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Ameliorative Potentials of *Bambusa vulgaris* Leaf Extract on Carbon tetrachloride Induced Toxicity in Albino Rats

Temidayo Ogunmoyole, Oluwatobi Afolayan, Toluwalase Ajayi, Omowunmi Jayeola Makun

ABSTRACT

Bambusa vulgaris has been widely utilized in folkloric medicine for the treatment several diseases. The present study evaluates the ameliorative effects of its leaves extract on carbon tetrachloride (CCl_4) induced organs' damage. This was done to provide a cost-effective and potent therapeutic option in the management of multi-organ disorders. Twenty Wistar rats were placed into five groups (I-V) of four animals each. All experimental animals, except group I were administered a single intraperitoneal dose of CCl_4 (3 ml/kg b.w.). Animals in groups III and IV were treated with *B. vulgaris* at 200 mg/kg b.w. and 400 mg/kg b.w. respectively, group V animals received 100 mg/kg b.w. silymarin after initial exposure, while group II animals were left untreated after initial exposure to CCl_4. Specific markers of liver, kidney and heart injury such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), catalase, glutathione peroxidase, urea, creatinine, bilirubin and cytokines were assayed in the serum and tissue homogenates. Results indicate that carbon tetrachloride caused a significant increase in the serum level of AST, ALP, ALT, LDH as well as bilirubin, urea and inflammatory cytokines relative to control. On the other hand, activity of glutathione peroxidase and catalase were diminished following exposure to CCl_4. However, treatment with the *B. vulgaris* extract restored to all deranged biochemical indices to values comparable with animals treated with silymarin. Histopathological evidence lends credence to the ameliorative potential of *B. vulgaris* leaf extract on damaged liver, heart and kidney. Hence, *B. vulgaris* can be considered a viable therapeutic agent for the management/treatment of renal, cardiac and hepatic diseases.

Keywords: *B. Vulgaris*, Liver, Restored, Serum, Diseases, Silymarin.

INTRODUCTION

Medicinal plants have been recognized as vital therapeutic agents. This is due to the presence of several secondary metabolites (phytochemicals) in their body parts. These plants serve as raw materials for the enhancing good health as well as in the treatment/management of diseases. Perhaps, this explains the global increase in the use of plant-based drugs in the management of diseases [1,2]. The use of traditional herbal preparations for the treatment of diseases has been since antiquity. Medicinal herbs are relatively safe as cheap but potent therapeutic interventions in the treatment of disease [3,4]. *Bambusa vulgaris* Schraw. Ex J.C. Wendl. (Poaceae) is widely distributed and grows in tropical and subtropical areas. Its shoots have been exploited as traditional medicine for the treatment of sexually transmitted infectious diseases [5,6]. Leaves of *B. vulgaris* has been used for treating fever, fidgeting and lungs inflammation, while its stem extract has been used for purifying blood and treating leukoderma and inflammations [7,8]. Its root and bark extract are useful in the treatment of ringworm infection, bleeding gums, painful joints, and eruptions. *B. vulgaris* is rich in phytochemicals with proven analgesic, antipyretic, anti-inflammatory, antimicrobial and antidiabetic properties [9].

Information on the use of *B. vulgaris* leaves in the management of multiorgan toxicity is scanty. Although, *B. vulgaris* leaves have been widely used in folkloric medicine to manage ailments individually. The present study therefore investigates the potential usefulness of *B. vulgaris* leaves in the simultaneous management of liver, kidney and heart diseases. This effort might proffer a cheaper, locally available and more efficient alternative to the conventional drugs used for the treatment of these diseases especially in patients with multiple organ complications.

MATERIALS AND METHODS

Collection and identification of *B. vulgaris* leaves

Fresh leaves of *B. vulgaris* were collected from a farm in iWork Ekiti. The leaves were identified at the Department of Plant Science, Ekiti State University, Ado – Ekiti, with herbarium number,

UHAE2021037. The leaves were air-dried, pulverized and stored airtight.

Preparation of plant extract

Seventy five percent homogenate of the powdered leaves was prepared in 80% methanol and left for three days for optimal extraction. With the aid of cheese cloth, the mixture was separated into supernatant/filtrate (that was used for further analysis) and residue (that was discarded). The filtrate was dried to a constant weight (crude extract) and refrigerated. Subsequently, the crude extract was reconstituted with distilled water and administered as outlined in Table 1.

Table 1: Treatment of experimental animals

Description	Treatment
I (Normal control)	Distilled water only for 14 days
II (Negative control)	CCl_4 (3 ml/kg b.w.) single administration
III	CCl_4 (3ml/kg b.w.) + 200 mg/kg b.w. *B. vulgaris*
IV	CCl_4 (3ml/kg b.w.) + 400 mg/kg b.w. *B. vulgaris*
V (Standard control)	CCl_4 (3ml/kg b.w.) + 100 mg/kg b.w. silymarin

Reagents and chemicals

Reduced glutathione, hydrogen peroxide, potassium hydrogen phosphate, phosphotungistate, malondialdehyde (MDA), creatine phosphate, ethylenediaminetetraacetate (EDTA), magnesium acetate and Ellman's reagent were products of Sigma Aldrich. Other reagents and chemicals were purchased from standard commercial suppliers. All biochemical kits used in the study were purchased from Rando Chemical Ltd. England.

