

Nephroprotective Activity of Ethanolic Leaves Extract of *Vachellia origena* (Hunde) Kyal.

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Research Article

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Abstract

Vachellia origena is a medicinal plant distributed in J. Saber, Alturba, Ibb, Dhamar, Sanaa, Haraz, Shibam, and Yafea. They are used medically to treat many diseases, such as antimicrobial, antifungal and antioxidant. The aim of this study was to investigate the effect of *V. origena* ethanolic leaves extract on biochemical parameter activities and protection against histopathological changes of kidneys induced by paracetamol in male guinea pigs. 28 male guinea pigs (350–650 g) were randomly assigned into seven groups of five guinea pigs each. Group I served as the control group. Group II received PCM (500 mg/kg) alone, Group III received PCM and proxamol (0.8 mg/kg), Group IV received 100 mg/kg *V. origena* leaves extract alone, and Group V received 200 mg/kg *V. origena* leaves extract alone. In group VI, we were administered PCM (500 mg/kg) and 100 mg/kg *V. origena* leaves extract. Meanwhile, in group VII were administered with PCM (500mg/kg) and 200 mg/kg *V. origena* leaves extract. The treatment period lasted for ten days, after which sera were harvested and assayed for serum kidney indices using standard methods. Obtained results showed that the PCM-only group increased level of serum urea and creatinine and decreased levels of total protein and albumin. Also, it caused many histopathological changes. While treatment with the ethanolic leaves extract of *V. origena* (100 mg/kg and 200 mg/kg) prevented the kidney from intoxication induced by PCM and decreased the level of histopathological lesions in guinea pigs. Data from our study suggest that the ethanolic extract of the leaves of the plant *V. origena* has protective effects against PCM-induced kidney toxicity.

1. INTRODUCTION

Paracetamol (PCM) or acetaminophen ($C_8H_9NO_2$) (N-acetyl-p-aminophenol) is an analgesic and antipyretic drug, and it has almost no anti-inflammatory properties ^[1]. Paracetamol is generally considered safe for human use at recommended doses; potentially fatal liver damage occurred when an acute overdose was used or even, in rare cases, when normal doses were taken by certain individuals ^[2]. An overdose of PCM is known to cause hepatotoxicity and usually triggers nephrotoxicity. Renal insufficiency is reported to occur in 1–2% of patients exposed to PCM toxicity ^[3]. After oral administration, about 63% of paracetamol is metabolized via glucuronidation and 34% via sulfation, primarily in the liver. The water-soluble metabolites consisting of these metabolic pathways are excreted via the kidney. N-acetyl-p-benzoquinone imine (NAPQI) is a reactive intermediate that occurs when oxidation of 55 percent of PCM takes place by the microsomal P-450 enzyme system. NAPQI is detoxified by intracellular GSH in therapeutic doses ^[3]. Accordingly, NAPQI has been implicated as the responsible metabolite of PCM toxicity ^[4]. In PCM overdosing cases, glutathione stores are depleted, and a rapid increase in the concentration of NAPQI causes necrosis ^[5]. Additionally, this condition results in the production of massive metabolites, causing large amounts of unbound reactive species. PCM toxicity creates acute tubular necrosis, which is one of the main causes of acute renal failure. Serum urea and creatinine levels may be indicators of acute tubular necrosis induced by PCM ^[3]. Free radicals are produced by exposure to drug toxicity in an organism, and oxidative damage plays an important role

in PCM-induced hepatorenal injuries ^[6]. Therefore, natural compounds having antioxidant activity could be used for alternative treatments of PCM toxicity ^[3].

The genus *Vachellia* Wight & Arn. (*Acacia* Mill.) is one of the largest genera in the family Fabaceae. It includes about 1350 species, which are grouped into three subgenera (*Acacia*, *Aculeiferum* and *Phyllodineae*). The *Acacia* is essentially a tropical tree, but it also extends into the subtropical. Around 1000 species are found in Australia, and 170 species were found to be native to Africa and among them, 18 species are widespread and 152 are endemic to the African continent ^[7–8], Eritrea, Ethiopia and Saudi Arabia ^[9]. The genus *Acacia* Mill. is represented in Yemen by 32 species: 5 introduced and cultivated as ornamental trees (*A. auriculiformis*, *A. calcicola*, *A. cyanophylla*, *A. Cyclops* and *A. farnesiana*); 25 natives to the mainland, including *A. origena*; and 2 endemics to Socotra Island, *A. sarcophylla* and *A. pennivenia* ^[10].

