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Protective effects of *Alchornea cordifolia* and *Byrsocarpus coccineus* leaf extracts against diclofenac-induced oxidative stress and hepatorenal injuries in Wistar rats

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Background: Traditional medicine practitioners claimed that plant extracts are effective in the management of liver and kidney diseases in humans without scientific evidence. Thus, this study evaluated the protective effect of *Alchornea cordifolia* extract (ACE) and *Byrsocarpus coccineus* extract (BCE) against diclofenac sodium (DCF) induced oxidative stress and hepatorenal injuries in rats and compared their efficacies. Twenty four rats were divided into 4 groups with 6 rats per group. Normal saline was given to the rats in group 1 while those in groups 3 and 4 were treated with 250 mg/kg ACE and BCE respectively for 28 days by oral gavages. The rats in groups 2 to 4 were injected with 10 mg/kg DCF in the last 7 days of treatment. Blood was collected from rats, serum was separated from the blood and used for estimations of hepatorenal injury markers while the homogenized tissue supernatants were used for assays of oxidative stress markers.

Results: There was a significant ($p<0.01$) increase in the levels of ALT, AST, GGT, MDA, creatinine and BUN but a significant ($p<0.01$) decrease in the levels of SOD, CAT, GPx, GST, GSH and G6Pase of DCF-exposed rats when compared with normal control. However, treatment of DCF-injected rats with ACE and BCE significantly ($p<0.01$) elevated the levels of SOD, CAT, GPx, GST, GSH, and G6Pase but significantly ($p<0.01$) reduced the levels of ALT, AST, GGT, MDA, creatinine and BUN when compared with DCF control.

Conclusion: These findings showed that treatment with ACE and BCE may have protective effects against DCF-induced hepatorenal damage in rats, attributed to their phytochemicals but ACE has greater activity than BCE.

Key words: Antioxidants, Hepatotoxicity, Inflammation, Nephrotoxicity, Oxidative stress

Non-steroidal anti-inflammatory drugs (NSAIDs) are in a class of drugs used in the treatment of musculoskeletal disorders, inflammation and pain but found to have adverse effects on living tissues (Altman *et al*., 2015). Diclofenac sodium (DCF) is one of the NSAIDs, and a derivative of phenyl acetic acid, which may cause adverse effects in the liver and kidney of mammals (Ogbe *et al*., 2019). It can inhibit an enzyme called cyclooxygenase (COX) with anti-inflammatory, analgesic and antipyretic properties but has greater potency against COX-2 than COX-1 (Altman *et al*., 2015). The adverse effects of DCF was attributed to the production of highly reactive metabolites, leading to the accumulation of oxidants and free radicals, known as reactive oxygen species (ROS). The resultant condition is known as oxidative stress, and may lead to hepato-renal injuries in rats (Adeyemi and Olayaki, 2018). Oxidative stress occurs as a result of disruption in the balance between the ROS such as nitric oxide radical ($NO^{\cdot}$), hydrogen peroxide (H_2O_2), super oxide anion radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), and the antioxidants that counteract their effects such as reduced glutathione (Nwanna, 2021; Ogbe *et al*., 2022).

The biochemical transformation of xenobiotics or therapeutic drugs by the liver, and to a lesser extent by the kidneys of animals, make the organs susceptible to the toxic effects of these foreign compounds (Ogbe *et al*., 2019). These organs are vulnerable to tissue damage due to oxidative stress caused by the excess of reactive nitrogen species (RNS) or ROS, which attack polyunsaturated fatty acids (PUFAs) of cellular membrane lipids, leading to lipid peroxidation, that may cause functional loss and modification of proteins (Nwanna, 2021; Agu *et al*., 2022). Hepatic and renal disorders caused by drugs occur as a result of adverse events in the animal tissues, which are commonly referred to as hepatotoxicity and nephrotoxicity respectively (Hussain *et al*., 2019). Presently, liver and kidney diseases are major global health challenges, affecting all nations of the world but the incidence of each disease varies from one country to another (Owumi and Dim, 2019; Raghuvanshi *et al*., 2020).