Animals protocol

Albino rats were used in compliance with internationally recognized guideline (Revised NIH Publications 1978, No. 8023) while ethical approval (ORD/AD/EAC/19/0083) was obtained from the Committee on Care and Use of Laboratory Animals, Office of Research and Development, Ekiti State University, Ado Ekiti, Nigeria. Twenty Wistar albino rats with mean weight of 170 g were purchased from the Animal Breeding Colony, College of Medicine, Ekiti State University, Ado - Ekiti. Choice of doses of toxicant, extract and silymarin administered was based on available literature. Albino rats were kept in separate iron cages at temperature (24 ± 1℃), relative humidity and 12 / 12-h light and dark cycle. Rats were allowed to have an unrestricted access to food and drinking water *ad libitum*. Good hygiene was maintained by daily routine change of rat beddings.

Preparation of organs' homogenates

Albino rats were decapitated under very light cold ether anesthesia. Dissection was done to surgically remove the liver, heart and kidney which were carefully defatted. A 10% homogenate of the organs were prepared in 100 mM potassium phosphate buffer (pH 7.4). The homogenates were subjected to centrifugation for 30 min at 3000 rpm and decanted to obtain a clear supernatant that was refrigerated. Cardiac puncture was used to obtain whole blood which was kept in EDTA bottle for an hour at 25℃. The whole blood was then centrifuged at 25℃ for 15 mins and decanted to obtain the serum which was immediately refrigerated.

Phytochemical screening of B. vulgaris extract

Established protocols were followed to detect the presence/absence of phytochemicals such as flavonoids, terpenoids, cardiac glycosides saponins, alkaloids, tannins and phenolics.

Determination of Biochemical Parameters

Assay for enzymatic activity of aspartate aminotransferase (AST)

Enzymatic activity of aspartate aminotransferase (AST) was determined according to Reitman and Frankel [10]. One hundred microliter of organs homogenates and serum. was added in separate test-tubes to 100 mM potassium phosphate buffer (pH 7.4) containing 2 mM α-oxoglutarate and 100 mM L-aspartic acid and incubated for 30 min at 37℃. Immediately, 500 µl of 20 mM 2,4-dinitrophenylhydrazine then added and re-incubated at 25℃ for 20 min. Finally, 5.0 ml of 400 mM M NaOH was introduced to the reaction mixture and left to stand for 5 min. Absorbance at 546 nm of the resulting solution was against the blank.

Determination of alanine amino transferase (ALT) activity

Activity of alanine aminotransferase in the serum and organs' homogenates was measured according to the method of Reitman and Frankel [10]. Five hundred microliter of a reagent (R1), made up of 100 mM potassium phosphate buffer pH 7.4, 2.0 M α-oxo glutaric acid and 200 mM L-alanine was added to 100 µl of each of serum and organs' homogenates and incubated at 37℃ for 30 min. Five hundred microliter of another reagent (R2) containing 20 mM 2,4-dinitrophenylhydrazine was then added to the mixture and re-incubated at 20℃ for 20 min. Finally, 5.0 ml of 400 mM M NaOH was added to the mixture and left for 5 min at 25℃. Absorbance at 546 nm of the resulting solution was read against the blank.

Measurement of alkaline phosphatase (ALP) activity

The method described by Engelhardt [11]. was adopted in assaying for alkaline phosphatase using ρ-nitrophenyl phosphate as substrate. One milliliter of a reagent (A) consisting of 0.5 mM magnesium sulphate, 1.0 M bicarbonate buffer (pH 10.1) and 19 mM p-nitrophenol phosphate, was added separately to 20 µl ml of serum and organs' homogenates followed by thorough mixing. Absorbance at 400 nm of the resulting mixture was then monitored for 180 s at 60 s intervals.

Assay for Antioxidant Enzymes

Determination of glutathione peroxidase (GPx) activity

Rot ruck *et al* [12]. was followed in the determination of GPx activity. Ten microliters of sodium azide, 200 µl reduced glutathione (GSH) and 100 µl hydrogen peroxide were added to two separate test tubes each made up of 500 µl of potassium phosphate buffer and labelled reagent blank and sample respectively. Exactly six hundred microliter (600 µl) of distilled water was added to sample test tube, while 110 µl was added to the blank, mixed thoroughly and incubated for 180 s, after the addition of trichloroacetic acid. The mixture was centrifuged for 5 min at 3000 rpm. Thereafter, 2 ml of K_2HPO_4 and 1 ml of DTNB was added to 1 ml of supernatant obtained, and absorbance at 412 nm was read against the blank. Glutathione peroxidase activity was obtained by interpolation from the standard curve.

Measurement of catalase activity

Enzymatic activity of catalase was measured following the method of Sinha [13]. Five-fold dilution of the sample was prepared in distilled water. A reaction mixture made up of 2 ml of 0.8 mM hydrogen peroxide and 2.5 ml of 0.1 M potassium phosphate buffer pH 7.4 was prepared in a volumetric flask. Appropriate volume of diluted enzyme (sample) was quickly added to the reaction mixture at 25℃ and gently mixed. One milliliter of the resultant mixture was measured and added

to 1 ml of a reagent containing a mixture of dichromate and acetic acid every 60 s. Finally, the H_2O_2 content in the sample withdrawn was measured according to Sinha [13]. The rate constant, K, for the catalytic degradation of H_2O_2 was measured using the first order equation of the reaction: $K = 1/t \log S_0/S$, where S_0 represents $[H_2O_2]$ at the start of the reaction, while S represents $[H_2O_2]$ as the reaction progresses at time t (min). A plot of the rate constant K against time (min) generates a curve from which the rate constant of catalase $K_{(0)}$ at time 0 was extrapolated.

Determination of Kidney Function Parameters

Urea determination

The concentration of serum urea was determined using the method of Tietze [14]. according to manufacturer's instructions.

