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Original Research Article

Ameliorative Effect and *In-Vivo* Antioxidant Properties of Methanol Extract of *Burkea africana* Stem Bark On Acetaminophen-Induced Hepatotoxicity in Rats

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ABSTRACT

Burkea africana (Wild Syringa) is a wildly used plant in traditional medicine in Africa. It has been used for the treatment of hepatitis and other disorders. This study was aimed at investigating the liver and kidney protective potentials of the plant in acetaminophen-induced hepatic and nephrotoxicity in rats. Extraction was by cold maceration using 80% methanol. Thirty-six (36) rats were assigned into six groups of six rats each. Group 1 received distilled water, group 2 received silymarin and acetaminophen, group 3 received only acetaminophen. Groups 4-6 received plant extract at 200, 400 and 600 mg/kg b.wt and acetaminophen. Blood samples were taken for estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), bilirubin, total protein, albumin, serum urea, creatinine, malondialdehyde (MDA), catalase and glutathione. Significant increases ($P<0.05$) in the serum liver enzymes activities, bilirubin (total and direct), urea and creatinine were observed, while total proteins and albumin were significantly reduced ($P<0.05$) in the acetaminophen-treated group when compared to normal control. The extract-treated groups revealed significant ($P<0.05$) reduction in the activities of serum liver enzymes, bilirubin (total and direct), urea and creatinine values, with a significant ($P<0.05$) improvement in serum total proteins and albumin concentrations. The hepatoprotective and nephroprotective effects observed are probably due to the antioxidant activity of the extract. In conclusion methanol extract of *Burkea. africana* stem bark may possess hepatoprotective and nephroprotective activities and could be useful in the treatment of hepatitis and nephritis.

***Keywords*:** Antioxidant, Hepatoprotection, Acetaminophen, *Burkea africana*, Nephroprotection.

Introduction

The liver is a very important organ in the body owing to its numerous functions. This vital organ is however exposed to attack by toxic substances in its attempt to bio-transform and or detoxify the chemical substances in the body. Scientists have paid attention in finding drug that can help preserve the functional integrity of the liver parenchyma in the face of toxic challenges.[1] Acetaminophen, an analgesic, and antipyretic drug has been known to cause severe liver damage when overdose is ingested.[2] Its toxicity results from the accumulation of a toxic metabolite known as N-acetyl-P-benzoquinone imine (NAPQI), which is produced by the activity of cytochrome P450 enzyme involved in its metabolism.[3] NAPQI is detoxified by conjugating with glutathione. Excess NAPQI binding to membrane lipids and proteins causes lipid peroxidation and cross-linking of membrane proteins.[4] Liver damage is seen as cell necrosis of hepatocytes and liver cirrhosis. Hepatoprotection results from the ability to prevent oxidative activity, inhibition of cytochrome P450 enzymes, reduced Lipid peroxidation, an increase in level of glutathione or its reducing equivalent.[1] Antioxidants enzymes include superoxide dismutase (SOD), peroxidases (Glutathione peroxidases),[5] glutathione reductase, glutathione S-transferases, ascorbate peroxidase and catalase.[6] They act by inhibiting or neutralizing reactive oxygen species (ROS) formation and also detoxify products of lipid peroxidation such as malondialdehyde (MDA). Glutathione and its reducing equivalent, ascorbate, together with enzymes (mono and dihydroascorbate reductase, dehydroascorbate reductase and glutathione reductase) are useful for the removal of ROS. Synthetic liver and or kidney protective drugs are bewildered with a lot of side effects and are also expensive. Scientists have undertaken to find better, safer and cheaper liver protective drugs from natural sources such as medicinal plants. *Burkea africana* (Ceaesalpiniaceae) also known as Wild Syringa is a deciduous flat-topped plant which is readily found in tropical and sub-tropical region of Africa.[7] It has been widely used in folkloric medicine in Africa for the treatment of several disease conditions including hepatitis and other liver related disorders. It is also used for the treatment of fever, scabies, stomach aches, abscesses, edema, syphilis, ulcers, wounds, cough, catarrh, menorrhea, pneumonia, epilepsy, bloody diarrhea, gonorrhea, toothache, headache, poisoning, skin diseases and inflammation of the tongue and gums.[8,9] Studies have revealed that *Burkea africana* stem bark has in-vitro antioxidant,[10,11] molluscicidal,[12] antibacterial, antifungal,[13] and antiviral (anti-influenza) activities.[14] The anti-cancer, antidiarrheal, sedative and anxiolytic properties of the plant have been reported.[7] *Burkea africana* stem bark have been shown to have significant cell protecting effect *in-vitro*.[15] Phytochemical screening of the methanol and aqueous stem bark extract of *Burkea africana* revealed constituents such as tannins, saponins, cardiac glycosides, alkaloids, flavonoids, terpenoids, coumarins, plobatannins, sterols and terpenes in the bark.[13] The presence of two major proanthocyanidins components (fisetinidol-(4α- 8)-catechin 3-gallate and bisfisetinidol-