Alchornea cordifolia (Schumach. & Thonn.) Müll.Arg. - belonging to the family, Euphorbiaceae, is a sprawling, much-branched short tree, which grows to about 8 m high, and distributed throughout tropical Africa. It is called ‘Christmas bush’ (English), ‘Owii’ (Idoma), and ‘Bambani’ in Hausa (Agishi, 2010). The leaves are simple, triangular, about 1.5 mm long, acute, with petiole 5-15 cm long, blade ovate to elliptical-ovate 10-25 cm × 7-15 cm (Mavar-Manga *et al*., 2007). The parts of this plant such as leaves, stem, and roots are used as pain killers, sedatives, for treatment of stomach aches, naso-pharyngeal infections, diarrhoea, dysentery, haemorrhoids, parasitic and bacterial infections (Okeke *et al*., 1999). It was reported that ACE has anti-inflammatory (Osadebe and Okoye, 2003), hepatoprotective (Olaleye *et al*., 2006), antimicrobial (Ebi, 2001) and antioxidant (Olaleye and Rocha, 2008) activities.

Byrsocarpus coccineus Schum. & Thonn., belonging to the family, Connaraceae - is a scandent shrub of tropical Savana found across West Africa, and grows wildly but could be cultivated (Akindele *et al*., 2014). It is also called “Short-pod” or “Crimson thyme” (English), “Onyilakwechi” (Idoma), “Tsaamiyar-kasa” (Hausa), “Oke-abolo” (Igbo), “Anune-chigh” (Tiv), and “Orikoteni” (Yoruba) (Agishi 2010). The preparations from leaves, roots, and whole plant are used in the African traditional medicine for the treatment of jaundice, ear ache, dysentery, and sexually transmitted diseases (Akindele *et al*., 2014). Scientific evaluation of BCE showed that the aqueous leaf extract has analgesic (Akindele and Adeyemi, 2006), anti-inflammatory (Akindele and Adeyemi, 2007), antimicrobial (Ahmadu *et al*., 2006), anxiolytic and sedative (Akindele and Adeyemi, 2010) activities.

The traditional medicine practitioners claimed that the extracts from these plants under study can cure liver and kidney diseases in humans but lack scientific evidence. Our literature search showed that there is paucity of scientific reports on the protective effects of these two plant extracts against hepatorenal oxidative injury in drug-exposed rats. Therefore, this study was designed to evaluate the protective effects of ACE and BCE against DCF-induced oxidative stress and

hepatorenal injuries in Wistar rats, and to compare their efficacies.

MATERIALS AND METHODS

Chemicals

The DCF is an injectable liquid purchased from the North China Pharmaceutical Co. Ltd, 115 Hainan Road, Shijiazhuang, Hebei, China while 2, 2 - diphenyl-1-picryl hydrazine (DPPH) was purchased from Sigma-Aldrich, U.S.A. All the reagents used are of analytical grade. The biochemical tests reagent kits were purchased from Randox Laboratories Ltd., United Kingdom.

Preparation of diclofenac sodium

Each 3 ml ampoule contains 75 mg of DCF solution. A single dose of the drug was suspended in 0.2 ml of normal saline (0.9 g/dL NaCl), which served as a vehicle for the drug.

Preparation of *A. cordifolia* and *B. coccineus* leaf extracts

A. cordifolia and *B. coccineus* leaves were harvested from a forest in Otukpa, Benue state, Nigeria. They were identified and authenticated by Mr. Mark Uleh, a Lecturer/Taxonomist in the Department of Forestry and Forest Products, Federal University of Agriculture, Makurdi, Nigeria. The voucher specimens have been deposited in the College of Forestry herbarium, and given the voucher numbers; *A. cordifolia* - FH/0206 and *B. coccineus* - FH/0138. The plant leaves were dried at room temperature for about three weeks, crushed to fine particles with mortar and pestle, and sieved with a porcelain sieve. Plant extracts were prepared according to a method previously described by Abu and Uchendu (2010). Briefly, the aqueous ethanol solvent was prepared by mixing 800 ml distilled water with 200 ml absolute ethanol to produce a mixture with ratio 4:1. Thereafter, 200 g of pulverized sample was macerated in 1200 ml of aqueous ethanol and left for 72 hours. The mixture was sieved with a clean piece of white cloth, and filtered with Whatman no. 1 filter papers. The filtrate was placed in a water bath at 50 ^{0}C, and the extract was dried to a constant weight in a desiccator.