Vachellia origena Hude (synonym: *Acacia origena*) ^[11] is a flat-topped tree up to 12 m tall with a yellow-brown, papery peeling of layers; paired, straight spines; yellow-brown branchlets; bipinnate leaves; 10–25 pairs of leaflets; flowers in globular cream heads; and glabrous green-brown pods ^[10]. It is widespread on the escarpment above 1900 m and also located on mountains around the high plateau from 2300 m to 2900 m; thus, it grows at altitudes higher than any other *Acacia* in Yemen ^[10]. Based on previous studies, *Vachellia origena* trees have medicinal value since they contain several phytochemicals, such as phenols, flavonoids, tannins, saponins, alkaloids, steroids, and carbonyls ^[12]; therefore, they are used medically to treat many diseases, such as antioxidants ^[13] and antibacterial ^[14]. In conventional systems, *V. origena* is an interesting plant. Nevertheless, there have been no published scientific investigations on the nephroprotective benefits of *V. origena*, and its chemical components might be helpful in the management of nephrotoxicity. The current study was conducted to investigate the nephroprotective effect of ethanolic extracts of the *V. origena* leaves plant against nephrotoxicity induced by paracetamol.

2. MATERIALS AND METHODS

2.1. Collection of plant

Green leaves of *V. origena* were collected from Waqash Village, Jiblah, Ibb City, Yemen. The identification and authentication of plant specimens 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 University, under the code CM202115.

2.2. Plant extraction

The collected fresh plant leaf material was washed thoroughly with tap water to get rid of filth and dried in the oven at 40°C until the leaf became brittle. Once dried, the leaves were crushed coarsely and powdered using a blender. 30 g of leaf powder and 3000 mL of 70% ethanol were taken. The extraction

was placed in the electric shaker for 24 hours, then the extract was filtered and the ethanol was removed by evaporating the extract in an oven at 40°C to obtain the extract powder.

2.3. Preliminary phytochemical screening

Phytochemical screening was carried out according to standard methods described in literature sources [15].

2.4. Experimental protocol

All procedures involving the use of laboratory animals were reviewed and approved by the Institutional Animal Ethics Committee of Aljazeera University-Yemen. 28 male guinea pigs (350–650 g) were housed in a controlled environment with room temperature and a 12-h light-dark cycle to accommodate free access to food and water ad libitum. Guinea pigs were randomly divided into seven groups containing 5 animals each, and all treatments were given daily for ten days. Paracetamol (PCM) and plant extracts at 100 and 200 mg/kg of body weight were administered orally. Guinea pigs in group I served as the control group and were administered distilled water only. Group II received PCM (500 mg/kg) alone, Group III received PCM (500 mg/kg) and proxamol (0.8 mg/kg), Group IV received 100 mg/kg *V. origena* ethanolic leaves extract alone, and Group V received 200 mg/kg *V. origena* ethanolic leaves extract alone. In Group VI, we were administered 500 mg/kg PCM and 100 mg/kg *V. origena* ethanolic leaves extract. Meanwhile, Group VII was administered 500 mg/kg PCM and 200 mg/kg *V. origena* ethanolic leaves extract. On day 11, all animals were anesthetized with chloroform and blood was collected.

2.5. Sample preparation

The blood sample put in tubes does not contain any anticoagulant to allow the blood to clot at room temperature for thirty minutes. Then, to get the serum, the blood was centrifuged at 3000 rpm for 15 minutes. The serum samples were taken into clean tubes, and they were saved in the deep freezer for biochemical analysis. The kidney was obtained after the guinea pig was sacrificed, washed for histological studies, and preserved in 10% formalin.

2.6. Biochemical analysis

Serum samples were used to estimate total proteins, albumin, blood urea nitrogen, and creatinine. For all estimations, a diagnostic kit (Span Diagnostics Ltd., India) was utilized following the manufacturer's instructions [16].

2.7. Histological analysis

Examination of kidney histology was performed according to routine histology techniques. Briefly, after the animal was sacrificed, the kidney section was harvested, rinsed in normal saline, and sectioned into small pieces. The sectioned tissue was then fixed in 10% formalin, dehydrated stepwise with increasing concentrations of ethanol solution (50–100%), 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) and then observed under a light microscope (Olympus BX41, Japan).

2.8. Statistical analysis

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

3. RESULTS

3.1. Phytochemical's screening of *Vachellia origena* leaves

Qualitative tests for various phytochemical constituents were carried out on the leaves extracts and the results were presented in Table 1.

Table 1
Phytochemical screening
of alcoholic extracts of *V.
origena* leaves

Phytocjchemical	Result
Flavonoids	+
Tannins	+
Alkaloids	+
Steroids	+
Glycosides	
Triterpenoids	+
Saponins	
(+) Present; (-) Absent	

The preliminary phytochemical screening of *V. origena* ethanolic leaves extracts showed the presence of flavonoids, tannins, alkaloids, steroids, and triterpenoids, while glycosides and saponins were absent.

