Effect of pre-treatment with methanol leaf extract of *Commelina diffusa* on paracetamol-induced liver damage

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Abstract

The present study investigated the effect of pre-treatment with methanol extract of the leaves of *Commelina diffusa* on paracetamol-induced liver damage on experimental rats. Wistar rats were used and grouped; groups 1 and 2 as controls, groups 3 and 4 were administered 200 mg/kg and 400 mg/kg of *Commelina diffusa* leaves extract, and group 5 received silymarin 100 mg/kg (standard drug). Hepatotoxicity was induced with 3 g/kg of paracetamol (PCM) on the 7th day in all the animal groups except the positive control group (Group 1). At the end of the experiment, blood samples were collected from the anaesthetized rats for biochemical indices (liver enzymes and antioxidants). Both doses (200 mg/kg and 400 mg/kg) of *Commelina diffusa* leaves extract significantly reduced the liver enzymes level, and also increased antioxidant enzymes level when compared with the PCM-treated (negative) control group. The methanol leaf extract of *Commelina diffusa* possesses preventive effect on liver damage evident via its antioxidative and hepatoprotective actions.

Keywords: *Commelina diffusa,* antioxidant, hepatotoxicity, paracetamol, silymarin.

Introduction

Commelina diffusa (family, Commelinaceae), the climbing dayflower, is an herbaceous plant in the dayflower family found in the tropics. It is an herb which may be annual or perennial.[1] It is a flowering plant with its flowering occurring between May to November.[2] *Commelina diffusa* is useful as a medicinal plant as it exhibits antioxidant activity by preventing generation of free radicals and hence is alternatively beneficial to alleviate inflammatory diseases. Also in most cases it acts as hepatoprotective agent.[3]

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Oxidative stress is a harmful state wherein oxygen free radicals attack biological molecules such as lipids, proteins and DNA.[4] Free radicals are atoms or molecules that have unpaired electrons usually unstable and highly reactive.[5] Free radicals can affect intracellular signal transduction and gene regulation, resulting in cytokine production essential to the inflammatory process. Oxidative stress plays a major role in many liver diseases.[6] The liver is a major organ that is attacked by reactive oxygen species (ROS). The mammals possess sophisticated antioxidant system which maintains the redox homeostasis in the liver. With an excess in ROS, the homeostasis is disturbed, resulting in oxidative stress which plays a critical role in liver disease and other chronic degenerative disorders.[7]

The liver is a frequent target organ for drug toxicity. The production of radical species specifically ROS and RNS has been postulated as an early event of drugs hepatotoxicity and as an indicator of hepatotoxic potential.[8] Numerous exogenous drugs could induce oxidative stress including increase of cellular oxidants and lipid peroxidation, depletion of antioxidant in the liver, and such drugs include analgesic drugs, anti-cancer drug, anti-inflammation drugs, and anti-depressants. For example, liver oxidative damage has been found to be induced by sulfasalazine a drug used to treat inflammatory bowel diseases,[9] while paracetamol induced hepatotoxicity may result in DNA damage and dysfunction of the mitochondria.[10]

Paracetamol, a common and widely used analgesic and antipyretic agents can cause hepatotoxicity, lead to acute liver failure. Very high doses of paracetamol (2.5 g/kg and above) can induce hepatic damage in animals, as its toxic metabolite (N-acetyl-p-benzoquinineamine) binds to macromolecules of hepatocytes to cause cell necrosis.[11,12]

This present study aimed at investigating the extent of protection on the liver following pre-treatment with methanol extract of *Commelina diffusa* prior to paracetamol-induced hepatic damage on wistar rats. Liver enzymes level and antioxidant status of the experimental animals were investigated.

Materials and Methods

Collection of Plant Material and Preparation of extract

Fresh leaves of *Commelina diffusa* were collected from

the school environment of Delta State University, Abraka, Nigeria, and were identified and authenticated by Dr. H.A. Erhenhi, a taxonomist of the Department of Botany, Faculty of Sciences, Delta State University, Abraka, Nigeria. Dried leaves of *Commelina diffusa* were powdered with the aid of a mechanical grinder. Three hundred and forty-two grams (342 g) of the powdered leaf were dissolved in 2.6 L of methanol (70%) and extracted using Soxhlet evaporator at 25 ^{0}C. The filtrate was further concentrated to dryness with the aid of a water bath set as 40 ^{0}C. The final extract was stored in the refrigerator prior to the study.