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(4α-6, 4α- 8)-catechin 3-gallate 93) as well as *β*-sitosterol, Harman-type alkaloids, tryptamine and monomeric flavan-3-ols (catechin, epicatechin and fisetinidol) have been reported.[10] Empirical evidence is lacking on the liver and kidney protection as well as the *in-vivo* antioxidant properties of this plant to substantiate its use in folkloric medicine. Therefore, this study was undertaken to investigate possible scientific evidence of the hepatoprotective, nephroprotective and in-vivo antioxidant properties of this plant.

Materials and Methods

Chemicals and reagents
Commercial assay kits used for the determination of serum biochemical parameters were obtained from Dialab, Wiener Neudorf, Australia. Spectrophotometer (CHEM-5V3; Erba, Mannheim, Germany) was used to take the absorbance reading. A clinical refractometer (Atago; Bellview, WA, USA) was used to estimate serum total protein. Silymarin (Megalex 70® Alexandria co. Pharmaceuticals and Chemical Industries, Alexandria-Egypt), Acetaminophen (M & B Paracetamol® May and Baker Nigeria Plc.), Pentobarbitone sodium (Kyron Laboratories Ltd, Benrose) where the standard drugs used. The solvent reagent used was methanol of good analytical grade.

Collection and Identification of Plant Material
Burkea africana stem barks were freshly collected from the wild in Makurdi Local Government Area of Benue State, Nigeria and identified by a plant taxonomist of the Department of forestry, Federal University of Agriculture, Makurdi and a voucher specimen with the number UAM/FH/0326 was deposited in their herbarium.

Preparation of extract
The stem bark of *Burkea africana* was air-dried at room temperature and pulverized using a grinding machine. The powdered plant material was stored in an air-tight polythene bags at room temperature until needed for use. Extraction was done by cold maceration at room temperature using 80% methanol and the mixture was vigorously shaken at every 2 hours interval. It was then filtered after 48 hours using Whatman filter paper size 1. The filtrate was concentrated *in vacuum* using a Rotary evaporator (Buchi, UK). The extract was stored in the refrigerator at 4^0C until required for use.

Experimental Animals
Albino rats of both sexes weighing 120-180 g were obtained from a private commercial farm in Nsukka, Enugu State, Nigeria. The rats were acclimatized in aluminum cages and housed in the animal house of the Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka for a period of 7 days. They were fed with commercially prepared poultry feed pellets (Topfeed®), produced by Premier Feed Mill Nigeria (FMN) and clean drinking water provided *ad-libitum*. The experimental design used in this investigation was approved by the Ethical Committee of the Department of Veterinary Physiology and Pharmacology of the University of Nigeria Nsukka (Approval Reference Number: FVM-VPP-UNN-IACUC-2019-039) in line with good laboratory animal practice regulations as well as the principles of laboratory animal use and care as enshrined by the Natural Research Council guidelines of 2011.