3.2. Effect of treatment with ethanolic extract of *V. origena* leaves on serum biochemical parameters

PCM administration significantly ($P < 0.05$) elevated the level of blood urea nitrogen and creatinine, which were 36.67 ± 1.69 and 0.73 ± 0.09 mg/dl when compared with the control, 19.00 ± 1.41 and 0.38 ± 0.05 mg/dl. Treatments with Proxamol (standard drug) and *V. origena* ethanolic extracts at 100 and 200 mg/kg significantly decreased ($P < 0.05$) the blood urea nitrogen level to 21.33 ± 0.94 , 24.00 ± 1.94 and 20.67 ± 1.05 mg/dL and creatinine to 0.60 ± 0.01 , 0.50 ± 0.01 and 0.45 ± 0.05 mg/dL, respectively. The levels of total proteins and albumin were significantly reduced ($P < 0.05$) by PCM administration at 3.60

± 0.29 g/dL relative to the control group at 5.18 ± 0.09 g/dL. However, treatment with Proximol (standard drug) and *V. origena* ethanolic extracts at 100 and 200 mg/kg increases the total protein level to 4.93 ± 0.12 , 4.83 ± 0.33 and 4.80 ± 0.08 g/dL and the albumin level to 3.20 ± 0.16 , 3.40 ± 0.28 and 3.40 ± 0.21 g/dL, respectively.

Table 2
Effect of *V. origena* extracts treatment on the biochemical parameters involved in nephrotoxicity brought on by paracetamol.

	Urea (mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Control	19.00 ± 1.41	0.38 ± 0.05	5.18 ± 0.09	3.60 ± 0.29
Paracetamol (PCM)	$36.67 \pm 1.69^{\#}$	$0.73 \pm 0.09^{\#}$	$3.60 \pm 0.29^{\#}$	$2.70 \pm 0.16^{\#}$
PCM + Proximol(0.8 mg/kg)	$21.33 \pm 0.94^*$	$0.60 \pm 0.01^*$	$4.93 \pm 0.12^*$	$3.20 \pm 0.16^*$
<i>V. origena</i> (100 mg/kg)	$19.67 \pm 1.05^*$	$0.38 \pm 0.05^*$	$5.08 \pm 0.38^*$	$3.60 \pm 0.21^*$
<i>V. origena</i> (200 mg/kg)	$18.33 \pm 1.35^*$	$0.38 \pm 0.05^*$	$5.05 \pm 0.26^*$	$3.53 \pm 0.23^*$
PCM + <i>V. origena</i> (100 mg/kg)	$24.00 \pm 1.94^*$	$0.50 \pm 0.01^*$	$4.83 \pm 0.33^*$	$3.40 \pm 0.28^*$
PCM + <i>V. origena</i> (200 mg/kg)	$20.67 \pm 1.05^*$	$0.45 \pm 0.05^*$	$4.80 \pm 0.08^*$	$3.40 \pm 0.21^*$

All value represents mean $\pm$ SD of five animals. $\# P < 0.05$ compared with normal control value. $* P < 0.05$ compared with non-treated control values.

3.3. Effect of *V. origena* extracts on kidney histopathology in PCM- induced hepatotoxicity in guinea pigs

Histopathological examination of kidney sections of the control group showed normal morphology of glomeruli, tubules, blood vessels, and interstitium (Figs. 5.a, b and c). After 10 days of PCM administration, acute renal damage was caused, which included severe congestion of the glomerular tuft and renal blood vessels as well as interlobular blood capillaries. Perivascular and periglomerular leukocytic infiltration were also seen (Fig. 5.d and e). The examination of kidney sections obtained from guinea pigs administered with PCM and the proximal treated group for 10 days showed a normal view with just a little congestion of the renal blood vessels and interlobular blood capillaries. The glomeruli showed congestion of the glomerular tuft with vacuolation of the glomerular endothelium. Shrinkage of some of the renal glomeruli was also seen. The lining epithelium of the renal tubules may be showing necrotic changes compared to the PCM-only group (Fig. 5.f).

The kidney tissue section of the guinea pigs treated with 100 mg/kg ethanol leaves extract of *V. origena* administered with PCM for 10 days showed little histological change when compared to animals of the PCM-only group, such as mild congestion of the glomerular tufts and interlobular blood capillaries. The glomeruli show a mild increase of the glomerular tuft with the presence of eosinophilic debris in the glomerular space. The lumen of renal tubules contained eosinophilic debris (Fig. 5.g). The examination of kidney tissue sections obtained from guinea pigs administered with PCM and 200 mg/kg of *V. origena* ethanol extract for 10 days showed little histological change when compared to animals in the PCM-only group, such as mild congestion of the glomerular tufts and interlobular blood capillaries. The glomeruli show a mild increase of the glomerular tuft with the presence of eosinophilic debris in the glomerular space. The lumen of the renal tubules contained eosinophilic debris (Fig. 5.h.).