Animals

Twenty-five (25) Wistar rats (180 g average weight) were procured from the Animal House of the Faculty of Basic Medical Science, Delta State University Abraka, Nigeria. The animals were acclimatized for a period of one week prior to the study, and fed commercial rat feed and portable water *ad libitum*. Guidelines followed in the handling of animals were in accordance with global standard adopted by the Ethical Committee of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria (FBS/CT/091720).

Experimental design

The rats were randomly placed into five groups, n = 5:

Group 1 – Normal Saline (NS) 10 ml/kg

Group 2 – Normal Saline (NS) 10 ml/kg + Paracetamol (PCM) 3000 mg/kg

Group 3 – *Commelina diffusa* (CD) 200 mg/kg + Paracetamol (PCM) 3000 mg/kg

Group 4 – *Commelina diffusa* (CD) 400 mg/kg + Paracetamol (PCM) 3000 mg/kg

Group 5 – Silymarin 100 mg/kg + Paracetamol (PCM) 3000 mg/kg

The experimental animals were administered the extracts or silymarin once orally daily for 6 days. All the animals except the normal control group were administered PCM 3000 mg/kg on day 7 and observed for 24 hours.[13] At the end of the treatment (8th day), the animals were subjected to chloroform anesthesia. Blood samples were collected, centrifuged at 4000 rpm for 10 minutes, and the supernatant used for biochemical assays.

Biochemical and Histological Studies

The biochemical studies included analyses on liver enzymes (aspartate aminotransferase - AST, alanine transaminase - ALT, and alkaline phosphatase – ALP), and antioxidants such as superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels.[14,15,16.17,18]

The liver organs of the rats were harvested and persevered in formal saline for histopathological studies. Results are presented as mean ± standard error of mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P-values < 0.05 were taken as significant.

Results

Effect of Commelina diffusa (CD) extract on serum liver enzymes in paracetamol induced hepatotoxicity

Statistically significant ($p<0.05$) increase was observed in all the liver enzymes (AST, ALT, and ALP) of experimental animals in PCM control (negative) group as compared with those in the normal (positive) control group. Administration of 200 mg/kg and 400 mg/kg of *Commelina diffusa* prior to induction of liver damage resulted in decrease in liver enzyme of experimental rats as compared to the PCM treated group. The reduction was significant ($p<0.05$) at a dose of 400 mg/kg of *Commelina diffusa*. (Table 1). Also, significant decreased liver enzymes were detected in rats administered silymarin (standard drug) as compared with the PCM control group.

Effect of Commelina diffusa (CD) extract on serum antioxidant levels in paracetamol induced hepatotoxicity

Comparative significant decrease in antioxidant enzymes (SOD, CAT) and increased lipid peroxidation biomarker (MDA) were observed in PCM control group as against those in normal control group. Non-significant ($p>0.05$) increase in the levels of superoxide dismutase (SOD) and catalase (CAT), and a significant ($p<0.05$) decrease in malondialdehyde (MDA) were observed in serum of rats administered 200 and 400 mg/kg of *Commelina diffusa* as compared with the PCM control group (Table 2).

Table 1: The effect of *Commelina diffusa* on liver enzymes in paracetamol induced hepatotoxicity

	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal control	46.41 ± 1.51	15.53 ± 1.80	31.23 ± 3.27
PCM control	69.72 ± 0.94 [a]	26.92 ± 3.18 [a]	55.77 ± 1.18 [a]
CD-200 + PCM	59.49 ± 2.32	19.13 ± 1.56	42.88 ± 5.23
CD-400 + PCM	47.02 ± 0.96 [b]	15.01 ± 2.31 [b]	32.63 ± 2.20 [b]
Silymarin + PCM	50.86 ± 0.75 [b]	13.94 ± 1.85 [b]	40.42 ± 2.04 [b]

[a] = *P<0.05 was taken to be significant when compared with the normal control group.*

[b] = *P<0.05 was taken to be significant when compared with the PCM control group.*

Table 2: The effect of *Commelina diffusa* on serum antioxidant parameters in paracetamol - induced hepatotoxicity

	SOD (IU/L)	CATALASE (IU/L)	MDA (IU/L)
Normal control	0.31 ± 0.03	0.52 ± 0.06	0.58 ± 0.06
PCM control	0.27 ± 0.06 [a]	0.45 ± 0.09 [a]	0.72 ± 0.07 [a]
CD-200 + PCM	0.41 ± 0.04	0.57 ± 0.04	0.52 ± 0.02 [b]
CD-400 + PCM	0.30 ± 0.02	0.64 ± 0.01	0.48 ± 0.04 [b]
Silymarin + PCM	1.00 ± 0.51 [b]	1.51 ± 0.04 [b]	0.42 ± 0.05 [b]

[a] = *P<0.05 was taken to be significant when compared with the normal control group.*
[b] = *P<0.05 was taken to be significant when compared with the PCM control group.*

Discussion

Oxidative stress results from abnormal production of reactive oxygen species (ROS) or depletion of antioxidant defenses due to an imbalance in intracellular prooxidant/antioxidant.[19] Over production of reactive oxygen species, often leading to peroxidation of membrane phospholipids and production of reactive aldehydes.[20] Progressive oxidative stress leads to cell damage, which results in the alteration of cellular enzymes.[21] The liver is considered to be highly sensitive to toxic agents in living systems.

