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Research Article

Water Extract of Three Aromatic Plants Mixture Ameliorates Paracetamol-Induced Renal-Hepato Damage in Male Albino Rats

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I N F O

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A B S T R A C T

Background and Objectives: This study examined the effects of fruit water extracts of mixture from selected three plants (*Xylopia aethiopica, Coriandrum sativum* and *Anethum graveolens*) on some indices of liver and kidney function tests in the male albino rats, and also to evaluate the antioxidant power of its water extract and the protective effect against paracetamol-induced hepatic and renal toxicity in male albino rats.

Materials and Methods: Three groups of rats were used (control, paracetamol-treated and protected group), which were supplemented with mixture water extract for four weeks followed by intraperitoneal injection of paracetamol. Levels of ALT, AST, ALP, gamma-glutamyl transferase, CAT, GP_x, serum direct bilirubin, serum total bilirubin, serum albumin, serum total globin, total serum urea and serum creatinine, as well as histopathological changes in the liver and kidney were investigated. Quantitative determination of the total phenolic content (TPC) was performed using the Folin-Ciocalteu method, quantitative determination of antioxidant activity was performed according to the β-carotene bleaching method and Diphenylpicrylhydrazyl (DPPH) free radical scavenging assay. The experiment was conducted for two weeks. Statistical analysis was carried out by analysis of variance.

Results: Oral administration of paracetamol recorded significant decrease in TAC, CAT and GPx compared to normal control rats whereas rats supplemented with water extract and then intoxicated with paracetamol showed a significant increase in TAC, CAT and GPx levels compared with paracetamol intoxicated rats, and compared to normal control. AST, ALT, ALP, GGT, total bilirubin, direct bilirubin and indirect bilirubin levels of rats treated with paracetamol were quite higher than that of control group whereas the rats treated with mixture extract and paracetamol had significant reduction when compared with the paracetamol group. Paracetamol induced significant increase in the concentration of plasma urea and creatinine in intoxicated rats as compared to normal control rats, whreas there was no significant difference between mixtures

extract supplemented rats and protected rats as compared with control.

Conclusion: The aqueous extract mixture dose dependably offered potential renal-hepato protection from paracetamol induced hepatic damage, normalizing biochemical parameters in rats.

Keywords: *Xylopia aethiopica, Coriandrum sativum, Anethum graveolens,* Hepato-Renal Protective Activity, Rats

Introduction

Herbal remedies have been used in the management of various diseases from time immemorial. These remedies which are commonly self-medicated are more often with no proper dose regimen. Such indiscriminate use of medicinal plant simply because herbs are natural in origin, without recourse to safety and or adverse effects on biological system is worrisome.[1] Certain medicinal plants and herbs are believed to enhance health and improve resistance against infection through conditioning the body tissues and re-establishing body equilibrium.[2]

Liver diseases have to become one of the major causes of morbidity and mortality all over world. The manifestation of drug- induced hepatotoxicity are highly variable ranging from asymptomatic elevation of liver enzyme to fulminate hepatic failure.[3] Paracetamol, taken in overdoses can causes severe hepatotoxicity and nephrotoxicity.[4] Searching for effective and safe drugs for liver disorder are continues to be area of interest. The hepato-renal activities of many Sudanese medicinal plants have been well investigated.[5-9]

The objectives of this research work were to (i) To assess in vivo and in vitro antioxidant activity of aqueous extract of the three plants and their mixture investigated. (ii) Investigate the hepatoprotective activity of aqueous extract of *Xylopia aethiopica, Coriandrum sativum* and *Anethum graveolens* and their mixture against over dose paracetamol –induced hepatotoxicity. (iii) To observe alteration in the levels of biochemical markers of hepatic damage like GOT, GPT, ALP and GGT in both paracetamol treated and untreated rats. (iv) To confirm the hepatoprotective effect of water extract of the three plants and their mixture studied by histological studies.

Material and Methods

Dry fruits of *Xylopia aethiopica, Coriandrum sativum* and *Anethum graveolens* were purchased from Omdurman market in Khartoum State, Sudan. The dried fruits were grounded and blended at a mixture: (2g Xylopia *aethiopica*, 0.5g *Anethum graveolens* and 0.5g *Coriandrum sativum*)/ 100ml water).