Effect of pretreatment with methanol extract of Burkea africana stem bark on acetaminophen-induced liver damage
Thirty-six (36) rats of both sexes, weighing between 120-180 g were randomly assigned to six (6) groups of six (6) rats each. Groups 1 (Normal control) received distilled water only for seven (7) days. Groups 2 (Positive control) was pretreated with Silymarin once daily for the same period, after which acetaminophen (2000 mg/kg b.wt) was administered one hour post administration of silymarin. Group 3 (negative control) was also administered distilled for seven (7) days, after which acetaminophen (2000 mg/kg b. wt) was administered. Groups 4, 5 and 6 were pretreated with methanol extract of *Burkea africana* stem bark (MEBASB) at 200, 400 and 600 mg/kg body weight respectively, once daily, for seven (7) days. At day 7 one-hour post administration of MEBASB, acetaminophen was administered at 2000 mg/kg body weight by oral gavage. A single dose of pentobarbitone sodium (35 mg/kg body weight) was thereafter given to all groups intraperitoneally (IP) using 1 mL syringe after 24 hours post acetaminophen administration. The time of injection, the time of sleep (when righting reflex is lost) and the time of awakening (when righting reflex is regaining) were recorded. The differences between the time of sleep and the time of awakening were calculated for each animal in the groups. Twenty four (24) hours after pentobarbitone injection, 2 ml of blood was collected from retro-bulbar plexus of the medial canthus of the eye by capillary tube puncture, for serum biochemistry (ALT, AST, ALP, urea, creatinine, serum protein profile, and bilirubin). Oxidative stress markers (malondialdehyde, catalase, and reduced glutathione) were also assayed.

Determination of serum biochemical parameters
Serum biochemical parameters were assayed following standard laboratory procedures using commercial diagnostic kits (Dialab Wiener Neudorf, Austria).
Liver enzymes (ALT, AST, and ALP) assays were carried out by the method described by Thomas.[16] Urea concentration was assayed by the method described by Burtis and Ashwood.[17] Creatinine was assayed by the method of Newman and Price.[18] Bilirubin (total and direct) were determined as described by Thomas, [16] while indirect bilirubin was calculated by finding the difference between total and direct bilirubin. Total protein was determined by the refractometry method using a clinical refractometer as described by Johnson *et al*.[19] Albumin level was determined by the method of Thomas, [16] while Globulin was determined by subtracting the concentration of albumin from the serum total protein concentrations.

Determination of oxidative stress markers
Catalase activity was determined according to the method described by Góth.[20] Reduced glutathione was determined by the potassium iodate method of Moron *et al*.[21] Serum malondialdehyde (MDA) concentrations was assayed by the thiobarbituric acid method of Stocks and Dormandy.[22]

Statistical analysis
Data from this study were analyzed using one-way analysis of variance (ANOVA) at significance level of 5% ($P< 0.05$). Results were expressed descriptively as mean ± standard error of the mean (S.E.M). The variant means were separated using Duncan's Multiple Range post hoc test.

Results and Discussion

Methanol extract of *Burkea africana* stem bark was investigated for its hepatoprotective, nephroprotective, and *in-vivo* antioxidant properties in rats. Hepatic injury as well as oxidative stress induced by acetaminophen were assessed by estimation of serum liver enzymes, bilirubin and serum total protein and some oxidative stress markers such as malondialdehyde (MDA), catalase and glutathione (GSH). Pentobarbitone induced hypnosis (sleep) is a functional parameter that assess the ability of the liver cells to effectively metabolize barbiturates. The duration of sleep induced by barbiturates is directly proportional to the rate of barbiturate metabolism.[3] In this study, the duration of sleep was significantly prolonged ($P<0.05$) in the group administered only acetaminophen (Negative control) when compared to the normal control (Figure 1). This suggests hepatic impairment which is due to toxic effect of acetaminophen overdose, as was also shown in the work of Gupta and Dixit.[23] Damage to the liver results in a decrease in microsomal drug metabolizing enzymes (MDME), which decreases the metabolism of barbiturate, therefore producing prolonged hypnosis. The silymarin (a known hepatoprotective drug) pre-treated group showed significantly reduced ($P<0.05$) sleeping time when compared to the acetaminophen group. The methanol extract of *Burkea africana* stem bark showed a significant ($P<0.05$) dose-

dependent decrease in the duration of hypnosis, compared to acetaminophen group. The group administered 600 mg/kg of the extract had the lowest duration of hypnosis. This is an indication that the extract had no inhibitory effect on microsomal drug metabolizing enzymes (MDME). MDME (Cytochromes P-450) are responsible for metabolism of barbiturate in the liver.[3] Decreased sleeping time at all doses is a clear indication of the ability of the extract to protect the liver against acetaminophen poisoning. Since barbiturates are largely metabolized in the liver, a healthy liver will lead to a shortened duration of hypnosis induced by barbiturates at a given dose. This is because the amount of hypnotic broken down per unit time by MDME will increase.[23]