Creatinine determination

The concentration of serum creatinine was determined using the method of Tietze [14]. according to manufacturer's instructions.

Determination of Cardiac Parameter and Cytokines

Lactate dehydrogenase

Lactate dehydrogenase (LDH) activity was determined by the method of Deck *et al* [15]. as outlined in the manufacturer's instructions. Increase in absorbance at 340 nm was measured at 1-min interval for 3 min. Serum LDH activity was calculated as units per liter (U/L).

Determination of Cytokines

ELISA Assay: Competitive ELISA was employed in the quantification of IL-1β and IL-6 following established principle of competitive immunoassay. TNF-α was quantified using an amplification ELISA with high sensitivity. ELISA experiments were conducted in accordance with the instructions contained in the manual accompanying the kit. Seven standard dilutions of the cytokines (IL-6= 1.37-1,000 pg./mL, IL-1β= 0.48-100 pg./ mL, and TNF-α= 0.31-20 pg./mL) were prepared from which a standard curve was plotted. Amount of each of the cytokines in the serum was measured with the aid of a Multi-Reader (Biotech Citation 3, Biotech, Winooski, VT) coupled with Gen5 software.

Lipid Peroxidation

Lipid peroxidation was determined by measuring the amount of thiobarbituric acid reactive substances (TBARS) according to the method of Okawa [16]. An aliquot of 0.4 ml of the serum and organs' homogenates was mixed with 1.6 ml of Tris-KCl buffer and 0.5 ml of 30% TCA. Five hundred microliter of 0.75% TBA was added and incubated for 45 min at 80°C, cooled in ice and centrifuged at 3000 rpm for 5 min. Absorbance of the clear supernatant obtained was read against a reference blank of distilled water at 532 nm. Lipid peroxidation was expressed in units/mg tissue using a molar absorptivity of $1.56 \times 10^{-5} M^{-1} CM^{-1}$

Histopathological examination of organs

A standard laboratory protocol for paraffin embedding was used to treat the formalin-preserved liver, kidney and heart tissues. Appropriate thin sections (4 mm) of the organs were fixed on slides, deparaffinized using *p*-xylene followed by rehydration in graded percentages of ethanol (100, 80, 70 and 50%) and rinsing with distilled water. Staining of slides was done with hematoxylin for 300 s followed by rinsing with distilled water. The resulting slides were then counter stained using eosin, mounted and for microscopical viewing.

Data analysis

Data obtained were presented as mean ± SEM. One Way Analysis of Variance (ANOVA) was adopted for statistical analysis. Duncan's Multiple Range Test (DMRT) on SPSS was then conducted. Values were considered significant at $p = 0.05$.

RESULTS OF ANALYSIS

Phytochemical screening of B. vulgaris extract

Preliminary phytochemical screening of *B. vulgaris* extract indicates the presence of saponins, flavonoids, phenolics, terpenoids and alkaloids while tannin and cardiac glycosides were not detected (Table 2).

Biochemical Parameters

Effect of B. vulgaris extract on aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase

Biomarkers of liver (AST, ALT, ALP, Total bilirubin) (Table 3) were markedly elevated in animals exposed to CCl_4. When treated with *B. vulgaris* leaf extract, levels of these biomarkers in experimental animals were restored in a manner similar to CCl_4 - intoxicated animals treated with silymarin (Table 3).

Effect of B. vulgaris extract on selected antioxidant enzymes

Exposure to CCl_4 caused a significant in inhibition of catalase and glutathione peroxidase in serum of experimental animals. Treatment with *B. vulgaris* caused a dose dependent restoration of catalase and GPx activity (Table 4).

Effect of B. vulgaris on selected kidney function parameters

Table 5 indicates that administration of CCl_4 resulted in a marked increase in urea and creatinine level in the serum and kidney homogenates of experimental animals relative to control. However, treatment with graded doses of *B. vulgaris* reversed the toxicity in a dose dependent manner.

Effect of *B. vulgaris* extract on Cardiac Parameter and Cytokines

Effect of B. vulgaris on lactate dehydrogenase

Table 6 showed the effect of *B. vulgaris* extract on lactate dehydrogenase activity in the serum and heart of animals exposed to CCl_4. It revealed that CCl_4 caused a significant increase in serum level of lactate dehydrogenase in the experimental animals. Administration of graded doses of *B. vulgaris* relieved the toxicity imposed by the toxicant in manner comparable with the negative control animals.

Effect of B. vulgaris extract on inflammatory cytokines

Similarly, administration of CCl_4 caused a marked derangement of selected inflammatory markers in the serum of experimental animal (Table 6). Treatment with *B. vulgaris* extract caused a reversal of the derangement. The reversal was similar to animals that were treated with silymarin.

Effect of B. vulgaris on lipid peroxidation

Finally, lipid peroxidation which had been induced in selected organs of albino rats following their exposure to CCl_4, was significantly prevented by treatment with *B. vulgaris* extract (Figures 1a and b).

