of acetaminophen has been known to be a depleting agent of the glutathione pool, the body's antioxidants defense system. The administration of aquous extracts of *C. rueppellii* and *R. staddo* mimics the replenishing potentials of glutathione, thus, preventing free radical generation following overdose of PCM.

The increased HDL-C levels noticed in treatment groups indicates a possibly lower risk of developing coronary heart diseases and other related cardiovascular events. **Nichols *et al*. (2007)** reported that a moderate rise in HDL-C levels, resulting from the use of statin drugs, has been linked to a corresponding decline in the risk of developing coronary atherosclerosis. **Stocker and keaney (2004)** also reported that lowering LDL-C was a more effective method of reducing the risk of developing cardiovascular diseases than surgical methods. However, the abundance of (flavonoids) antioxidants in the plants (*C. rueppellii* and *R. staddo*) could prevent the oxidation of LDL-C within the blood vessels. It is a known fact that LDL-C is virtually harmless till it is oxidized by reactive species in the blood vessels, resulting in atherosclerosis **(William Boden, 2007).**

Obtained results are in agreement with the hypolipidemic effect of *V. calvoana* extracts in a diabetic rat model as reported by **Iwara *et al*. (2015),** which implicated flavonoids and other bioactive principles for the effects.

Conclusion

The current findings showed that the aqueous extract of *C. rueppellii and R. staddo* reduced levels of serum total cholesterol, triglyceride, LDL in the animal model. However, it was unable to produce significant effect on HDL concentration–very important cholesterol required in high level to maintain homeostasis inside the body this may be due to the challenge on the liver as a result of the PCM abuse. Conclusively, it may be adduced that the presence of bioactive constituents viz; luteolin, triterpenes, lupeol, flavonoids, anthraquinone and free radical scavenging properties in *C. rueppellii and R. staddo* plants enabled a hypolipidemic effect on the animals despite challenge on the liver. However, it is recommended that future studies be undertaken to ascertain the mechanism of action of this extract as the scope of this study was limited to lipid profile level estimation.

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التأثير الخافض لدهون الدم للمستخلص المائي لنبات المرار ونبات الشخط على السمية الكبدية التي يسببها الأسيتامينوفين في خنازير غينيا

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يتم استخدام عقار الباراسيتامول (الأسيتامينوفين) كمسكن للألم للمرضي في البلدان متوسطة الدخل بدون وصفة طبية وقد تكون الجرعات اعلي من الموصي بها. في هذه الدراسة تم تقييم التأثير الخافض للدهون للمستخلص المائي لأوراق نبات المرار ونبات الشخط المستحدث بواسطة الباراسيتامول في ذكور خنازير غينيا. تم استخدام 44 من ذكور خنازير غينيا بوزن يتراوح بين 350−650 جرام، حيث تم تقسيمها الي 11 مجموعة في كل مجموعة 4 من ذكور خنازير غينيا ، المجموعة الاولي مجموعة الكونترول, المجموعة الثانية اعطيت الباراسيتامول (2جم/ كجم) فقط , المجموعة الثالثة اعطيت الباراسيتامول (2جم/ كجم) والسليمارين (100ملجم/كجم), المجموعة الرابعة اعطيت المستخلص المائي لأوراق نبات المرار بتركيز(100ملجم/كجم), المجموعة الخامسة اعطيت المستخلص المائي لأوراق نبات المرار بتركيز(200ملجم/كجم), المجموعة السادسة اعطيت الباراسيتامول (2جم/ كجم) و المستخلص المائي لأوراق نبات المرار بتركيز(100ملجم/كجم),المجموعة السابعة اعطيت الباراسيتامول (2جم/ كجم) و المستخلص المائي لأوراق نبات المرار بتركيز(200ملجم/كجم), المجموعة الثامنة اعطيت المستخلص المائي لأوراق نبات الشخط بتركيز(100ملجم/كجم), المجموعة التاسعة اعطيت المستخلص المائي لأوراق نبات الشخط بتركيز (200ملجم/كجم), المجموعة العاشرة اعطيت الباراسيتامول (2جم/ كجم) والمستخلص المائي لأوراق نبات الشخط بتركيز(100ملجم/كجم),المجموعة الحادية عشر اعطيت الباراسيتامول (2جم/ كجم) و المستخلص المائي لأوراق نبات الشخط بتركيز(200ملجم/كجم), فترة التجربة سبعة ايام ثم تم اخذ مصل الدم لإجراء فحوصات دهون الدم. توصلت دراستنا الي ان المستخلص المائي لأوراق نبات المرار والشخط ذو قيمة معنوية ($P<0.05$) حيث قلل من مستويات الكولسترول الكلي والدهون الثلاثية والبروتينات الدهنية منخفضة الكثافة والجلوكوز في مصل الدم لحيوانات التجربة. بينما لم تلاحظ اي تأثيرات على مستوي البروتينات الدهنية عالية الكثافة. من خلال نتائجنا في الدراسة نجد ان المستخلص المائي لأوراق نبات المرار والشخط ذات تأثيرات معنوية خافضة لدهون الدم.