The group administered only acetaminophen (negative control) had significantly increased serum AST, ALT and ALP activities of 214.73 ± 13.78, 540.84 ± 0.34 and 671.32 ± 0.83 IU/L, respectively. They were significantly (P<0.05) higher than that of the normal control with values 83.40 ± 1.45 IU/L, 67.86 ± 0.77 IU/L and 464.84 ± 7.72 IU/L, respectively. Animals that received silymarin (100 mg/kg b.wt) had serum AST, ALT, and ALP activities significantly reduced (P<0.05) when compared to the acetaminophen-treated group (Table 1). Pre-treatment with MEBASB showed significant (P<0.05) and dose-dependent decreases in serum activities of AST, ALT, and ALP at the dose levels of 200, 400 and 600 mg/kg b.wt when compared with the acetaminophen-treated groups. The extract at 600 mg/kg b.wt had more significant (P<0.05) effect on serum AST, ALT and ALP activities of 144.00 ± 1.50, 131.26 ± 0.80 and 563.54 ± 2.93 IU/L, respectively. Although acetaminophen is widely used as an antipyretic, analgesic, and anti-inflammatory agent without toxicity at recommended dose, it causes severe liver injury at higher doses (Overdose), and has been used by many researchers in hepatoprotective study model.[24,25] The acute severe liver necrosis which is as a result of excess production of the toxic metabolite, N-acetyl-P-benzoquinone imine (NAPQI) of acetaminophen, leads to a fall in glutathione concentration.[26,27] Consequently, excess NAPQI not conjugated for excretion by glutathione, binds to the sulfhydryl group of proteins forming adduct proteins, resulting in hepatocellular necrosis.[26,2] A common feature in hepatocellular necrosis is the leakage of cellular enzymes which are found within the cytoplasm as well as increase in serum bilirubin and decrease in serum total protein concentrations.[28] This results in an increase in the activity of serum liver enzymes such as ALT, AST, and ALP, which researchers have often used as markers of hepatic injury.[29] Increases in serum liver enzymes are estimated by measuring their activity which is seen in the increase in the concentration of the products of the biochemical reactions they catalyze.[30] In this study therefore, the significant (P<0.05) increase in the serum activities of ALT, AST, ALP and serum bilirubin concentration {total bilirubin (0.43 ± 0.01 mg/dL), direct bilirubin (0.04 ± 0.00 mg/dL) and indirect bilirubin (0.39 ± 0.01 mg/dL)} with a decrease in serum total protein value (6.18 ± 0.10 g/dL) of acetaminophen-treated group (Table 1) as well as the significantly (P<0.05) prolonged pentobarbitone sleeping time (Figure 1), were indications of severe liver necrosis caused by acetaminophen overdose.[30] The hepatoprotective effect *Burkea africana* stem bark was hence evident in the significant (P<0.05) reduction in the activities of serum ALT, AST, ALP (Table 1). Pre-treatment with the extract showed significant (P<0.05) increase in TP and globulin values, 6.72 ± 0.17 g/dL and 3.44 ± 0.34 g/dL, respevtively at 600 mg/kg b.wt when compared with the acetaminophen-treated group. Total bilirubin and indirect bilirubin values of MEBASB (200, 400 and 600 mg/kg b.wt) pre-treated groups showed significant reductions (P<0.05) when compared with the acetaminophen-treated group. Bilirubin a by-product of hemoglobin metabolism, is conjugated and excreted by the liver.[31] Bilirubin assay is a highly sensitive test that is used to evaluate the functional status and extent of necrosis of the liver.[31] In a condition of hepatic necrosis, it has been reported in previous studies that hepatic parenchymal cells abnormalities are the causes of increased total and indirect bilirubin because of its inability to conjugate and excrete bilirubin into bile.[2] In this study, the decrease in total and indirect bilirubin, with a corresponding increase in direct bilirubin is an indication of the maintenance of hepatic function by the extract. The improvement in serum total protein and globulin concentration indicates the ability of the extract to maintain stable and functional hepatocytes, therefore retaining their capacity to synthesize proteins. This is in agreement with the findings on silymarin, a hepatoprotevtive agent, which has been known to stimulate protein synthesis.[32]