Experimental animals and management

The Wistar strain of albino rats were obtained from the Animal house, College of Health sciences, Benue State University, Makurdi, Nigeria, and were fed to grow up to maturity before being used for this study. They were kept to acclimatize for about two weeks in the Department of Veterinary Physiology and Biochemistry research laboratory, Federal University of Agriculture, Makurdi, Nigeria; under environmental conditions of 12 h dark and 12 h light cycle, with an average temperature of 29 ^{0}C. They were fed with animal feeds, produced by the Grand Cereal and Oil Mills Ltd, Jos, Nigeria, and clean water *ad libitum.* The rats were handled with care according to the International guidelines and principles for biomedical research involving animals (CIOMS, 1985).

Phytochemical analyses of *A. cordifolia* and *B. coccineus* leaf extracts

Aqueous ethanol extracts of *A. cordifolia* and *B. coccineus* leaves were prepared by maceration of 10 g of pulverized sample in a mixture of 100 ml distilled water and 50 ml ethanol (2:1) in a conical flask, covered with aluminum foil. After 48 h the mixture was filtered with Whatman no. filter papers and the filtrate tested for phytochemical components, according to the methods previously described by Edeoga *et al*. (2005), and Kumar *et al*. (2007).

Scavenging effect of *A. cordifolia* and *B. coccineus* leaf extracts on 2, 2-diphenyl-1-picryl hydrazyl radicals

The radical scavenging effects of ACE and BCE on DPPH radicals were evaluated according to a modified form of a method previously described by Ayoola *et al*. (2008). The ACE and BCE concentrations were prepared as 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/ml in methanol. Similar concentrations of ascorbic acid were prepared, and used as the antioxidant standard. Then, 1 ml of plant extract or ascorbic acid was placed in a test tube and 3 ml of methanol was added, followed by 0.5 ml of 1 mM DPPH in methanol. The decrease in absorption was measured by UV-Visible spectrophotometer at 517 nm after 10 minutes. A blank solution was prepared containing the same quantity of methanol and DPPH. The actual decrease in absorption was measured against the blank, and the percentage inhibition was calculated. All the tests were performed in duplicates and the mean values were determined. The

scavenging effects of ACE and BCE were expressed as percentage inhibition of DPPH radicals using the equation below:

Percentage inhibition (%) =

$$\frac{Absorbance\ of\ blank - Absorbance\ of\ test}{Absorbance\ of\ blank} \times 100$$

Animal groups and treatments

Twenty four adult Wistar rats of either sex, weighing 200 - 250 g were randomly divided into 4 groups, with 6 rats in a group.

Group 1 rats received 0.2 ml of normal saline by intramuscular (i.m.) route for 7 days, and served as normal control.

Group 2 rats were given 10 mg/kg body weight DCF in 0.2 ml normal saline per day by i.m. for 7 days, and served as DCF control.

Groups 3 and 4 rats were treated with 250 mg/kg b. wt. ACE and BCE respectively by daily oral gavages for 28 days, and injected with DCF in the last 7 days of treatment just as in group 2.

Collection and preparation of serum and tissue samples

About 24 h after treatment, blood was collected from rats by intra-cardiac puncture, under ether anesthesia. The blood was allowed to stand for at least one hour, and centrifuged at 3000 rpm for 10 minutes. Thereafter, serum was separated with clean Pasteur pipettes and used for biochemical assays. The rats were euthanized, their liver and kidney were immediately excised, rinsed and placed in ice-cold dextrose saline solution overnight before being used. The liver and kidney were separately homogenized in 0.1 M phosphate buffer (pH 7.4), and the homogenate centrifuged at 10,000 rpm for 15 minutes in a cold ultracentrifuge at 4 ^{0}C. Then, the supernatant was separated and used for the estimation of oxidative stress markers.

