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Comparative study of the effects of *Cassia spectabilis* and *Newbouldia laevis* leaf extracts on diclofenac-induced hepatorenal oxidative damage in rats

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Abstract

Background: There is high incidence of liver and kidney diseases worldwide but medicinal plants may provide remedy. This study evaluated and compared the protective effects of *Cassia spectabilis* extract (CSE) and *Newbouldia laevis* extract (NLE) against diclofenac (DF) induced hepatorenal oxidative damage in rats.

Methods: Twenty four rats were divided into 4 groups with 6 rats in each. Normal saline was given to the rats in group I while those in groups III and IV were treated with 250 mg/kg b. wt. CSE and NLE respectively for 28 days by oral gavages. Those in groups II to IV were exposed to 10 mg/kg DF in the last 7 days of treatment. 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 DF-exposed rats when compared with normal control. However, treatment of DF-exposed rats with CSE and NLE significantly ($p < 0.01$) increased 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 DF control.

Conclusion: The current findings showed that treatments with CSE and NLE may have protective effects against DF-induced hepatorenal oxidative damage in rats, attributed to certain phytochemicals, but CSE has greater bioactivity than NLE.

Keywords: Hepatoprotective, Lipid peroxidation, Phytochemicals, Radicals, Renoprotective

Introduction

Liver and kidney diseases are major global health problems, posing great challenges to several countries, especially the developing nations of the world [1, 2]. Several hepatorenal disorders are caused by drug-induced toxicities in animals [2, 3]. Diclofenac sodium (DF) is among the drugs reported to cause adverse effects in the liver and kidney of animals [2, 4]. It is a phenyl acetic acid derivative, developed as a non-steroidal anti-inflammatory drug; used for the treatment of pain and musculoskeletal diseases in animals and humans [5]. The toxic effect of DF was attributed to the production of reactive metabolites, 5-hydroxy diclofenac and N, 5-dihydroxy diclofenac. These metabolites may increase the levels of oxidants and free radicals known as reactive oxygen species (ROS), which result in oxidative stress and cause damage to hepatorenal tissues in rats [6]. Oxidative stress occurs when there is disruption in the balance

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between ROS such as superoxide anion radicals, hydroxyl radicals, nitric oxide and hydrogen peroxide and the antioxidant like reduced glutathione which neutralizes them [7].

The liver is the main organ which performs the function of xenobiotic metabolism, assisted by the kidneys, lungs and gastrointestinal tract [8]. The biochemical modifications of foreign compounds, drugs and environmental pollutants by the liver of animals, make it susceptible to toxic effects [9]. The kidney is the major organ responsible for excretion of metabolic waste products in animals [10]. Thus, it is vulnerable to oxidative damage because of oxidative stress caused by ROS, which attack polyunsaturated fatty acids in the renal membrane lipids, resulting in lipid peroxidation [11]. Drug-induced liver injury is estimated to have an annual incidence of 10 to 15 per 10,000 to 100, 000 persons exposed to prescription drugs [12]. The global prevalence of chronic kidney disease (CKD) is estimated to be 11–13% [13]. The 2010 global burden of disease study reported that CKD was ranked 27th in the list of diseases which caused total number of deaths worldwide in 1990 but moved to 18th in 2010 [14]. It was estimated that nearly two million people die of acute kidney injury (AKI) every year worldwide [15].

Cassia spectabilis (DC.) Irwin & Barn. - is a leguminous plant species in the family, Fabaceae, and subfamily, Caesalpinioideae [16]. It is a tropical plant known by the common name, "Golden cassia", but in Nigeria it is called "Okpehekwu", by the Idoma people of Benue state. The trees are rounded in shape, have evergreen foliage, with the height of 15–20 ft. The leaves are compound green, flowers are showy and yellow, but the bark is gray and smooth [17]. The plant products are used as laxative and purgative, and as traditional medicines for treatment of flu and cold [18]. Scientific studies have shown that the plant extract has radical scavenging activity [16], antifungal and antimicrobial activities [18].