Table 2: Preliminary phytochemical screening of *B. vulgaris* leaf extract in aqueous solvent

Phytochemical	Present/absent
Saponins	++
Alkaloids	+
Flavonoids	++
Phenolics	+
Terpenoids	+
Tannins	–
Steroids (cardiac glycosides)	–

Table 3: Effect of *B. vulgaris* leaf extracts on selected liver parameters in rat exposed to CCl_4 toxicity

Parameter	Tissues	Control	CCl_4 only	CCl_4+ *B. vulgaris* (200 mg/kg bwt)	CCl_4 + *B. vulgaris* (400 mg/kg bwt	CCl_4+silymarin (100 mg/kg bwt)
ALT U/L	Serum	60.25 ± 2.76^{a}	84.85 ± 2.62^{b}	54.25 ± 2.90^{a}	$48.69\pm10.77^{a*}$	$48.20\pm2.69^{a*}$
	Liver	39.05 ± 0.50^{c}	64.00 ± 1.56^{d}	34.15 ± 1.63^{c}	$31.55\pm1.49^{c*}$	37.00 ± 3.25^{c}
ALP U/L	Serum	44.84 ± 2.14^{a}	62.12 ± 2.99^{b}	45.04 ± 1.82^{a}	$36.23\pm0.33^{a*}$	44.70 ± 1.84^{a}
	Liver	54.25 ± 2.79^{c}	75.19 ± 1.22^{d}	$38.19\pm2.62^{c*}$	$32.29\pm1.24^{c**}$	$42.46\pm1.92^{c*}$
AST U/L	Serum	52.15 ± 2.48^{a}	67.20 ± 1.70^{b}	$38.70\pm2.26^{a*}$	$34.23\pm3.15^{a*}$	$36.15\pm3.18^{a*}$
	Liver	51.30 ± 1.84^{c}	64.72 ± 1.12^{d}	$39.74\pm1.49^{b*}$	$32.70\pm0.42^{b**}$	47.10 ± 1.27^{b}
T.BIL mg/dl	Serum	80.00 ± 4.89^{a}	109.80 ± 0.28^{b}	$52.30\pm3.25^{a*}$	$47.51\pm2.96^{a*}$	91.80 ± 2.55^{a}
	Liver	76.40 ± 0.57^{b}	116.70 ± 1.27^{d}	$43.60\pm3.96^{b*}$	$38.00\pm2.26^{b**}$	$55.47\pm0.38^{b*}$

Each data point represents the mean ± standard error in mean of an experiment conducted in triplicate. 'a' and 'c' stand for a marked difference from the control's 'b' and 'd' in that order. * and ** represent very significant and far more significant difference from the control

Table 4: Effect of *B. vulgaris* leaf extract on selected antioxidant enzyme in rat exposed to CCl_4 toxicity

Parameter	Tissues	Control	CCl_4 only	CCl_4+ *B. vulgaris* (200mg/kg bwt)	CCl_4 + *B. vulgaris* (400 mg/kg bwt of	CCl_4+silymarin (100 mg/kg bwt)
GPX	Kidney	68.34 ± 2.95^{a}	36.55 ± 2.68^{b}	38.28 ± 3.22^{b}	71.33 ± 3.78^{a}	$85.22\pm1.89^{a*}$
U/mg protein	Heart	52.85 ± 0.86^{c}	32.34 ± 0.93^{d}	55.06 ± 2.40^{c}	60.61 ± 3.20^{c}	53.20 ± 1.80^{c}
	Liver	70.35 ± 2.34^{e}	26.27 ± 1.82^{f}	51.96 ± 1.73^{e}	60.99 ± 3.20^{e}	59.26 ± 1.05^{e}
	Serum	77.61 ± 1.49^{g}	36.79 ± 1.22^{h}	63.94 ± 1.50^{g}	66.94 ± 1.66^{g}	55.35 ± 2.11^{g}
Catalase U/mg protein	Kidney	0.44 ± 0.03^{a}	0.04 ± 0.01^{b}	0.15 ± 0.01^{b}	0.18 ± 0.01^{b}	0.19 ± 0.00^{b}
	Heart	0.18 ± 0.00^{c}	0.05 ± 0.01^{d}	0.27 ± 0.02^{c}	0.31 ± 0.03^{c}	0.13±0.02
	Liver	0.13 ± 0.00^{e}	0.05 ± 0.01^{f}	0.21 ± 0.01^{e}	0.26 ± 0.01^{e}	0.17 ± 0.02^{e}
	Serum	0.09 ± 0.04^{g}	0.03 ± 0.01^{h}	0.16 ± 0.00^{h}	0.20 ± 0.03^{h}	0.18 ± 0.01^{h}

Each data point is the mean ± standard error in mean of an experiment replicated thrice. 'a', 'c', 'e', 'g' represents significant difference from the control's 'b', 'd', 'f', 'h' respectively. * Represents very significant difference from the controls

Table 5: Effect of methanolic extract of *B. vulgaris* leaf on urea and creatinine in the serum and kidney of rats exposed to CCl_4 toxicity

Parameter	Tissues	Control	CCl_4 only	CCl_4+*B. vulgaris* (200mg/kg bwt)	CCl_4 + *B. vulgaris* (400 mg/kg bwt	CCl_4+silymarin (100 mg/kg bwt)
Urea (mg/dl)	Kidney	0.15±0.01[a]	1.38±0.00[b]	0.25±0.00[a]	0.43±0.01[a]	0.44±0.01[a]
	Serum	0.21±0.01[c]	0.76±0.00[d]	0.17±0.00[c]	0.33±0.00[c]	0.12±0.01[c]
Creatinine (mole/l)	Kidney	51.52±1.50[a]	92.44±3.45[b]	43.61±1.80[a]	31.40±1.32[a]	33.16±3.80[a]
	Serum	38.49±1.80[b]	86.63±2.47[d]	37.10±2.47[b]	42.19±1.71[b]	58.02±1.81[b]

Each data point stands for the mean ± standard error in mean an experiment replicated thrice. 'a' and 'c' represent marked difference from the control's 'b' and 'd' in that order.