Malondialdehyde (MDA) showed significant (P<0.05) increase in acetaminophen-treated group (0.08±0.00 mg/mL) when compared with normal control (0.04 ± 0.00 mg/mL). There was also a significant decrease (P<0.05) in MDA of MEBASB treated groups at all doses (0.05 ± 0.00, 0.05 ± 0.00 and 0.05 ± 0.00 mg/mL) when compared with the acetaminophen group (0.08 ± 0.00 mg/mL) (Table 2). Acetaminophen toxicity also gives rise to reactive free radicals from oxidative reaction of cytochrome P450. The free radicals react covalently with proteins and polyunsaturated membrane fatty acids causing lipid peroxidation, cellular deformity, mitochondrial dysfunction and cell apoptosis.[33] The extract exerted its protective effect probably by eliciting hepatic regeneration[25] which accounts for the improvement in protein synthesis and also enhanced the capacity of the liver to metabolize and excrete excess acetaminophen. *Burkea africana* stem bark have been known to have in-vitro antioxidant activity owing to the presence of wild range of phyto-constituents such as phenols, flavonoids, and alkaloids with free radical scavenging ability.[13,7] This, therefore, could prevent peroxidation of membrane lipids which are rich in polyunsaturated fatty acids thereby preserving the structural integrity of hepatocyte.[25] This was substantiated by the in-vivo antioxidant activity observed as MDA decreased. Catalase and glutathione (GSH) decreased significantly (P<0.05) in acetaminophen-treated group with values of 5.41 ± 0.77 IU/L and 5.51 ± 0.11 mg/mL, respectively when compared with the normal control with values of 26.10 ± 2.69 IU/L and 7.32 ± 0.07 mg/mL. There was a dose-dependent significant (P<0.05) increase in catalase (5.95 ± 0.20, 6.47 ± 0.53 and 8.89 ± 0.16 IU/L) at 200, 400 and 600 mg/kg b.wt of MEBASB respectively, when compared with the acetaminophen-treated group (5.41 ± 0.77 IU/L). Serum GSH values of 6.75 ± 0.29 and 6.88 ± 0.21 mg/mL at the dose of 400 and 600 mg/kg b.wt were significantly higher (P<0.05) when compared with the acetaminophen-treated group (5.51 ± 0.11 mg/mL). The highly reactive metabolite of acetaminophen (NAPQI) is, in normal condition, detoxified by conjugation to form a nontoxic compound known as mercapturic acid, by reduced glutathione (GSH), which is a natural antioxidant present in the liver.[34] In this study, glutathione was elevated on treatment with MEBASB at all doses (Table 2) which could substantiate the reduction in MDA indicative of the ameliorative effect of the extract on oxidative stress induced by acetaminophen toxicity.

Figure 2 and Figure 3 represents serum urea and creatinine concentrations, respectively. Serum urea and creatinine levels of the acetaminophen-treated group (45.26 ± 0.35 mg/dL and 0.72 ± 0.02 mg/dL, respectively) were significantly ($p < 0.05$) increased compared with the normal control (40.86 ± 0.35 and 0.52 ± 0.00 mg/dL respectively). This result points to nephrotoxic effect of acetaminophen. There was a significant ($p < 0.05$) decrease in serum urea (43.08 ± 1.00, 36.50 ± 0.82 and 35.92 ± 0.49 mg/dL) and creatinine (0.58 ± 0.02, 0.41 ± 0.03 and 0.37 ± 0.01 mg/dL) at 200, 400 and 600 mg/kg b.wt of MEBASB, respectively when compared with acetaminophen-treated group. Acetaminophen induces both acute and chronic nephrotoxicity especially at very high doses of 10-15 g and it is seen as necrosis and damage to proximal convoluted tubules.[35,36] At lower doses (0.5-1 g), it has been demonstrated to cause nephropathy sequel to hepatic damage.[35] Acetaminophen-induced nephrotoxicity often results in wide range of metabolic disorders with serum electrolytes, urea and creatinine changes.[33] Elevations in the serum urea and creatinine are reliable and well-documented parameters for investigating renal insufficiency in animals and man.[37] Blood urea nitrogen (BUN), a product of protein/amino acid metabolism is excreted in urine. An increase in serum levels of urea (uremia) occurs as a result of an imbalance in urea production and elimination, other causes or elevated serum urea include high protein diet, increased catabolism due to starvation, tissue damage, amongst others.[37] Creatinine, therefore, is elevated in serum due to increased tissue creatine breakdown. Serum creatinine concentration is determined by many factors including muscle mass,