Newbouldia laevis P. Beauv: (Family: Bignoniaceae) - is a medium sized angiosperm or tree, which can grow to a height of about 10 m. It is a tropical plant and widespread from Guinea Savanna to the rain forests of Africa. The English/trade names are "African border tree", "Smooth Newbouldia" or "Fertility tree", but in Nigeria it is called "Ogblichi" (Idoma), "Akoko" (Yoruba), "Ogirisi" (Igbo) and "Aduruku" (Hausa) [19]. The leaves are waxy, dark green, while the flowers are tubular, pink and come in bunches [20]. The roots and leaves are used for treatment of diseases such as fever, headache, convulsion and epilepsy [21]. Scientific studies have shown that its stem bark has radical scavenging effect on certain free radicals [22], while different parts of the plant possess antimicrobial activities [23].

There seems to be paucity of scientific reports on the protective effects of these plant leaf extracts against hepatorenal oxidative damage in drug-exposed rats, but traditional medicine practitioners claimed they are effective in the management of liver and kidney disorders in humans. Therefore, this study was designed to evaluate the protective effects of *C. spectabilis* and *N. laevis* leaf extracts against DF-induced hepatorenal oxidative damage in rats and compare their bioactivities.

Materials and methods

Chemicals and reagents

Diclofenac sodium is an injectable liquid purchased from 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. Biochemical tests reagent kits were purchased from Randox Laboratories, UK. All the reagents used are of analytical grade.

Preparation of diclofenac sodium

Each 3 ml ampoule contains 75 mg of diclofenac sodium. 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 Cassia spectabilis and Newbouldia laevis leaf extracts

The *C. spectabilis* and *N. laevis* leaves were harvested from a forest in Obu, Otukpa, in Benue state. 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 were deposited in the College of Forestry herbarium, with voucher numbers given as: *C. spectabilis* - FH/0258 and *N. laevis* - FH/0202. The leaves were dried at room temperature for at least three weeks, pulverized to fine particles with mortar and pestle, and sieved with a porcelain sieve. The preparation of plant extracts was done according to the method of Abu and Uchendu [24]. Aqueous ethanol mixture was prepared by adding 800 ml distilled water to 200 ml absolute ethanol and mixed thoroughly. Then, 200 g of the powdered sample was macerated in 1200 ml of aqueous ethanol solvent and allowed to stand for 72 h. The mixture was sieved with a clean piece of cloth and filtered with Whatman no. 1 filter papers. The filtrate was placed on water bath for the solvent to evaporate at 50 °C, while the extract was dried to a constant weight in a desiccator and the weight was determined.

Experimental animals and management

Albino Wistar rats, *Rattus norvegicus,* were purchased from the Animal house, College of Health sciences, Benue State University, Makurdi, Nigeria. Equal numbers of male and female rats, weighing between 200 and 250 g were

used for this study. They were allowed to acclimatize for at least three weeks in the Department of Veterinary Physiology and Biochemistry research laboratory, Federal University of Agriculture, Makurdi, Nigeria; under normal environmental conditions of 12 h dark and 12 h light cycle, with an average temperature of 29 °C. They were fed with standard animal feeds, produced by Grand Cereal and Oil Mills Ltd., Jos, Nigeria and clean water ad libitum. The rats were treated with care according to the International guidelines and principles for biomedical research involving animals [25].

Phytochemical analyses of *C. spectabilis* and *N. laevis* leaf extracts

Aqueous ethanol (2:1) extracts of *C. spectabilis* and *N. laevis* leaves were prepared by maceration of 10 g pulverized sample in a mixture of 100 ml distilled water and 50 ml ethanol in a conical flask, covered with aluminum foil. After 48 h, the mixture was filtered with filter papers and the filtrate tested for phytochemical constituents, according to the methods earlier described [26, 27].

Scavenging effects of CSE and NLE on 2, 2-diphenyl-1-picryl hydrazyl radicals

The radical scavenging effect (RSE) of CSE and NLE on DPPH radicals were determined by a method previously described [28]. Briefly, the concentrations of CSE and NLE 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 also prepared. Then, 1 ml of plant extract/ascorbic acid was placed in a test tube and 3 ml methanol added, followed by 0.5 ml of 1 mM DPPH in methanol. A blank solution was prepared which contains the same quantity of methanol and DPPH. After 10 min, the decrease in absorption was measured against the blank by UV-Visible spectrophotometer at 517 nm, and the percentage inhibition was determined. All the tests were performed in duplicates and the mean values were calculated. The RSE of CSE and NLE were expressed as percentage inhibition of DPPH radicals using the equation below:

$$\text{Percentage inhibition } (\%) = \frac{\text{Absorbance of blank-Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