Chemical Tests

- Quantitative determination of the Total Phenolic Content (TPC) was performed using the Folin-Ciocalteu method.[10]
- Quantitative determination of antioxidant activity was performed according to the β-carotene bleaching method[11] and Diphenyl picrylhydrazyl DPPH) free radical scavenging assay.[12]

Biochemical Study

Experimental Design

Twenty one adult male Swiss albino rats with initial weights ranging from 120 to 150g were used as experimental animals for biochemical and histological studies for studying the effect of plant infusion on panadol (paracetamol)-induced free radicals and hepatotoxicity.[13] All animals housed and reared at the premise of the NCR in metal cages for acclimatization perod of a week, and maintained under controlled conditions with temperature (28 ºC), food and water ad libitum. The rats were equally divided into three groups (7 rats in each group) as follows: Group (1) Served as normal control were received distilled water. Group (2) rats were intoxicated after 4 weeks by orally administered with paracetamol (2 g/kg. b.w). Group (3) where rats supplemented with freshly prepared, mixture water extract (30 mg/ ml boiled water) for four weeks (28 days), Then rats were intoxicated by orally administered with paracetamol (2 g/ kg. b,w).

Blood and Tissue Sampling

At the end of the experimental period, blood samples were drawn into heparinized tubes. Plasma was used for determination of liver and kidneys function and for the presence of antioxidant biomarkers. The RBCs were washed several times with cold saline solution. The packed RBCs were stored at -20°C for determination of Glutathione Peroxidase (GPx). The liver was excised and rinsed with cold saline, dried on filter paper, andweighed. A portion of the liver tissue of each rat waskept in 10% formalin for histological and histochemical examinations.

Biochemical Assays

In this study, assay kits were purchased from Elitek Diagnostic (Spain), Boehringer-Mannheim (Germany), Lincer Chemicals (Italy), Stanbio (Spain), Sigma Diagnostic (USA), and RANDOX (USA). Plasma Total Antioxidant

Capacity (TAC), Plasma Catalase (CAT) and cellular GPx levels were determined using the methods of Koracevic*et al*.,[14] Aebi,[15] Paglia and Valentine,[16] respectively. Plasma total protein, albumin, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), γ-Glutamyl Transferase (GGT), total bilirubin and direct bilirubin activities were determined according to the methods described by Gornall*et al.,*[17] Doumas*et al*.,[18] Reitman and Frankel,[19] Belfield and Goldberg,[20] Persijn and Van der Slik,[21] and Walters and Gerarde,[22] respectively. Kidney functions (plasma creatinine and urea) were also assessed according to the method of Bartles*et al.*[23] and Fawcett and Soctt.[24]

Histological Study

To examine the extent of cellular damage caused by paracetamol, the liver samples of experimental and control rats were fixed in 10% formalin saline for 24 h. Following a rinse with water, the tissues were dehydrated in graded series of alcohol, cleanedin xylol and embedded in paraffin wax (58-60°C). Using a rotary microtome, 6-µm-thick sections wereobtained. The sections were deparaffinized in xyleneand hydrated in graded series of alcohol ranging from 100 to 90, 70, 50 and 30% and then in distilled water. Thereafter, the sections were stained with hematoxylin and counterstained with aqueous eosin for microscopicinvestigations.[25] The stained sections were mounted in DPX.

Histochemical Study

The mercury bromophenol blue method was used for the histochemical demonstration of total proteins.[26] The polysaccharide inclusions the periodic acid Schiff (PAS) method was applied for visualization of polysaccharide materials.[27]

Statistical Study

The data presented in the study were statistically evaluated as mean ± SE for each group. Statistical evaluation of the difference between the group mean values was carried out by analysis of variance (ANOVA) analysis. P values less than 0.05 were considered significant.[28]

Results and Discussion

Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) in the mixture aqueous extract was determined by Folin-Ciocalteu reagent and compared with the standard solutions of Gallic Acid Equivalents (GAE). The result is presented as mg GAE/l. The TPC value of the mixture aqueous extract was 1580 mg/ l (Table 1).