4. DISCUSSION

PCM-induced nephrotoxicity has been previously documented by a number of studies^[17]. In this study a clear elevation of urea and creatinine in the PCM-alone group provided evidence that the administration of 500 mg/kg of PCM induced kidney injury. Creatinine is produced from the metabolism of protein in muscles, with most creatinine being filtered out of the blood by the kidney and excreted in urine. In renal disease, serum urea accumulates and causes uremia because the rate of serum urea production exceeds the rate of clearance^[18]. The significantly high blood urea in the PCM-treated group suggests kidney injury. PCM-induced nephrotoxicity is caused by the toxic effect of N-acetyl-p-benzoquinone imine (NAPQI). PCM is oxidized by cytochrome p-450 and produces the reactive intermediate metabolite NAPQI^[19]. Another factor in PCM toxicity is the formation of reactive oxygen species (ROS), especially superoxide anions. The nephrotoxicity caused by ROS and NAPQI is largely counteracted by glutathione in the early stages of toxicity (Miettinen and Bjorklund, 2014). However, after the depletion of glutathione, NAPQI covalently binds with sulfhydryl groups of proteins in later stages of toxicity^[20]. The significant decrease ($P < 0.05$) in serum total protein and albumin in the PCM-treated group (Table 2) could be due to arylation of protein by NAPQI^[20]. Protein content in the blood in *V. origena*-treated groups (100 and 200 mg/kg) was significantly increased compared to the PCM-treated group, providing evidence that *V. origena* may be able to minimize the toxic effect of PCM. Also, the creatinine clearance in urine improved with *Acacia senegal* administration^[21].

The present study showed that proxamol has similar effects in controlling serum biochemical parameters in PCM-induced toxicity in guinea pigs. Also, administration of *V. origena* at 100 mg/kg and 200 mg/kg concurrently with PCM significantly inhibited the rise in kidney injury markers, i.e., urea and creatinine, compared to the PCM-treated group, which revealed the ability of *V. origena* to eliminate creatinine from blood into urine, eventually normalizing creatinine content in the blood, which may be attributed to its protective effect on the cell by the prevention of free radical production. Lipid peroxidation is characterized as the procedure of oxidative debasement of polyunsaturated fatty acids and leads to weakened membrane function and structural integrity. *V. origena* may diminish the level of free radicals responsible for lipid peroxidation and thus decrease the level of malondialdehyde. This reveals that *V.*

origena has the potential of scavenging free radicals and lessening PCM-instigated free-radical damage, which is confirmed by the histological results. Administration of *V. origena* extract improves oxidative stress via numerous mechanisms that incorporate a decreased level of free radicals like superoxide and preservation of total antioxidant capacity through maintaining near-normal activity levels of endogenous enzymatic/non-enzymatic antioxidants. The later impacts might be credited to a higher level of total phenolic contents, flavonoids, tannins, saponins, alkaloids, steroids, and carbonyl in the *V. origena* extract, as displayed by phytochemical examination ^[12].

The biochemical results were also confirmed by the histological findings, which showed that, the oral administration of PCM caused severe damage to the kidney, with tubular degeneration, wide lumen, damaged glomeruli, interstitial vascular congestion, and epithelial degeneration, whereas *V. origena* pretreatment resulted in significant dose-dependent nephroprotection against PCM- induced nephrotoxicity. Most drugs induce renal injuries that affect the proximal tubules, glomerulus, or more distal parts of the nephron ^[22]. The results of this study were in agreement with the results of another study carried out on *Acacia* sp. by Osman *et al.*, (2022) ^[23], who showed that there was a significant ($p < 0.05$) increase in serum creatinine and blood urea levels in the PCM-treated group compared to the control groups due to nephrotoxicity. Treatment with *Acacia senegal* showed a significant decrease in serum urea levels compared to the PCM-treated group. Histopathological examination of the rat kidneys revealed severe degeneration in the PCM-treated group, while there was evidence of significant protection in the plant extract-treated groups against PCM-induced changes. The serum and urine biochemical results and histopathology analysis of the kidney indicated the nephroprotective potential of *Acacia senegal* extract against PCM-induced nephrotoxicity. Also, the results of the present study were in agreement with Hala *et al.*, (2022) ^[24], who found that the administration of *A. senegal* aqueous extract followed by treatment with gentamicin improved the activity of protein and albumin towards the normal values in plant extract-treated groups, while it was reduced significantly in the group treated only with gentamicin. There was a significant elevation in urea and creatinine levels in the gentamicin-treated group, and there was a significant reduction in urea and creatinine levels in the *Acacia senegal*-treated groups at 250 and 500 mg/kg.

The results of the present study provided the first experimental evidence that *V. origena* ethanol extract prevents the kidney parameters, such as urea, creatinine, and total proteins, as well as albumin concentrations, from gradually increasing after induction by PCM and they were kept at mean normal values in comparison with the positive control, which was induced by PCM and caused kidney cell damage.

5. CONCLUSION

According to the results of this study, it is concluded that PCM-induced nephrotoxicity. The treatment of guinea pigs with *V. origena* ethanolic leaf extract and proxamol showed a significant protective effect against PCM-induced kidney injury, which was reflected in the biochemical and histological parameters, providing evidence of the beneficial effect of *V. origena* extract in mitigating the chronic PCM

intoxication in male guinea pigs and might be a potential therapeutic candidate for PCM-induced nephrotoxicity. The ethanolic extracts of the leaves of the plant *V. origena* contain nephroprotective ingredients (flavonoids, tannins, alkaloids, steroids and triterpenoids) that protect from PCM-induced kidney damage, which are rich in antioxidant properties, and in turn may quench the free radicals generated by nephrotoxicity.