Biochemical analyses

Biochemical assays of serum aspartate aminotransferase and alanine aminotransferase (ALT and AST) (Reitman and Frankel, 1957), gamma glutamyl transferase (GGT) (Szasz, 1969), total protein (Gornall *et al*., 1949), albumin (Doumas *et al*., 1971), creatinine (Bartels *et al*., 1972) and urea (Fawcett and Scott, 1960) were carried out based on the standard methods previously described, and according to the procedures in the reagent kit manuals produced by Randox Laboratories Ltd, U.K. Absorbance was determined with UV-VIS spectrophotometer at appropriate wavelengths and time. Globulin level was estimated by taking the difference between total protein and albumin values of a sample, as earlier described by He *et al*. (2017).

Standard methods were used to determine the biochemical oxidative stress markers in the serum, liver, and kidney tissues of rats. The superoxide dismutase (SOD) activity was determined by estimation of the amount of enzyme required to produce a 50% inhibition of adrenaline oxidation in animal tissues (Misra and Fridovich, 1972). The catalase (CAT) activity was determined by estimating the amount of enzyme needed to break down 1 µmole H_2O_2/min/mg protein, according to the method by Sinha (1972). The glutathione peroxidase (GPx) activity was determined by the estimation of the amount of enzyme that oxidizes 1 µmole GSH/min/mg protein, according to the method by Tappel (1978). The glutathione S-transferase (GST) activity was estimated following a procedure provided by the reagent kits manufacturer, based on the method described by Chi-Yu *et al*. (1981). The level of lipid peroxidation product, malondialdehyde (MDA), was determined by the measurement of the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation, following the method by Satoh (1978). The amount of malondialdehyde produced was calculated from the data obtained, and the level of lipid peroxidation is expressed as nmol of MDA per mg protein. The reduced glutathione (GSH) level in animal tissues was determined by estimating the amount of reduced glutathione present in sample, following a method described by Ellman (1959). This method is based on the development of a relatively stable coloured complex when Ellman's reagent is added to sulfhydryl compounds. The absorbance of this complex at 412 nm is proportional to the amount of reduced glutathione in the sample. The glucose 6-phosphatase (G6Pase)

activity was determined according to a method earlier described by Koide and Oda (1959).

Statistical analyses

The Statistical Package for Social Sciences (SPSS version 21) software produced by IBM Corp. Ltd was used for all the data analyses. The data was expressed as Mean ± Standard Error of mean (SEM), with n = 6. They were analyzed by one-way analysis of variance (ANOVA), and the level of significance was determined by Fischer's least significant difference (LSD) in a Post Hoc test. The differences between mean values were considered significant at $p<0.01$.

RESULTS

Effect of *A. cordifolia and B. coccineus* extracts on antioxidant status and hepatorenal oxidative stress markers in rats

There was a significant ($p<0.01$) decrease in the liver and kidney antioxidant enzymes (CAT, SOD, GPx and GST) activities of DCF-injected rats when compared with the normal control. However, treatment of DCF-injected rats with 250 mg/kg body weight ACE and BCE significantly ($p<0.01$) increased the activities of these antioxidant enzymes compared with DCF control, with the exception of liver GST, kidney CAT and SOD activities of BCE-treated rats which were not significantly ($p>0.01$) different from the DCF control (Table 1).

There was a significant ($p<0.01$) increase in the levels of liver and kidney malondialdehyde (MDA), a lipid peroxidation product, and a significant ($p<0.01$) decrease in the liver and kidney glucose 6-phosphatase (G6Pase), protein, and reduced glutathione (GSH) levels of DCF-injected rats when compared with the normal control. However, the treatment of DCF-exposed rats with 250 mg/kg b. wt. ACE and BCE significantly ($p<0.01$) reduced the liver and kidney MDA levels, and significantly ($p<0.01$) increased the liver G6Pase, protein and GSH levels compared with DCF control, but the kidney G6Pase and GSH levels of BCE-treated rats were not significantly ($p>0.01$) different from the DCF control (Table 2).