protein consumption, and breakdown.[37,38] Serum creatinine concentration is, therefore, a more reliable marker of renal function.[37,2] The reduction in serum urea and creatinine on pre-treatment with the extract shows that it was able to stabilize the kidney parenchymal cells, thereby retaining the excretory function of the nephrons (glomeruli, the surrounding Bowman's capsule and tubules). Lowering of urea and creatinine has been used as a definite marker for the evaluation of nephroprotective effect of a drug.[39] Results of this work agree with that of Adeneye *et al*.[37]

Conclusion

It can be concluded therefore, that methanol extract of *Burkea africana* stem bark possesses possible hepatoprotective and nephroprotective potentials, which could be attributed to its antioxidant properties and amelioration of the oxidative effect of acetaminophen on the liver and kidney cells. This could be the rationale for the folkloric use of this plant by the Tiv people of Benue state. Further investigation is recommended to elucidate the active principles in this plant that are responsible for these activities and also the actual mechanisms of action by which these activities are brought about.

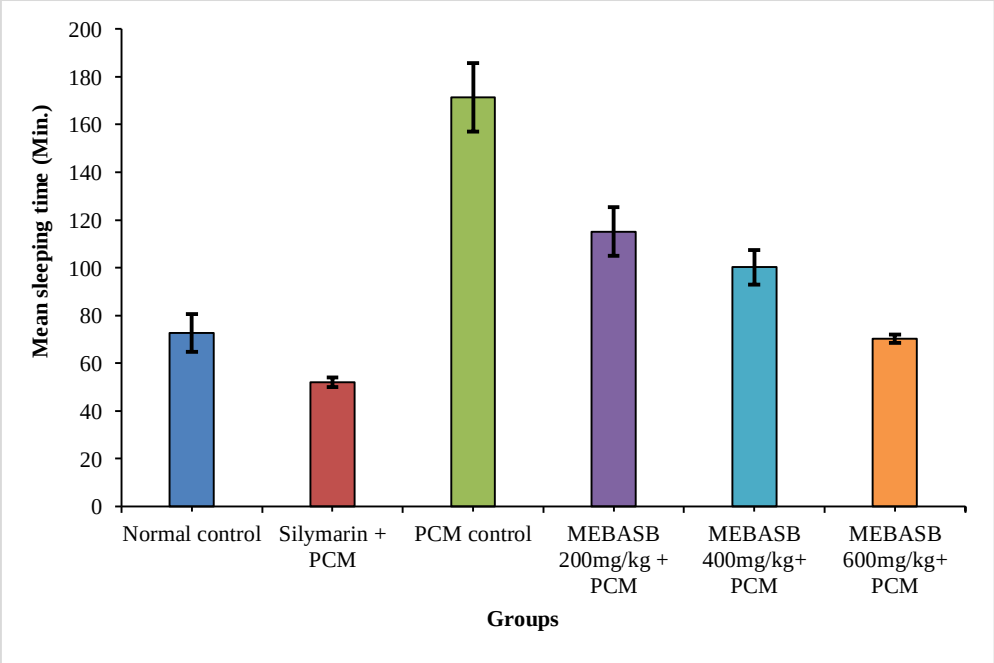

Figure 1: Pentobarbitone-induced hypnosis in acetaminophen-poisoned rats pretreated with MEBASB
Bars with different alphabet are significantly ($p < 0.05$) different. Normal control (Group 1), PCM = Paracetamol (Group 2), Silymarin (Group 3), MEBASB (Methanol extract of *Burkea africana* stem bark) 200 mg/kg (Group 4), 400 mg/kg (Group 5), 600 mg/kg (Group 6).