Declarations

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Author contributions

Mohammed S. M. Saif and Warda M. A. Kaidama contributed to writing the manuscript and interpreting the data. Abdulrahman A. Alahdal, Ahmed A.A. Al-Nawah, Ali M.A. Alshaweri, Ameen N.A. Al-Hasani, Ayman A.M. Al-Muntasir, Basem A.A. Al-Badwi, Hesham A.S. Shams Aldeen, Mohammed A.M. Mahdi, Osama E.A. Al-Duais, Taher E.S. Al faqeeh and Yasser A.A. Al-Hajj conceived the study. Mohammed S. M. Saif and Warda M. A. Kaidama supervised the study. All authors have read and approved the final version of the manuscript.

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Competing interests

The authors declare no competing interests.

Consent for publication

All authors have reviewed and approved the final manuscript and consented to its publication.

Additional information

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Figures

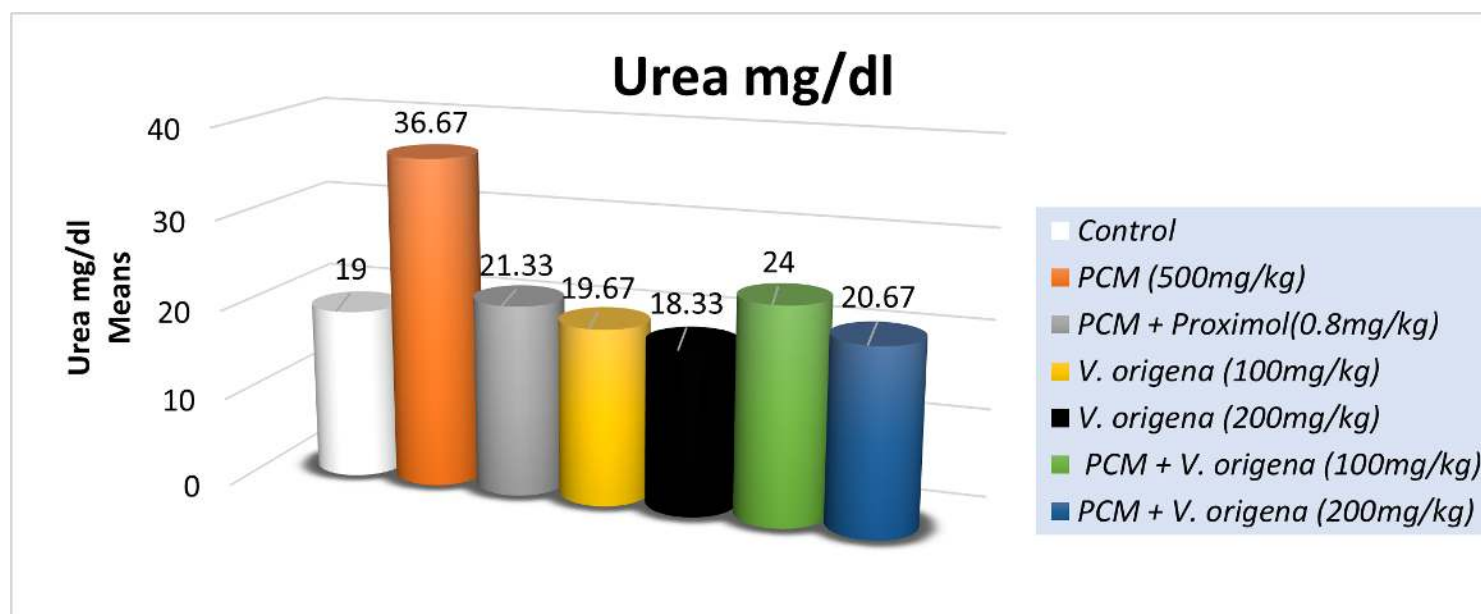


Figure 1

Effect of PCM and *V. origena* extract on urea levels (mg/dl) in PCM- induced

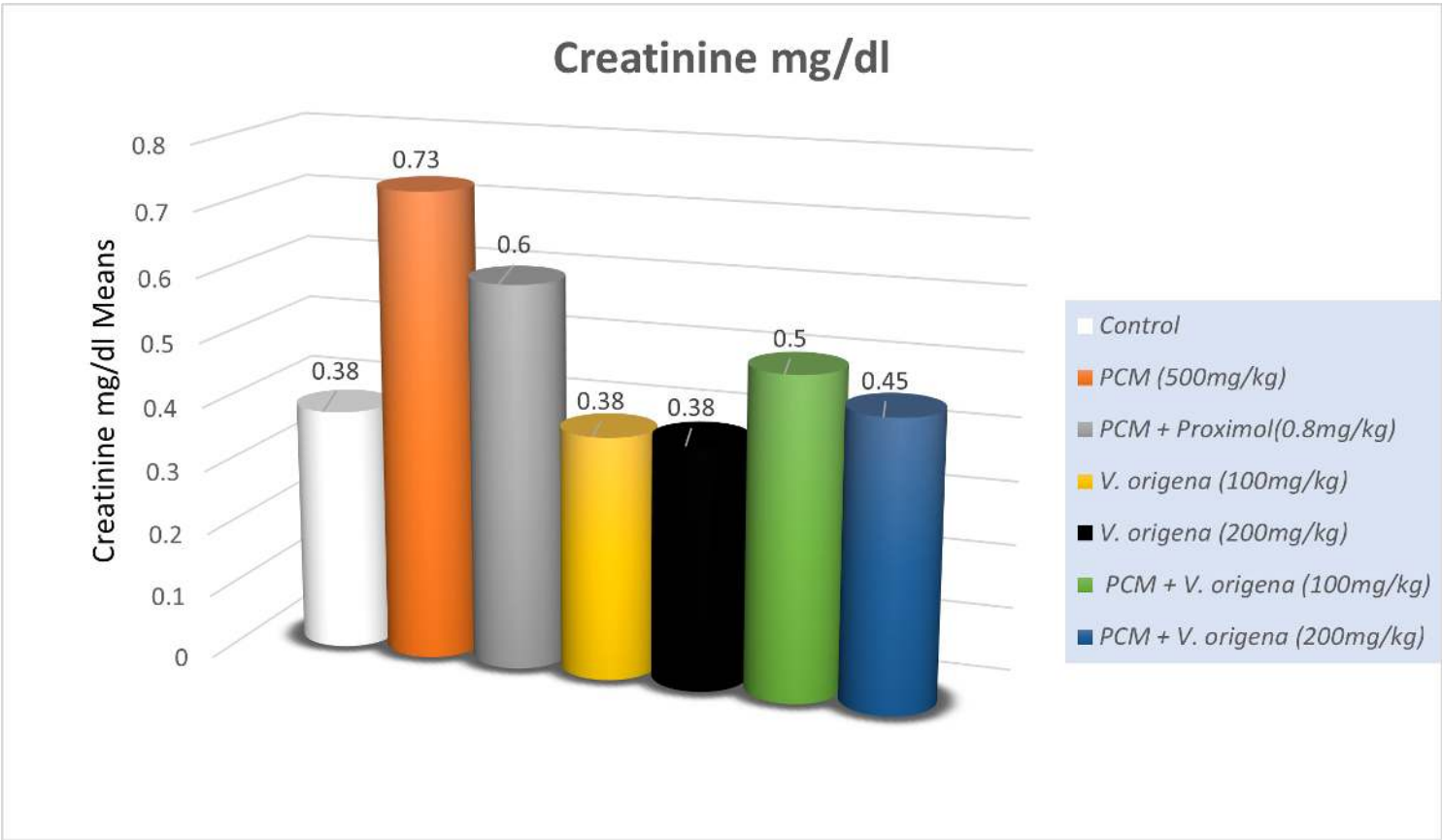


Figure 2

Effect of PCM and *V. origena* extract on creatinine levels (mg/dl) in PCM- induced nephrotoxicity in guinea pigs