Effect of *A. cordifolia* and *B. coccineus* extracts on serum markers of hepatorenal oxidative injuries in rats

There was a significant ($p<0.01$) increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), malondialdehyde (MDA), blood urea nitrogen (BUN) and creatinine levels but a significant ($p<0.01$) decrease in the levels of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glucose 6-phosphatase (G6Pase), total proteins, albumin and globulins of DCF-exposed rats when compared with normal control. However, treatment of DCF-exposed rats with 250 mg/kg b. wt. ACE and BCE significantly ($p<0.01$) reduced the serum ALT, AST, GGT, MDA, BUN and creatinine levels but significantly ($p<0.01$) increased the serum GSH, G6Pase, CAT, GPx, GST, total proteins, albumin and globulin levels when compared with the DCF control, except the SOD activity of ACE-treated rats which was not significantly ($p>0.01$) different from the DCF control, and the CAT activity of BCE-treated rats which was not significantly ($P>0.01$) different from the normal control (Table 3).

Phytochemical constituents and radical scavenging effects of *A. cordifolia* and *B. coccineus* extracts on DPPH radicals

The phytochemical components found in ACE are flavonoids, saponins, tannins, phenols, cardiac glycosides, reducing sugars, and anthraquinones. The phytochemical constituents of BCE include flavonoids, saponins, anthraquinones, tannins, reducing sugars and phenols.

At the highest concentration (5.0 mg/ml), the radical scavenging effects (RSEs) of ACE and BCE on DPPH radicals are 68.89% and 80.40% respectively while the value for vitamin C is 85.0%. At the lowest concentration (0.05 mg/ml), the RSEs of ACE and BCE on DPPH radicals are 4.27% and 35.84% respectively while the value for ascorbic acid is 90.19% (Table 4).

Table 1. Effect of *A. cordifolia* and *B. coccineus* extracts on hepatorenal antioxidant enzyme activities in DCF-injected rats

Treatment groups	Liver and kidney antioxidant enzyme activities in rats			
	Catalase (µmol/min/mg p)	SOD (µmol/min/mg p)	GPx (µmol/min/mg p)	GST (µmol/min/mg p)
Liver				
1. Normal saline	12.55±0.03	14.27±0.15	11.04±0.05	43.07±0.11
2. DCF + Saline	3.72±0.09[a]	4.42±0.02[a]	1.81±0.03[a]	18.11±0.11[a]
3. ACE + DCF	8.35±0.02[ab]	7.25±0.03[ab]	5.83±0.01[ab]	25.13±0.07[ab]
4. BCE + DCF	5.78±0.01[ab]	5.03±0.04[ab]	2.61±0.01[ab]	18.11±0.05[a]
Kidney				
1. Normal saline	3.12±0.01	11.24±0.13	9.46±0.05	16.91±0.25
2. DCF + Saline	0.89±0.02[a]	3.16±0.03[a]	1.28±0.05[a]	4.13±0.09[a]
3. ACE + DCF	1.94±0.03[ab]	5.38±0.01[ab]	4.58±0.02[ab]	9.98±0.05[ab]
4. BCE + DCF	1.04±0.02[a]	3.43±0.01[a]	3.05±0.03[ab]	6.23±0.06[ab]

Values are Mean ± SEM, n=6; P - Protein, DCF - Diclofenac sodium, [a]significantly different from normal control (p<0.01), [b]significantly different from DCF control (p<0.01), ACE -*Alchornea cordifolia* extract, BCE - *Byrsocarpus coccineus* extract

Table 2. Effect of *A. cordifolia* and *B. coccineus* extracts on liver and kidney levels of total Proteins, MDA, GSH and G6Pase in DCF-injected rats

Treatment groups	Levels of liver and kidney oxidative injury markers in rats			
	MDA (nmol/mg p)	Protein (g/L)	GSH (µg/mg p)	G6Pase (U/mg p)
Liver				
1. Normal saline	1.99±0.01	164.41±0.29	64.65±0.18	28.82±0.25
2. DCF + Saline	15.43±0.19[a]	121.07±0.06[a]	30.30±0.23[a]	14.62±0.02[a]
3. ACE + DCF	9.86±0.12[ab]	138.86±0.09[ab]	41.12±0.17[ab]	16.94±0.04[ab]
4. BCE + DCF	12.75±0.08[ab]	130.75±0.12[ab]	36.33±0.15[ab]	12.03±0.04[ab]
Kidney				
1. Normal saline	1.75±0.03	163.11±0.30	12.46±0.2	23.55±0.43
2. DCF + Saline	8.68±0.20[a]	82.73±0.47[a]	4.76±0.03[a]	9.59±0.12[a]
3. ACE + DCF	5.64±0.04[ab]	137.54±0.44[ab]	6.55±0.02[ab]	12.85±0.03[ab]
4. BCE + DCF	7.22±0.15[ab]	129.89±0.21[ab]	3.81±0.04[a]	10.73±0.21[a]