Table 1: Serum biochemical parameters of acetaminophen-poisoned rats pretreated with MEBASB

Parameters	Normal control	Silymarin + PCM	PCM Control	MEBASB + PCM		
				200 mg/kg	400 mg/kg	600 mg/kg
AST (IU/L)	83.40 ± 1.45^{a}	126.88 ± 1.89^{b}	214.73 ± 13.78^{e}	194.60 ± 1.57^{d}	148.64 ± 0.26^{c}	144.00 ± 1.50^{c}
ALT (IU/L)	67.86 ± 0.77^{a}	129.77 ± 1.21^{b}	540.84 ± 0.34^{e}	154.89 ± 1.14^{d}	134.37 ± 0.40^{c}	131.26 ± 0.80^{b}
ALP (IU/L)	464.84 ± 7.72^{a}	514.63 ± 0.89^{b}	671.32 ± 0.83^{e}	663.04 ± 0.82^{e}	612.88 ± 2.47^{d}	563.54 ± 2.93^{c}
T.P (g/dL)	7.25 ± 0.10^{c}	7.52 ± 0.14^{c}	6.18 ± 0.10^{a}	6.46 ± 0.12^{ab}	6.50 ± 0.09^{ab}	6.72 ± 0.17^{b}
ALB (g/dL)	3.78 ± 780.11^{b}	3.46 ± 0.11^{ab}	3.42 ± 0.09^{ab}	3.28 ± 0.09^{a}	3.52 ± 0.10^{ab}	3.58 ± 0.09^{ab}
GLB (g/dL)	3.47 ± 0.12^{b}	3.74 ± 0.30^{b}	2.94 ± 0.22^{a}	2.88 ± 0.22^{a}	2.98 ± 0.13^{ab}	3.44 ± 0.34^{b}
TBIL (mg/dL)	0.17 ± 0.01^{a}	0.17 ± 0.01^{a}	0.43 ± 0.01^{c}	0.28 ± 0.04^{b}	0.25 ± 0.02^{b}	0.18 ± 0.00^{a}
DBIL (mg/dL)	0.03 ± 0.00^{a}	0.05 ± 0.01^{bc}	0.04 ± 0.00^{ab}	0.03 ± 0.01^{a}	0.05 ± 0.01^{bc}	0.06 ± 0.00^{c}
InDBIL (mg/dL)	0.12 ± 0.01^{a}	0.14 ± 0.01^{a}	0.39 ± 0.01^{c}	0.25 ± 0.04^{b}	0.20 ± 0.02^{b}	0.12 ± 0.00^{a}

Values are Mean ± S.E.M, n = 6. Values with different superscripts along the same row are significantly different at P<0.05. PCM Paracetamol (Acetaminophen), MEBASB-Methanolic extract of *Burkea africana* stem bark.

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Table 2: Oxidative stress markers in acetaminophen-poisoned rats pretreated with MEBASB

Groups	MDA (mg/mL)	Catalase (IU/L)	GSH (mg/mL)
Normal control	0.04 ± 0.00^{a}	26.10 ± 2.69^{c}	7.32 ± 0.07^{b}
Silymarin + PCM	0.04 ± 0.01^{a}	19.09 ± 1.33^{b}	7.27 ± 0.13^{b}
PCM Control	0.08 ± 0.00^{d}	5.41 ± 0.77^{c}	5.51 ± 0.11^{a}
MEBASB 200 mg/kg + PCM	0.05 ± 0.00^{c}	5.95 ± 0.20^{a}	5.78 ± 0.26^{a}
MEBASB 400 mg/kg + PCM	0.05 ± 0.00^{bc}	6.47 ± 0.53^{a}	6.75 ± 0.29^{b}
MEBASB 600 mg/kg + PCM	0.05 ± 0.00^{ab}	8.89 ± 0.16^{a}	6.88 ± 0.21^{b}

Values are Mean ± S.E.M, n = 6. Values with different superscripts in the same column are significantly different at $p < 0.05$ MDA - Malondialdehyde, GSH-Glutathione, PCM-Paracetamol (Acetaminophen), MEBASB-Methanol extract of *Burkea africana* stem bark.