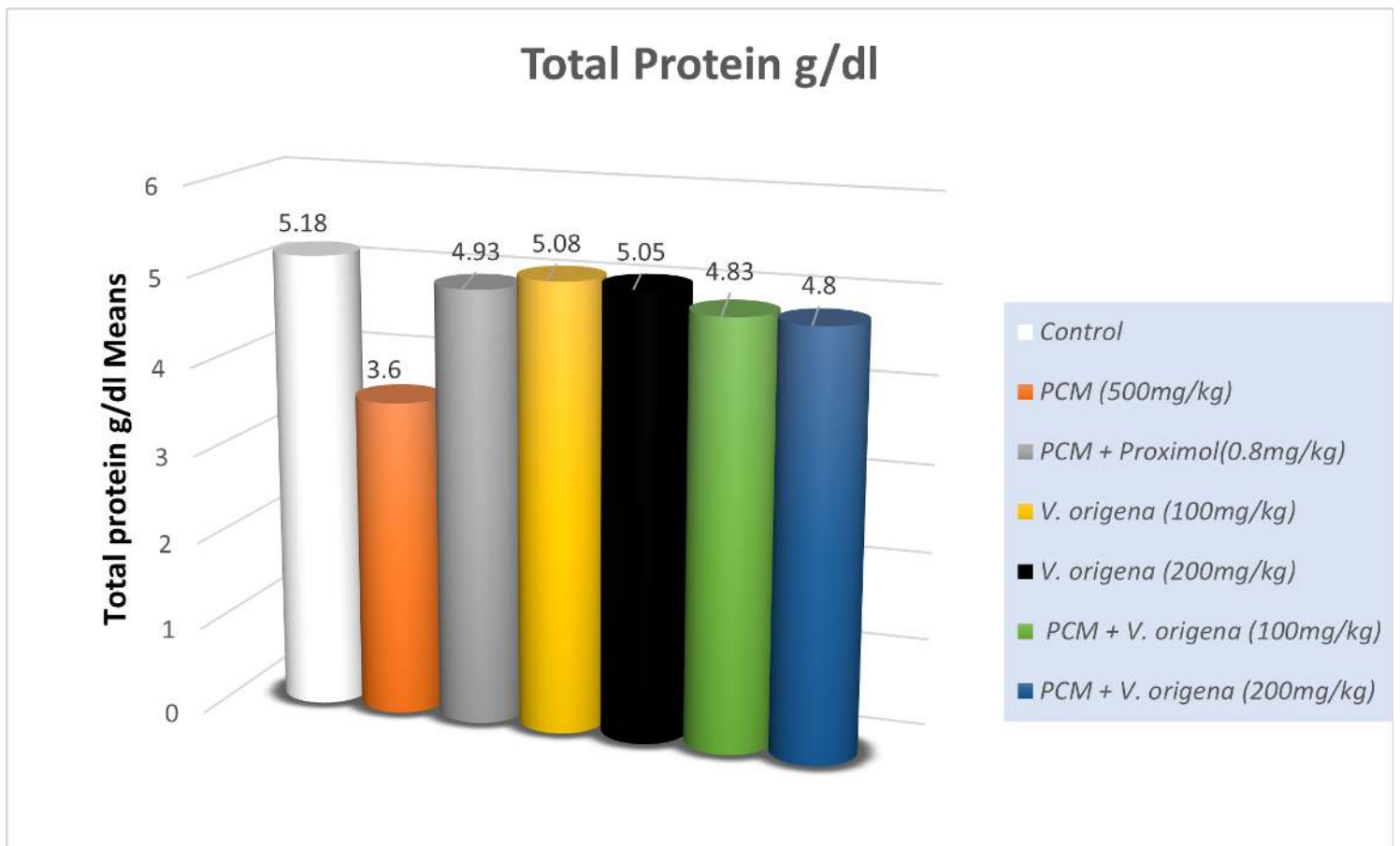


Figure 3

Effect of PCM and *V. origena* extract on total protein (g/dl) in PCM- induced nephrotoxicity in guinea pigs