In Vitro Antioxidant Activity of Mixture Extract

The radical scavenging activity of the mixture aqueous extract on β-carotene/ linoleic acid and DPPH free radicals increased with increasing concentration of aqueous extract from 50 to 400 µg/ ml. Table 2 illustrate the percentage inhibition of β-carotene linoleate and DPPH free radicals exhibited by the mixture aqueous extract. The mixture extract showed excellent radical scavenging activity, with percentage inhibition at the highest concentration of 400 µg/ ml being 78.2 and 79% according to the β-carotene-linoleate method and DPPH free radicals method, respectively.

In vivo Antioxidant Activity

The effect of water extract of mixture fruits and the administration of paracetamol on some antioxidant biomarkers (plasma Total Antioxidant Capacity (TAC), Catalase Activity (CAT) and cellular Glutathione Peroxidase activity (GPx)) are shown in Table 3. Statistical analysis indicated that oral administration of paracetamol recorded significant decrease in TAC, CAT and GPx compared to normal control rats. On the other hand rats supplemented with water extract and then intoxicated with paracetamol showed a significant increase in TAC, CAT and GPx levels compared with paracetamol intoxicated rats, and compared to normal control, the values of all parameters in the groups near control values.

Table 1.Total Phenolic Content (TPC) in different fruits and their mixture (100 µg/ml)

Parameter	TPC
Mixture	1580

Table 2.Comparison of In vitro Antioxidant activity of mixture water extracts

Scientific name		Concentration				IC_{50} values µg/ml
		50	100	200	400	
Mixture	DPPH method ±	43.5	56	70	79	141.58
	β-carotene method ±	43.5	58	63.5	78.2	73.79
Tertiary Butyl-hydroquinone	DPPH method	76.53	83.75	95.30	99.73	24.43
	β-carotene method	75.2	85	94	99.5	24.32

Liver and Kidney Functions

Table 3 shows activities of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphates (ALP), Gamma-Glutamyl Transferase (GGT) and plasma levels of total proteins, albumin, total bilirubin, direct bilirubin and indirect-bilirubin of all studied groups. The results of liver function tests of the rats revealed that AST, ALT, ALP, GGT, total bilirubin, direct bilirubin and indirect bilirubin levels of rats treated with paracetamol were quite higher than that of control group. In contrast, the rats treated with mixture extract and paracetamol had significant reduction in the levels of AST, ALT, ALP and GGT, total bilirubin, direct bilirubin and indirect bilirubin levels when compared with the paracetamol group. A tendency for decreasing concentrations for each of total proteins albumin were noted in paracetamol intoxicated-rats as compared to normal control. No significant change was found in total protein and albumin levels in rats treated with the mixture extract and paracetamol as compared with paracetamol intoxicated group. Results showed that paracetamol induced significant increase in the concentration of plasma urea and creatinine in intoxicated rats as compared to normal control rats. There was no significant difference in plasma urea and creatinine concentration between mixture extract supplemented rats and protected rats as compared with control.

Paracetamol overdose caused a significant increase (P<0.05) in liver biomarkers (AST, ALT, ALP, GGT, total bilirubin and direct bilirubin), indicating significant liver damage. This finding suggested that mega doses of paracetamol induce the production of free radicals, which cause damage to the hepatocytes of rats. This result correlates with the finding of[29] who demonstrated that the toxicity of paracetamol occurs when it is taken in high amounts. The elevations of plasma liver enzymes indicated liver damage and this correlates with the report of Sai *et al*.,[30] Increased plasma bilirubin levels in paracetamol-intoxicated rats could be looked upon as a compensatory/ retaliatory phenomenon in response to cellular peroxidative changes, which cause damage to the biliary gland. This is because bilirubin functions *in vivo* as a powerful antioxidant, antimutagen, and an endogenous tissue protector.[31]

The significant (P<0.05) reduction in total protein and albumin levels in paracetamol intoxicated rats also indicated cellular damage. The damage produced might be due to the functional failure of endoplasmic reticulum, which leads to a decrease in protein synthesis.[32] The administering albino rats with mixture aqueous extract then intoxicated with paracetamol caused a significant decrease (P≤0.05) in all previous liver function enzymes and they reached near control values. These findings indicated the ability of the extract to protect hepatocytes from oxidative damage