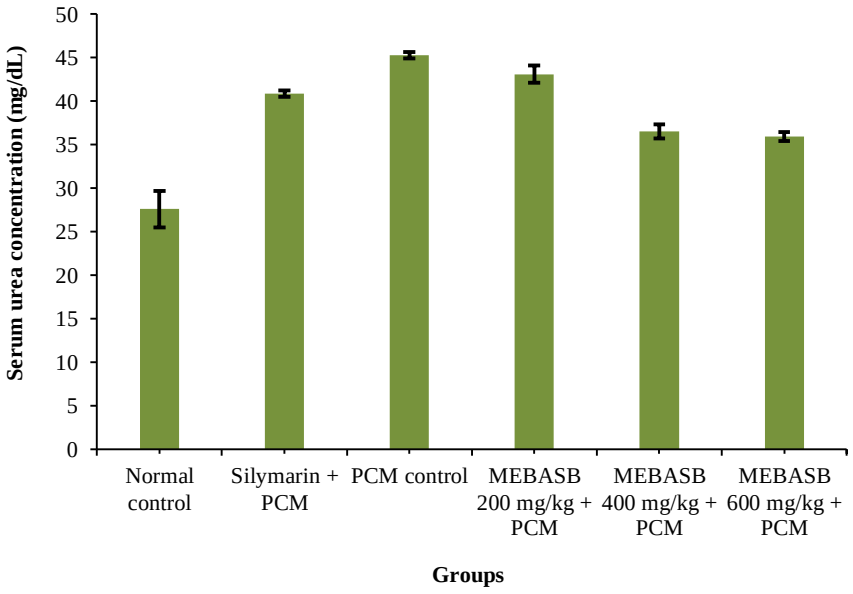

Figure 2: Serum urea concentration of acetaminophen-poisoned rats pretreated with MEBASB
Bars with different alphabet are significantly ($p < 0.05$) different. Normal control (Group 1), PCM = Paracetamol (Group 2), Silymarin (Group 3), MEBASB (Methanol extract of *Burkea africana* stem bark) 200mg/kg (Group 4), 400mg/kg (Group 5), 600mg/kg (Group 6).

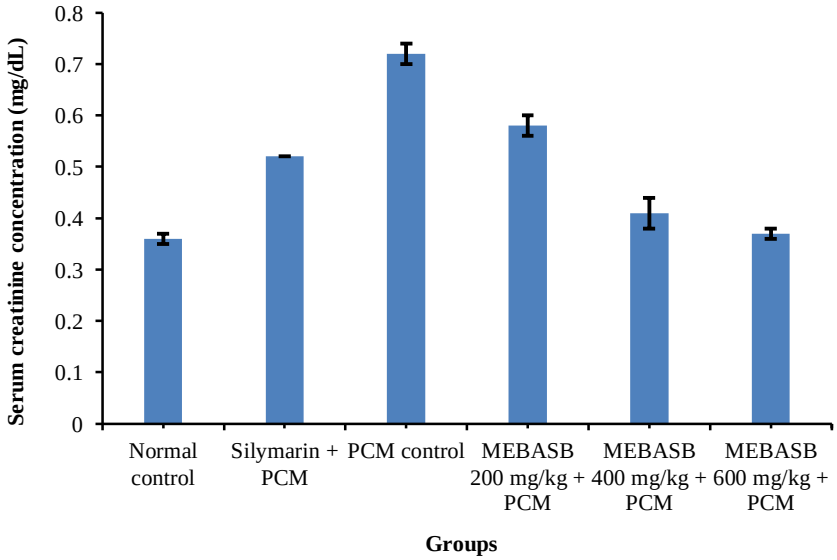

Figure 3: Serum creatinine concentration of acetaminophen-poisoned rats pretreated with MEBASB
Bars with different alphabet are significantly ($p < 0.05$) different. Normal control (Group 1), PCM = Paracetamol (Group 2), Silymarin (Group 3), MEBASB (Methanol extract of *Burkea africana* stem bark) 200 mg/kg (Group 4), 400 mg/kg (Group 5), 600 mg/kg (Group 6).

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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