Table 6: Effect of *B. vulgaris* leaf extracts on selected inflammatory markers in rat exposed to CCl_4 toxicity

Parameter	Tissues	Control	CCl_4 only	CCl_4+ *B. vulgaris* (200mg/kg bwt)	CCl_4 + *B. vulgaris* (400 mg/kg bwt of	CCl_4+silymarin (100 mg/kg bwt)
LDH (U/L)	Serum	122.64±4.81[a]	272.20±0.18[b]	198.27±3.10[a]	149.34±2.50[a]	153.55±2.69[a]
IL-4 (ng/L)	Serum	162.61±2.18[a]	112.21±1.55[b]	198.54±3.98[a]	202.05±11.14[a]	156.25±8.77[a]
IL-6 (pg/ml)	Serum	32.07±1.29[a]	18.57±0.79[b]	32.25±1.34[a]	41.66±1.18[a]	32.29±0.30[a]
IL-1β (pg/ml)	Serum	8.68±1.62[a]	1.28±0.13[b]	4.29±0.42[a]	4.91±0.53[a]	3.97±0.32[a]
TNF-a (ng/L)	Serum	25.39±2.56[a]	56.97±0.89[b]	31.35±1.51[a]	24.64±0.10[a]	16.36±1.70[a]

Each data point is the mean ± standard error in mean of an experiment replicated thrice. 'a' and 'c' stand for marked difference from the control's 'b' and 'd' in that order.

DISCUSSION

Integrity of critical organs such as the liver and kidney are often assessed by specific biomarkers. Aspartate aminotransferase has been suggested as a very useful indicator of organ damage. It occurs in the heart, erythrocytes, hepatocytes, renal and pancreatic cells. In normal cells, this biomarker is compartmentalized within the membrane of the hepatocytes, hence its activity in the serum is fairly constant. Preliminary phytochemical screening indicates the presence phenolics and flavonoids among other phytochemicals in the leaf extract of *B. vulgaris* (Table 2), suggesting that these phytochemicals with established antioxidant properties are responsible for the medicinal benefits of the plant. In the present study, a single intraperitoneal injection of CCl_4 resulted in a significant increase in the level of AST in the serum relative to normal animals (Table 3). This observation indicates a free-radical induced oxidative damage to the liver cell membranes, causing a leakage to the blood, thereby raising the AST level in the serum. Oral administration of *B. vulgaris* leaves extract caused the serum AST level to be restored to in a manner similar to normal rats. This suggest that the extract might play a role in the prevention of hepatic damage by enhancing the plasma membrane integrity thereby halting the enzyme leakage into the bloodstream. This observation is in agreement with Ogunmoyole *et al* [17]. The observed restoration of AST activity by treatment with *B. vulgaris* leaves extract may suggest that the plant has hepatoprotective potentials that could be of immense relevance in the management of hepatic disorders.

Alanine aminotransferase (ALT) is routinely employed as biomarker for diagnosing hepatic injury, perhaps due to its increased serum level whenever there is an injury in the hepatocytes [18,19]. In the present study, serum and liver ALT activity were significantly increased relative to the control in animals exposed to CCl_4 toxicity (Table 3). Treatment of exposed animals with *B. vulgaris* leaves caused a significant amelioration of liver hepatocytes. This was reflected in the restoration of ALT level to levels comparable with animals that were not exposed and those treated with silymarin (100 mg/kg b.w.). This suggests the safety of the extracts to the liver cells and its potential as hepatoprotective agent. Studies have shown certain antioxidant phytochemicals including terpenoids, flavonoids and cardiac glycosides can protect the hepatic and renal tissues against free radicals' attack [20]. Consequently, these phytochemicals could be responsible for the observed hepatoprotection. This observation can be linked to the presence of polyphenols, flavonoids and saponin in *B. vulgaris* (Table 2).

Total bilirubin is generally considered a true test of liver function since it reflects the hepatic ability to metabolize hemoglobin. Bilirubin level of rats exposed to CCl_4 was markedly increased relative to the control (Table 3), indicating that CCl_4 interfered with hepatic role in the metabolism of heme. Treatment with *B. vulgaris* extract brought back to normal, the level of bilirubin in the serum and liver in a manner that can favorably compete with silymarin at similar dose. This assertion is in agreement with Shah and Khan [21].

Antioxidant enzyme (catalase and glutathione peroxidase) activities were markedly increased in serum, kidney, liver and heart homogenates following exposure to CCl_4. Free radical scavenging enzyme like catalase and glutathione peroxidase protect the biological system from oxidative stress. Catalase is specific scavenging of O_2 formed by the enzymatic activity of superoxide dismutase [22]. The marked depletion in catalase activities in CCl_4-treated rats (Table 4) may be attributed to toxicant-induced rise in H_2O_2 level. Oral administration of *B. vulgaris* leaves extracts reactivated the activities of catalase and glutathione peroxidase suggesting the potential of the plant as hepatoprotective agent (Table 4). This observation agrees with the report of Shah and Khan [22]. This ability may be attributed to the polyphenolic and flavonoid component of the extract as suggested by earlier report of Imo *et al* [23].

Selected biomarkers such as urea and creatinine have been used to assess the integrity and functionality of the kidney. Urea is the primary metabolism derived from dietary protein and tissue protein turnover, while creatinine is the product of muscle creatine catabolism. Administration of CCl_4 to experimental rats caused a marked increase in urea and creatinine level and in the kidney homogenates and serum relative to control animals (Table 5). This suggests the nephrotoxic potential of the toxicant. Treatment with graded doses of *B. vulgaris* leaves extract caused a reversal of the trend in a dose-dependent manner, restoring the biomarkers level in the kidney homogenate and serum to similar level with control animals at the same dose. This observation is consistent with Shah

and Khan [22]. and can be linked to the antioxidant potential of flavonoids and polyphenolic components of *B. vulgaris* extract.