Table 3.The effect of the administration of paracetamol and water extract of the mixture and in vivo antioxidant activity and rat liver and kidney function tests (mean±SD)

Groups	GP 1 Control	GP2 Paracetamol treated	GP3 Protected group
TAC	1.15±0.13	0.92±0.06* (decrement)	1.64±0.25** (Increment)
CAT	386.1±28.02	198.91±19.54* (decrement)	286.94±21.26** (increment)
GPx	0.16±0.03	0.12±0.02* (decrement)	0.17±0.02** (Increment)
ALP	169.09±7.54	240.19±8.49* (increment)	220.29±9.32** (Decrement)
Gamma-Glutamyl Transpeptidase (GGT)	23.20 ±1.63	34.19 ±1.15* (increment)	31.57±0.89** (Decrement)
GPT (ALT)	16.43±1.51	39.57±2.64* (Increment)	32.00 ±2.16** (Decrement)
GOT (AST)	61.57±4.28	85.00±5.48* (increment)	73.29±2.43** (decrement)
Serum Direct Bilirubin	0.14±0.01	0.24±0.02* (Increment)	0.22±0.01** (decrement)
Serum Total Bilirubin	0.34±0.03	0.50±0.02* (increment)	0.43 ±0.02**
Serum Total Protein	6.74±0.39	5.97±0.18* (decrement)	6.09 ±0.22** (Increment)
Serum Albumin	3.15±0.21	2.88±0.19* (decrement)	3.02 ±0.12* (Increment)
Serum Total Globin	162.34±7.740	234.21±8.48* (increment)	214.20 ±9.37** (Decrement)
Total Serum Urea	37.78±1.59	42.69±1.08* (increment)	38.92±1.62
Serum Creatinine	0.71±0.04	0.73±0.03* Increment	1.72±0.03

*Significant at p≤0.05 Paracetamol group versus control group. **Significant at P≤0.05 Paracetamol group versus protected group. All the values were expressed as mean±SD

TAC= Total Antioxidant Capacity, CAT= Catalase, GP_x= Glutathione Peroxidase, ALP= Alkaline Phosphatase; GGT= Gamma-Glutamyl Transpeptidase, GPT (ALT)= Alanine Transferase, GOT (AST)= Aspartate Amino Transferase.

caused by paracetamol overdose. Reduction of bilirubin and elevation of total protein and albumin levels in protected rats indicated stabilized biliary cell function and endoplasmic reticulum leading to bile acid and protein synthesis.[33]

It was noted in the present study that the liver is not the only target organ of paracetamol; it causes free radical generation in other organs such as the kidneys as well. The significant increase in urea and creatinine in paracetamol-intoxicated rats revealed the toxic effect of paracetamol overdose on kidneys. Mixture aqueous extract contains powerful antioxidant components that serve as an extracellular neutralizer of free radicals.

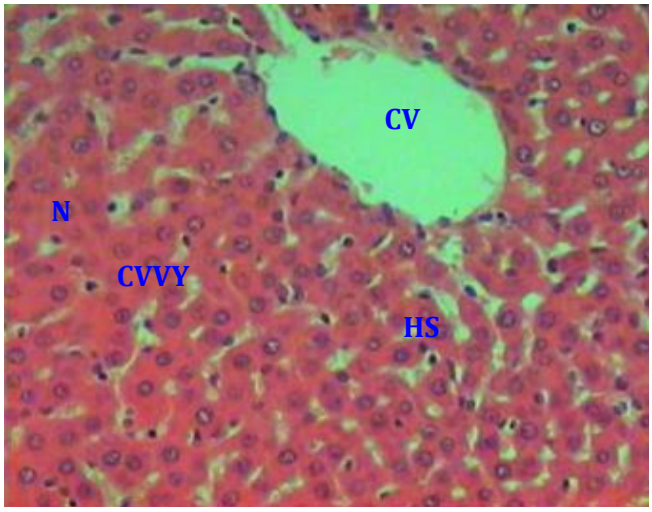

Figure 1.A micrograph of section of control liver showing the architecture of a hepatic lobule. The Central Vein (CV) lies at the centre of the lobule surrounded by the Hepatocytes (HC) with strongly eosinophilic granulated Cytoplasm (CY), and distinct Nuclei (N). Between the strands of hepatocytes the hepatic sinusoids are shown (HS) (H & E stain-X 300)