Values are Mean ± SEM, n=6; DCF - Diclofenac sodium, [a]significantly different from normal control at p<0.01, [b]significantly different from DCF control (p<0.01), ACE - *Alchornea cordifolia* extract, BCE - *Byrsocarpus coccineus* extract

Table 3. Effect of *A. cordifolia* and *B. coccineus* extracts on serum markers of hepatorenal oxidative injuries in DCF-injected rats

Biochemical parameters	Treatment groups			
	1. Normal control	2. 10 mg/kg DCF + Saline	3. 250 mg/kg b. wt. ACE + DCF	4. 250 mg/kg b. wt. BCE + DCF
ALT (U/L)	8.92±0.05	80.94±0.74[a]	23.88±0.23[ab]	36.17±0.31[ab]
AST (U/L)	14.06±0.05	124.91±0.53[a]	31.82±0.21[ab]	44.69±0.19[ab]
GGT (U/L)	39.97±0.10	125.39±0.22[a]	60.91±0.21[ab]	79.18±0.29[ab]
G6Pase (U/L)	33.35±0.46	14.07±0.10[a]	20.08±0.07[ab]	20.48±0.24[ab]
Protein (mg/dL)	156.58±0.78	69.75±0.44[a]	108.16±0.73[ab]	96.11±0.83[ab]
Albumin (mg/dL)	41.83±0.24	24.13±0.34[a]	33.44±0.09[ab]	28.17±0.37[ab]
Globulin (mg/dL)	113.3±0.91	45.39±0.19[a]	74.49±0.69[ab]	67.88±0.97[ab]
BUN (mmol/L)	5.03±0.04	34.23±0.81[a]	12.84±0.18[ab]	25.79±0.17[ab]
Creatinine (µmol/L)	100.6±0.43	481.2±5.64[a]	230.9±0.43[ab]	386.4±0.47[ab]
MDA (nmol/ml)	2.96±0.03	12.51±0.22[a]	6.99±0.18[ab]	9.04±0.03[ab]
GSH (µg/ml)	60.49±0.53	30.67±0.27[a]	38.02±0.14[ab]	34.63±0.16[ab]
SOD (U/mg p)	11.29±0.11	3.60±0.07[a]	3.53±0.01[a]	5.38±0.01[ab]
CAT (U/mg p)	8.99±0.08	2.02±0.05[a]	5.19±0.02[ab]	7.08±0.07[b]
GPx (U/mg p)	9.13±0.25	2.39±0.02[a]	4.75±0.03[ab]	3.56±0.03[ab]
GST (U/mg p)	41.05±0.59	15.17±0.19[a]	22.08±0.33[ab]	17.72±0.13[ab]

Values are Mean ± SEM, n=6; DCF - Diclofenac sodium, [a]significantly different from normal control at p<0.01, [b]significantly different from DCF control (p<0.01), ACE - *Alchornea cordifolia* extract, BCE - *Byrsocarpus coccineus* extract

Table 4. Radical scavenging effect of *A. cordifolia* and *B. coccineus* leaf extracts on DPPH radicals

Concentration (mg/ml)	Percentage inhibition of DPPH radicals		
	% Inhibition by Ascorbic acid	% Inhibition by *A. cordifolia* extract	% Inhibition by *B. coccineus* extract
5	85.0	68.89	80.40
3	90.01	67.69	34.04
2	90.41	68.55	39.04
1	91.33	70.69	56.52
0.5	91.32	71.16	61.75
0.1	90.47	65.75	62.95
0.05	90.19	4.27	35.84