Free radical induced oxidative damage has been suggested to involve inflammation. In the present study, exposure of experimental animals to a single intraperitoneal injection of CCl_4 resulted in significant upregulation of TNF-α (Table 6). The observed upregulation in this inflammatory cytokine suggests its role in hepatic and renal inflammation as a result of exposure to CCl_4. On the other hand, interleukin-1, interleukin-6, and interleukin-β appear not to be upregulated in the CCl_4- induced inflammation. Treatment with *B. vulgaris* leaf extract caused a dose-dependent amelioration of the inflammatory process resulting in the downregulation of TNF-α. This observation is linked to the avalanche of antioxidant agents present in the extract including polyphenols and flavonoids as earlier suggested. This also suggests the potential of *B. vulgaris* as anti-inflammatory agent that can be useful in the management of hepato-renal diseases.

Lactate dehydrogenase (LDH) level in the blood is a useful predictor of the integrity and functionality of the heart. Exposure to CCl_4 caused a marked increase in the serum level of LDH, suggesting the cardiotoxic potential of CCl_4. Treatment of CCl_4-exposed rats with *B. vulgaris* extract restored LDH activity in the serum to a level comparable with intoxicated rats that were administered silymarin (Table 6). This indicates the cardioprotective effect of *B. vulgaris* which can be attributed to its polyphenol and flavonoids content.

Exposure to CCl_4 resulted in an elevated lipid peroxidation (MDA) in all organs employed, suggesting its multi-organ toxicity and identifying its major mechanism of toxicity as lipid peroxidation. Administration of CCl_4 to experimental rats resulted in a marked elevation in MDA level relative to the control (Figures 1a and b). Reports have indicated that malondialdehyde (MDA) produced during the process of lipid peroxidation attacks critical macromolecules causing organ damage [24-26]. However, administration of *B. vulgaris* to animals exposed to CCl_4 markedly restored their antioxidant capacity by inhibiting lipid peroxidation (Figures 1a and b). Lodhi *et al* [26]. reported that the major component of the antioxidant mechanisms of *B. vulgaris* involves inhibition of lipid peroxidation. The antioxidant capacity of the plant can be attributed to its high polyphenolic content among other phytochemicals. Treatment with the plant produced comparable effect to silymarin, suggesting its potential in the management of multi-organ diseases.

Histopathological results indicate that administration of *B. vulgaris* extract reversed distortion of renal, hepatic and cardiac histoarchitecture (Figures 2a-c) in a dose dependent fashion, corroborating biochemical observations in the study.

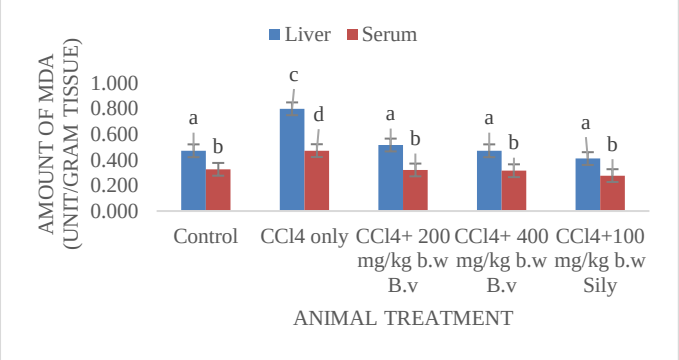

Figure 1a: Effect of *B. vulgaris* on MDA level in the serum and liver of CCl_4-intoxicated rats. Each data point is the mean ± standard error in mean of an experiment replicated thrice. 'a' and 'b' stand for marked difference from the control's 'c' and 'd' in that order. B.v - *B. vulgaris*, Silly- Silymarin

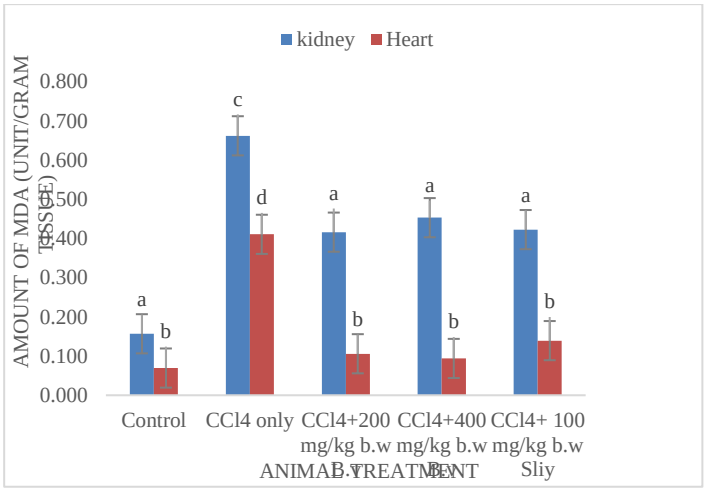

Figure 1b: Effect of *B. vulgaris* on MDA level in the heart and kidney of CCl_4-intoxicated rats. Each data point is the mean ± standard error in mean of an experiment replicated thrice. 'a' and 'b' stand for marked difference from the control's 'c' and 'd' in that order. B.v - *B. vulgaris*, Silly- Silymarin