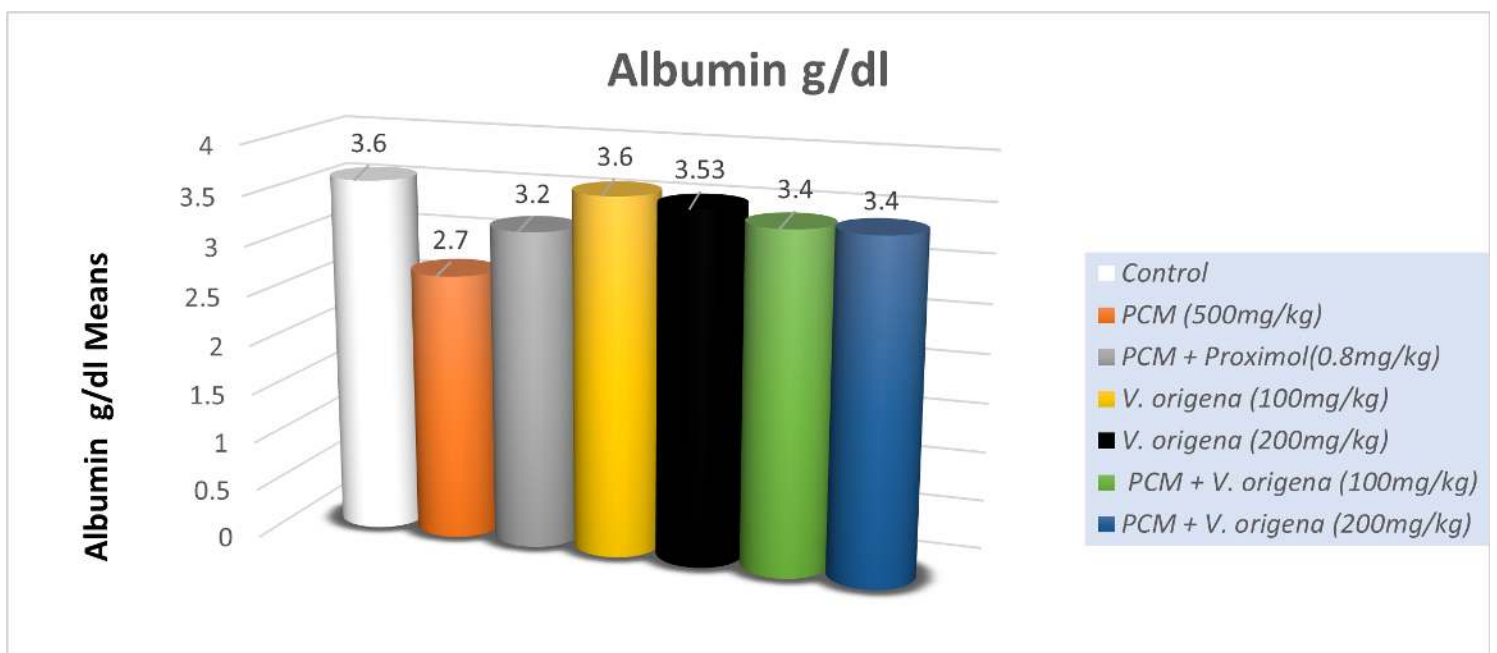


Figure 4

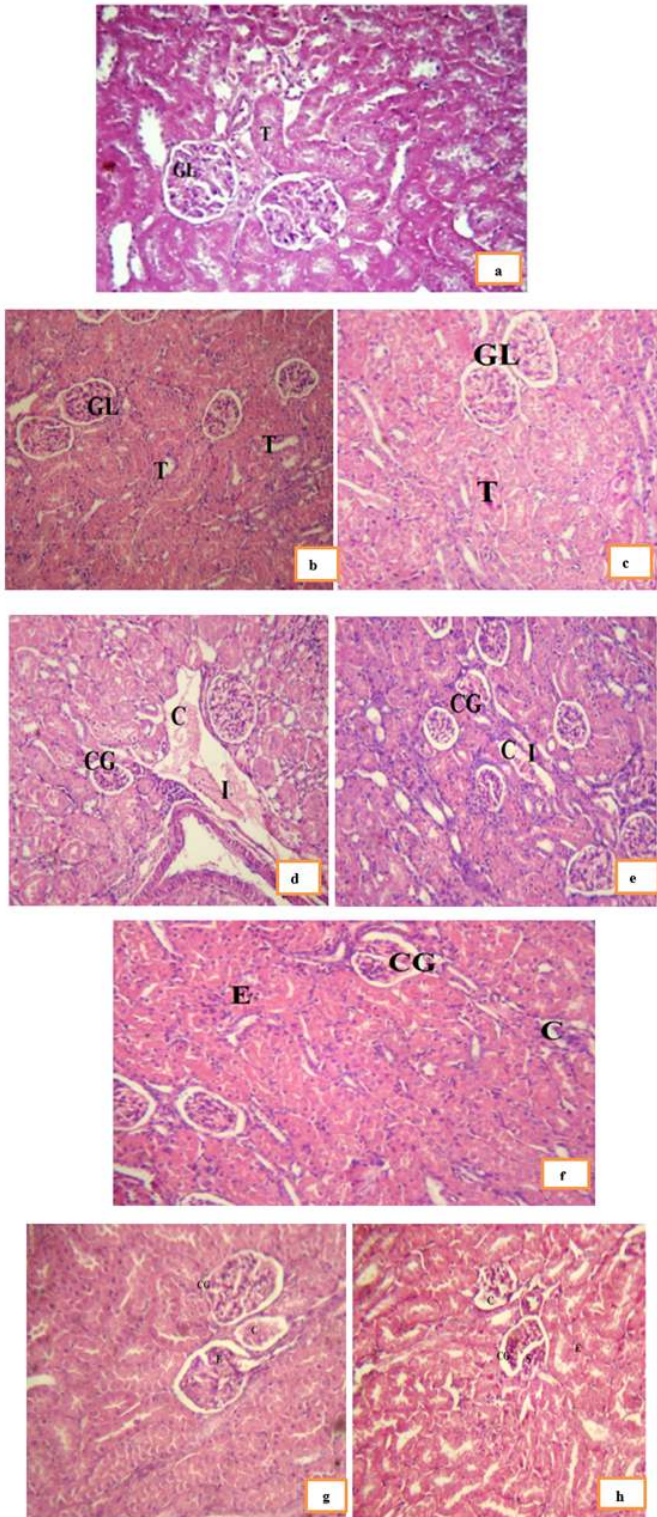


Figure 5

Histopathological study of kidney tissue of guinea pigs. (a) Control group given distilled water for ten days showed normal kidney architecture(H&E×100), (b and c) group given *V. origena* (100 and 200 mg/kg) showed the normal appearance of glomerulus (GL) and renal tubules (T) (H&E×100), (d and e) PCM intoxicated kidney tissue showed shrinking and congestion of the glomerular tuft (CG), renal

tubules and renal blood vessels (C) as well as interlobular blood capillaries. Also, mononuclear leukocytic infiltration(I), (f) effect of Proximol treatment on PCM intoxicated kidney tissue showing little congestion and shrinkage of some of the renal glomeruli (CG) and renal tubules (C). The glomeruli show presence of eosinophilic (E)debris in the glomerular space, (g) effect of 100 mg/kg *V. origena* treatment on PCM intoxicated kidney tissue showing little congestion and shrinkage of some of the renal glomeruli (CG) and renal tubules (C). The glomeruli show presence of eosinophilic (E)debris in the glomerular space, (h) effect of 200 mg/kg *V. origena* treatment on PCM intoxicated kidney tissue showing little congestion and shrinkage of some of the renal glomeruli (CG) and renal tubules (C). The glomeruli show presence of eosinophilic (E)debris in the glomerular space

Supplementary Files

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