Values are expressed as mean of duplicate readings

DISCUSSION

Plant extracts have continued to be investigated for the treatment of many human diseases because they have been found to exert pharmacological effect on the animal tissues and have therapeutic potentials (Hussain *et al*., 2019; Iserhienrhien and Okolie, 2022). The ACE and BCE used in this study contain phytochemicals such as alkaloids, flavonoids, tannins, and phenols. These secondary metabolites of plants may be responsible for the protective effects of these plant extracts against diclofenac-induced hepatorenal oxidative injuries in rats, as they are known to act as antioxidants which scavenge highly reactive metabolites and free radicals in animal tissues (Hussain *et al*., 2019, Ogbe *et al*., 2022). The findings of our *in vivo* study are supported by the results of our *in vitro* experiment, which showed that the two plant extracts have radical scavenging effects on DPPH radicals, and in agreement with the reports of previous studies (Jeyadevi *et al*. 2019; Ogbe *et al*. 2019). The medicinal values of plants have been shown to be as a result of the activities of their phytochemical components such as alkaloids, phenols, flavonoids, tannins and terpenoids (Hussain *et al*. 2019; Nwanna, 2021). Flavonoids are potent antioxidants which produce biological and pharmacological effects in animals, resulting in health-promotion or disease-prevention benefits. These health benefits of flavonoids and other phytochemical components are especially due to their anti-oxidative activities in the animal tissues (Ogbe *et al*., 2019; Iserhienrhien and Okolie, 2022).

Drug-induced hepatorenal oxidative injuries are commonly caused by oxidative stress, which is as a result of imbalance in the generation and elimination of ROS, RNS, oxidants or free radicals in the animal tissues (Hussain *et al*., 2019). The marked decrease in serum, liver and kidney superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities after exposure of rats to DCF may indicate oxidative stress, leading to the loss of enzymes activities; which is attributed to the deleterous effect of reactive hydroxylated metabolites produced by this drug. The DCF was reported to generate highly reactive metabolites (ROS/RNS), which may induce hepatorenal oxidative injuries in animals via the oxidative stress and lipid peroxidation processes (Alorabi *et al*., 2022). These findings are in agreement with earlier reports which showed that ROS and RNS generated by drugs may cause imbalance in the generation and elimination of ROS/RNS, leading to oxidative stress (Hussain *et al*., 2021; Ogbe *et al*., 2020; Alorabi *et al*., 2022). However, the marked elevation in the activities of these endogenous antioxidant enzymes, following the treatment of DCF-exposed rats with ACE and BCE may suggest that the plant extracts have protective effects against drug-induced oxidative stress, thereby preventing excessive loss of antioxidant enzymes' activities in rats. This may be attributed to the phytochemical components present in the plant extracts which can act as antioxidants, and may be utilized in the antioxidant defense system, consequently providing a shielding effect on the natural antioxidant enzymes of the animals (Ogbe *et al*., 2019). These findings are in agreement with reports of earlier studies which showed that plant extracts may promote tissues antioxidant status of drug-intoxicated rats (Hussain *et al*., 2019; Ogbe *et al*., 2020; Raghuvanshi *et al*., 2020).

The marked elevation in serum, liver and kidney levels of malondialdehyde (MDA) and marked decrease in reduced glutathione (GSH) levels after exposure of rats to DCF may indicate lipid peroxidation and oxidative stress, which may be attributed to the adverse effects of the ROS/RNS generated by this drug. These highly reactive metabolites attack cell membrane lipids or lipoproteins during oxidative stress, and cause lipid peroxidation, which is implicated in the development of several degenerative diseases including hepatic and renal tissue injuries in animals (Raghuvanshi *et al*., 2020). These findings are in agreement with Adeyemi and Olayaki (2018) and Ogbe *et al*. (2022) who reported marked elevation of MDA and decrease in GSH levels, following the exposure of animals to DCF. However, the marked decrease in the levels of MDA and elevation in GSH levels, following the treatment of DCF-exposed rats with ACE and BCE may suggest that the plant extracts have protective effects against oxidative stress, lipid peroxidation, and hepatorenal injuries induced by the adverse effects of ROS generated by this drug in rats. The protective effects of these plant extracts may be attributed to their antioxidant properties, which were utilized by the animal tissues, consequently providing a shielding effect on GSH, an important endogenous antioxidant, and prevented its depletion in the tissues. Reports of previous studies by Ogbe *et al*. (2019) and Al-Asmari *et al*. (2020) agreed with these findings.