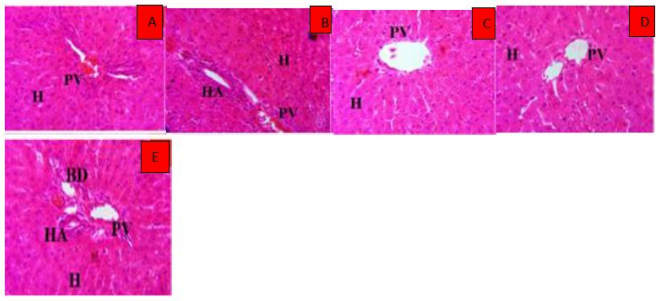

Figure 2a: Photomicrograph showing histoarchitecture of the liver tissue of experimental rats

A. photomicrograph of the liver slice of animals administered with basal feed and water only without toxicant and extract: it showed normal hepatocytic arrangement with mildly congested hepatic portal vein.

B. photomicrograph of rats administered with intraperitoneal injection of 3 ml/kg CCl_4: it showed severe vacuolar degeneration coupled with hyperplastic wall and noticeable shrinking in the portal area.

C. photomicrograph of rats administered with intraperitoneal injection of 3 ml/kg CCl_4 and treated with B. vulgaris leaf extract at 200 mg/kg bwt: it showed normal histomorphology of the hepatocytes coupled with slight hyperplastic wall.

D. photomicrograph of rats administered with intraperitoneal injection of 3 ml/kg CCl_4 and treated with B. vulgaris leaf extract at 400 mg/kg bwt: it showed normal hepatic histoarchitecture coupled with slight hyperplastic wall of the portal area.

E. photomicrograph of rats administered with intraperitoneal injection of 3 ml/kg CCl_4 and treated with Silymarin at 100 mg/kg bwt: it showed normal hepatic histoarchitecture. H represents the hepatocyte; PV stands for portal vein; BD represents bile duct; HA represents hepatic artery).

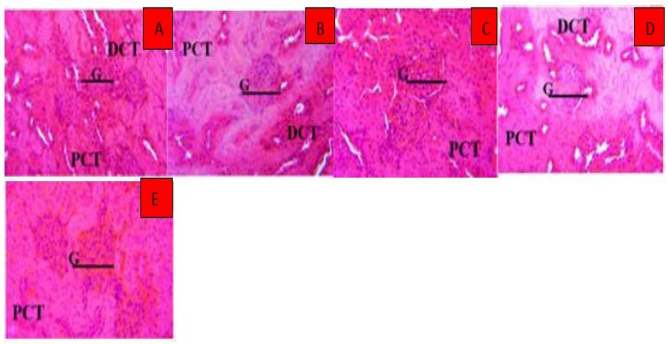

Figure 2b: Photomicrograph showing the histoarchitecture of the kidney tissues of experimental rats

A. photomicrograph of rats fed with basal feed and water only: it showed normal renal histoarchitecture with intact glomerulus.

B. photomicrograph of rats administered with intraperitoneal injection of 3ml/kg CCl_4: it showed distorted renal histoarchitecture with compromised glomerulus.

C. photomicrograph of rats administered with intraperitoneal injection of 3ml/kg CCl_4 and treated with B. vulgaris leaf extract at 200 mg/kg bwt: showed normal architecture of the glomerulus with vascular congestion.

D. photomicrograph of rats administered with intraperitoneal injection of 3ml/kg CCl_4 and treated with B. vulgaris leaf extract at 400 mg/kg bwt: it showed normal histoarchitecture of the glomerulus

E. photomicrograph of rats administered with intraperitoneal injection of 3ml/kg CCl_4 and treated with silymarin at 100 mg/kg bwt: it showed normal renal histoarchitecture.

(DCT= Distal convoluted tubules, VP= vascular pole; PCT= Proximal convoluted tubules; US= Urinary space; G= glomerulus)

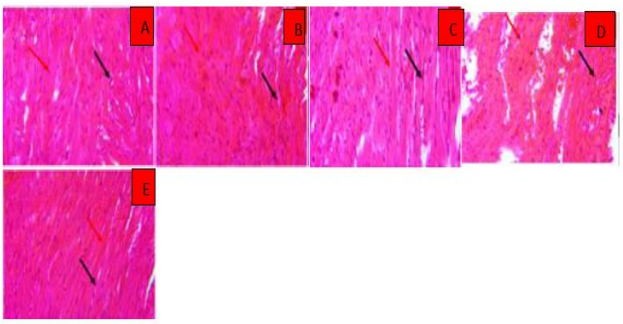

Figure 2c: Photomicrograph showing the histoarchitecture of cardiac tissue of experimental rats

A. photomicrograph of rats fed with basal feed and water only: it showed very distinct nuclei as well as very few myocardial degenerations.

B. photomicrograph of rats administered with intraperitoneal injection of 3 ml/kg bwt CCl_4 alone: it showed severe myocardial degeneration, distorted renal histoarchitecture with inflamed extracellular space.

C. photomicrograph of rats administered with intraperitoneal injection of 3 ml/kg bwt and treated with B. vulgaris leaf extract at 200 mg/kg: it showed less inflamed extracellular space with improved myocardium.

D. photomicrograph of rats administered with intraperitoneal injection of 3 ml/kg bwt and treated with B. vulgaris leaf extract at 400 mg/kg: it showed normal extracellular space, myocardium and renal architecture

E. photomicrograph of rats administered with intraperitoneal injection of 3 ml/kg bwt and treated with Silymarin at 100 mg/kg bwt: it showed normal slender muscle fibers, centrally placed nuclei and normal histoarchitecture.