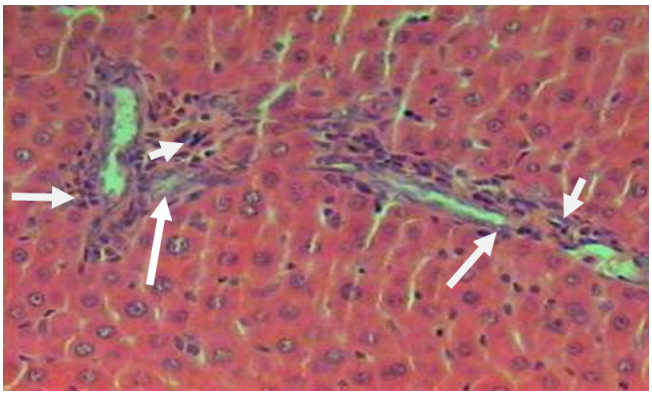

Figure 2.A micrograph of liver of rat treated with Paracetamol showing a portal tract with dilated and congested vein (arrow). Notice the periportal necrosis of the hepatocytes that surround the portal area (long arrow), and the inflammatory infiltration (arrowhead). (H & E stain-X 300)

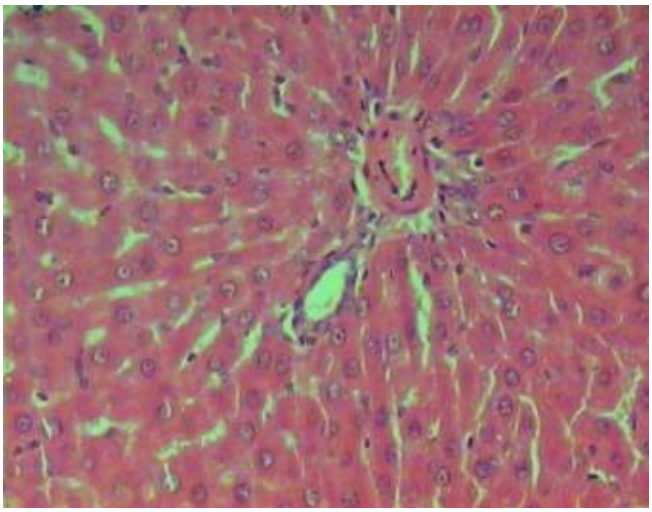

Figure 3.A photomicrograph of liver of rat treated with mixture of the three aromatic plants showing normal structure (H & E stain-X 300)

In the present study, the histopathological investigations (Figures 1-3), supported the biochemical findings. The paracetamol-treated rats showed necrosis, vacuoles, space formation and loss of cell boundaries in the liver. Oral administration of *mixure* before paracetamol administration reverted the above-mentioned changes. Plants produce bioactive compounds which act as defense mechanisms against predators and at the same time, may be toxic in nature.[34,35]

Conclusion

The inhibitory effect of the mixture aqueous extract (*Xylopia aethiopica*, *Coriandrum sativum* and *Anethum graveolens*on) hepatotoxicity was compared to that of positive control group. The hepatoprotective effect was confirmed by histopathological examination of liver of studied groups. The mixture aqueous extract was possess antioxidant properties and were found to be useful in the treatment of liver damage. Significant index and values were observed in acute assayed and effective alteration in all biochemical and histopathological sections was observed. It was concluded that the aqueous extract mixture dose dependably offered potential hepato protection from paracetamol induced hepatic damage, normalizing biochemical parameters in rats plausibly modulating lipid peroxidation and augmenting endogenous non-enzymatic antioxidant defense mechanism. So they have hepato protective activity which supports the hepatic cell protection. However the mechanism of action and the active component which is responsible for the actual hepato protectivity is not well known. Further exploration is needed in order to elucidate the components responsible for hepatic protection.

Further pharmacological & toxicological should be carried out on aqueous extract of plants to assets their safety,

therapeutic effectiveness & potential for commercial utilization. Bioassay-guided fractionation and purification lead to isolation of active ingredient responsible for activity.

Conflict of Interest: None

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