This study investigated the effect of pre-treatment of methanol leaf extract of *Commelina diffusa* on liver enzymes and antioxidant levels in rat model of paracetamol-induced liver damage. Paracetamol (PCM, acetaminophen) is a commonly and widely used analgesic and antipyretic agent. However, at hepatotoxic doses of paracetamol, the normal level of hepatic glutathione is depleted, due to the effect of the metabolite; N-acetly-p-benzoquiniamine (NAPQI) which covalently binds to cysteine groups on proteins to form 3-(cystein-S-yl) acetaminophen adducts.[22] Glutathione, normally protects hepatocytes by combining with the reactive metabolite of paracetamol thus preventing their covalent binding to liver proteins.[23]

Silymarin, an antioxidant and also a hepatoprotective agent which is widely distributed with its highest activity found in the red blood cells and in the liver,[24] was used at a dose of 100 mg/kg as standard drug treatment for liver damage.[25] Silymarin increases the levels of glutathione in the liver by more than 35% in healthy subjects and more than 50% in rats and protects the liver from damage.[24]

In this study, a significant hepatic damage was observed in the animals induced with paracetamol. This is evident by the elevated levels of serum markers. Serum AST, ALT, and ALP, which are biomarkers of hepatocyte damage and loss of functional integrity, showed a marked increase in activities following paracetamol administration at 3 g/kg. Also, paracetamol (3 g/kg) administered also caused a decrease in the serum levels of superoxide dismutase (SOD) and catalase (CAT), and also induced lipid perioxidation via an increased serum malondialdehyde (MDA) level.

Animal groups which received pretreatment with *Commelina diffusa* extract at both increasing doses (200 and 400 mg/kg) had their liver enzymes levels and antioxidant enzymes level slightly different from that of the normal rats which were not administered paracetamol. However, the difference was significant when compared with the rats that were dosed with paracetamol but did not receive *Commelina diffusa* leaves extract. This effect of *Commelina diffusa* leaves extract was similar to that produced by silymarin which had a much striking effect.

The decrease in the serum levels of liver enzymes biomarker and malondialdehyde, and increase in SOD and catalase as seen in the rats pretreated with *Commelina diffusa* extract reveals the hepatocyte-protecting and antioxidant activity of the *Commelina diffusa* leaves extract. The antioxidant activity of *Commelina diffusa* has been attributed to the presence of phytochemical compounds such as phenolic compounds as well as other phytochemicals such as tannins and flavonoids, and secondary metabolite such as alkaloids, phytosterols and triterpenoids.[26] Antioxidant property of this plant also supports its anti-inflammatory properties.[27]

Hepato-protective activities of *Commelina diffusa* have also been shown in studies by Sule and his colleagues.[19] The study showed that liver secretions, collected from albino rats that were treated with doxorubicin, showed infiltration of neutrophils with spotty inflammation whereas those pretreated with the leaves extract of *Commelina diffusa* at 200 and 400 mg/kg, had improved cell integrity, which was proved by normal hepatic stroma with hepatocytes, sinusoid and central vein.[19] Treatment with doxorubicin from the study caused a significant increase in the level of AST, ALT and total bilirubin when compared with the normal control whereas treatment of the positive control group with *Commelina diffusa* extract from the study demonstrated a significant decrease in the level of AST, ALT and total bilirubin.[19] These resultant effect following the treatment with *Commelina diffusa*

extract point to its free radical scavenging effect following oxidative damage caused by doxorubicin.[19] This was also validated in this present study in that PCM-induced hepatotoxicity was reduced by the effect of *Commelina diffusa* leaves extract.

Conclusion

The therapeutic administration of methanol leaves extract of *Commelina diffusa* on paracetamol-induced hepatotoxicity clearly suggests the hepatoprotective effect of *Commelina diffusa*. The herbal drug has equivalent therapeutic value with the positive control drug (silymarin). Further, this creates a hope in new drug discovery in controlling liver diseases using *Commelina diffusa* as a possible precursor. Elucidation of the active constituents responsible for its hepatoprotective ability is vital.

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