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transpeptidase (GGT) activities are biochemical markers commonly used to evaluate the degree of hepatocellular and hepatobiliary dysfunctions in animal tissues (Al-Asmari *et al*. 2020; Agu *et al*., 2022). The marked increase in serum activities of AST, ALT, GGT and the markedly decreased levels of total protein, albumin and globulin, following the exposure of rats to DCF may indicate hepatocellular and hepatobiliary injuries in the animals, which may be attributed to the adverse effects of the drug. These findings are in agreement with previous studies which showed that any change in the activities of these enzymes may be a signal of an underlying pathological process (Adeyemi and Olayaki, 2018; Ogbe *et al*., 2022). The blood levels of these enzymes are normally low but when there is necrosis of the hepatocytes or hepatocellular damage, they leak out into the blood, and their levels are drastically increased in serum (Owumi and Dim, 2019). Albumin and total protein have been reported to decrease as a result of hepatocellular injuries, and decreased metabolic functions of the liver, attributed to DCF-induced hepatotoxicity in animal tissues (Ogbe *et al*. 2019; Al-Asmari *et al*., 2020).

Alterations in the serum levels of creatinine, blood urea nitrogen (BUN), and glucose 6-phosphatase (G6Pase) are indicators of renal injury in animals (Hussain *et al*., 2019; Raghuvanshi *et al*., 2020). The markedly increased serum creatinine and BUN levels, following the administration of rats with DCF may indicate nephrotoxic injury in rats, which is attributed to the adverse effects of the drug. The marked reduction in serum, liver and kidney G6Pase activities, following the exposure of rats to DCF may indicate hepatorenal injuries, which lead to the inhibition of enzyme or loss of enzyme activity, attributed to the adverse effects of ROS/RNS produced by the drug. These findings are in agreement with the reports of previous studies by Raghuvanshi *et al*. (2020) who found that G6Pase activities were markedly reduced in rats after treatment with DCF, and Alorabi *et al*. (2022) who demonstrated that DCF markedly elevated the creatinine and urea levels in rats.

However, the marked reduction in serum AST, ALT and GGT activities, following the treatment of DCF-injected rats with ACE and BCE may suggest that these plant extracts have protective effects against DCF-induced hepatocellular and hepatobiliary injuries in rats. The reports of several studies demonstrated that some plant extracts have protective effects against drug-induced liver injury in animals while others caused a reversal in the elevated serum enzyme activities in animal tissues to near normal values (Ogbe *et al*. 2019; Al-Asmari *et al*., 2020; Raghuvanshi *et al*., 2020). The marked reduction of serum creatinine and BUN after treatment of DCF-exposed rats with ACE and BCE may suggest that the plant extracts have protective effects against nephrotoxic injury induced by DCF in rats. This may imply that the phytochemical components of

extracts prevented a rapid damage in renal tissue of the animals. The marked elevation in G6Pase activities, following the treatment of DCF-injected rats with the plant extracts may suggest that these plant extracts have protective effects against hepatorenal oxidative injuries in rats. The phytochemical components in the plant extracts may have prevented the inhibition of this enzyme (an important enzyme in glucose metabolism), by their action as antioxidants which scavenge ROS and prevent oxidative stress. Reports of previous studies have shown that plant extracts have protective effects against drug-induced hepatorenal oxidative injuries in rats (Jeyadevi *et al*., 2019; Hussain *et al*., 2019; Raghuvanshi *et al*., 2020).

In conclusion, the present study has shown that ACE and BCE may have protective effects against DCF-induced oxidative stress and hepatorenal injuries in Wistar rats but ACE has greater efficacy. The biological or pharmacological effects of these plant extracts may be attributed to their secondary metabolites such as alkaloids, flavonoids, tannins and other phenolic compounds, which act as antioxidants that can scavenge ROS, capable of causing oxidative damage to animal tissues. These findings have given credence to the use of these plant extracts in folklore medicine for the management of liver and kidney diseases in humans. However, there is a need to conduct the bioassay-guided isolation and characterization of the bioactive compounds responsible for the biological activities of these plant extracts, and determine their mechanism of action.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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