(Black arrow represents the nuclei; Red arrow represents the muscle fibers).

CONCLUSION

In conclusion, *B. vulgaris* leaves is rich in polyphenols and flavonoids that mitigate oxidative damage to critical organs such as kidney, liver and heart. All biochemical indices measured in the present study suggests its potential usefulness in the management of liver, kidney and heart diseases.

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Conflict of Interest

None declared.

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REFERENCES

1. Bhattarai NK. Biodiversity-People Interface in Nepal. Medicinal Plants for Forest Conservation and Health Care. 2005:78.
2. Chirumbolo, S. Role of quercetin in vascular physiology. Can. J. Physiol Pharmacol. 2012;90(12):1652-7.
3. Jantan I, Ahmad W, Bukhari, SN. Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. Front. Plant Sci. 2015;6:655.
4. Nurul H, Haruna A, Wira, S, Ibrahim, J. Immunomodulatory effects of selected Malaysian plants on the CD18/11a, expression and phagocytosis activities of leukocytes. Asian Pacific Journal of Tropical Biomedicine. 2015;5(1):48-53.
5. Ambika K, Rajagopal B. In Vitro Antimicrobial and Antiproliferative Activity of Bambusa vulgaris. Ijppr. Human. 2017;9(1):10-22.
6. Owolabi MS, Lajide L. Preliminary phytochemical screening and antimicrobial activity of crude extracts of Bambusa vulgaris Schrad. Ex JC Wendl. (Poaceae) from southwestern Nigeria. American Journal of Essential Oils and Natural Products. 2015;3(1):4245.
7. Sofowora EA. Medicinal Plants and Traditional Medicine in Africa. Ibadan – Owerri – Kaduna – Lagos: Spectrum Books Limited. 1993;pp.158.
8. Panee J. Potential Medicinal Application and Toxicity Evaluation of Extracts from Bamboo Plants. PMC. 2015;9(23):681–92.
9. Sharma P, Saikia P, Sarma K. Diversity, uses and in vitro propagation of different bamboos of Sonitpur District, Assam. Journal of Ecosystem & Ecography. 2016;6(2):1-9.
10. Reitman S, Frankel, S. Glutamic – pyruvate transaminase assay by colorimetric method. American Journal of Clinical Pathology. 1957;28:56–60.
11. Englehardt A. Measurement of alkaline phosphatase. Aerztl Labor. 1970;16: 42
12. Rotruck JT, Popeh, AL, Ganther E. Selenium: Biochemical Role as a Component of Glutathione Peroxidase. Science. 1973;179(4073):588-90.
13. Sinha AK. Colorimetric assay of catalase. Anal. Biochem. 1972;47:38994.
14. Tietz NW. Clinical guide to laboratory tests. InClinical guide to laboratory tests. 1995:pp.1096-96.
15. Deck, L.M., Royer, R.E., Chamblee, B.B., Hernandez, V.M. and Malone, R.R. (1998). Selective inhibitors of human lactate dehydrogenases and lactate dehydrogenase from the malarial parasite Plasmodium falciparum. J Med Chem, 41: 3879–3887.
16. Ohkawa, H., Ohishi, H. and Yagi, K. (1979). Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95:351–8.
17. Ogunmoyole T, Ola Awe AM, Fatile OG. Ethanolic extract of Mucuna pruriens leaves ameliorates carbon tetrachloride and rifampicin-induced hepatotoxicity and nephrotoxicity in wistar albino rat. BMC Complementary Medicine and Therapies. 2021;21:282.
18. Singh D, Naugler C. Cybil induced hepatotoxicity biomedical changes in wilstar rats. Journal of environmental biology. 2015;2(4):725-27.
19. Ogunmoyole T, Adeyeye RI, Olatilu BO, Akande OA, Agunbiade OJ, *et al*. Multiple organ toxicity of Datura stramonium seed extracts. Toxicology Reports. 2019;6:983-89.
20. Shirwaikar AK, Ranjendran CD, Bodla R. Antidiabetic activity of aqueous leaf extract of Bambusa vulgaris in streptozotocin nicotinamide type 2 diabetic rats. Journal of Ethnopharmacology. 2016;92:171-75.
21. Scott MD, Lubin BH, Kupers FA. Erythrocytes defense against hydrogen peroxide preeminent importance of catalase. Journal of Laboratory Medicine. 2010;118:7-16.
22. Shah, N.A. and Khan, M.R. (2014). Antidiabetic Effect of Sida cordata in Alloxan-Induced Diabetic Rats. BioMed Research International,
23. Imo C, Uhegbu FO, Ifeanacho NG, Egbeigwe O, Ezekwe AS. Biochemical and Histopathological Changes Associated with Methanolic Leaf Extract of Gongronema latifolia in Acetaminophen-Induced Hepatic Toxicity in Wistar Albino Rats. International Journal of Biomolecules and Biomedicine. 2014;4:1-7.
24. Das SK, Vasudevan DM. Effect of lecithin in the treatment of ethanol mediated free radical induced hepatotoxicity. Indian Journal of Clinical Biochemistry. 2006;21:62-69.
25. Arun, K, Balasubramanian U. Comparative Study on Hepatoprotective activity of Bambusa vulgaris and Eclipta prostrate against alcohol

induced in albino rats. International Journal of Environmental Science. 2011;2:373-91.
26. Lodhi P, Singh N, Kumar, M. Camellia sinensis kuntze extract ameliorates chronic Ethanol-induced hepatotoxicity in albino rats. Journal of Evidence Based Complementary and Alternative Medicine. 2018:787153.

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