Animal grouping and treatments

Twenty four adult rats were completely randomized into 4 groups, with 6 rats per group. Group I - rats received 0.2 ml of normal saline by intramuscular (i.m.) route for 7 consecutive days, and served as normal control. Group II - rats received 10 mg/kg body weight diclofenac (DF) in 0.2 ml normal saline by i.m. for 7 days, and served as diclofenac control. Groups III and IV - rats were treated with 250 mg/kg b. wt. CSE and NLE respectively by daily oral gavages for 28 days, and exposed to DF in the last 7 days of treatment as in group II.

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 min. Thereafter, serum was separated with clean Pasteur pipettes and used for biochemical assays. The rats were euthanized, their liver and kidneys were excised, rinsed to remove blood and placed in ice cold dextrose saline solution overnight before being used. The liver and kidney were homogenized separately in 0.1 M phosphate buffer (pH 7.4), and the homogenate centrifuged at 10,000 rpm for 15 min in a cold ultracentrifuge at 4 °C. Then, the supernatant was separated and used for estimation of oxidative stress markers.

Biochemical analyses

Biochemical assays of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [29], gamma glutamyl transferase (GGT) [30], total protein [31], albumin [32], creatinine [33], and BUN [34], were performed according to the procedures in the reagent kits manuals produced by Randox Laboratories Ltd., UK. The absorbance of each test was determined with UV-VIS spectrophotometer at appropriate wavelength and time. The Globulin level was determined by taking the difference between total protein and albumin values of the same sample as earlier described [4]. The activities of glucose 6-phosphatase (G6Pase) [35], super oxide dismutase (SOD) [36], catalase (CAT) [37], glutathione peroxidase (GPx) [38], glutathione S-transferase (GST) [39]; and the levels of malon dialdehyde (MDA) [40] and reduced glutathione (GSH) [41] in serum, liver and kidney tissues were determined by standard methods as previously described.

Statistical analyses

The Statistical Package for Social Sciences (SPSS version 21) software produced by IBM Corp. Ltd. was used for data analysis. Data were 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 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

Phytochemical constituents and radical scavenging effects of CSE and NLE on DPPH radicals

The phytochemical constituents of CSE include alkaloids, flavonoids, saponins, tannins, phlobatannins, reducing sugars, anthraquinones and phenols. The phytochemical

constituents of NLE include saponins, anthraquinones, tannins, reducing sugars and phenols.

The greatest radical scavenging effects of CSE and NLE on DPPH radicals were seen at 2.0 mg/ml, expressed as percentage inhibitions of radicals at 66.9% and 72.45% respectively, which are close to the standard, ascorbic acid, with percentage inhibition of 90.4% (Table 1). The IC_{50} (concentration of extract which produced 50% inhibition of DPPH radicals) of CSE and NLE are 5.0 mg/ml and 4.9 mg/ml respectively.

Effect of CSE and NLE on antioxidant status and oxidative stress markers in liver and kidney of rats

There was a significant ($p < 0.01$) decrease in the activities of liver and kidney CAT, SOD, GPx and GST of DF-exposed rats when compared with normal control. However, treatment of DF-exposed rats with CSE and NLE significantly ($p < 0.01$) elevated the activities of these antioxidant enzymes compared with DF control (Table 2).

There was a significant ($p < 0.01$) elevation in the levels of liver and kidney tissues lipid peroxidation product, malon dialdehyde (MDA), and significant ($p < 0.01$) reduction in protein, G6Pase and GSH levels of DF-exposed rats when compared with normal control. However, treatment of DF-exposed rats with CSE and NLE significantly ($p < 0.01$) reduced the levels of MDA, and significantly ($p < 0.01$) increased the levels of protein, G6Pase and GSH compared with DF control (Table 3).

Effect of CSE and NLE on serum markers of hepatorenal oxidative damage in rats

There was a significant ($p < 0.01$) elevation in serum ALT, AST, GGT, MDA, BUN and creatinine levels but a significant ($p < 0.01$) reduction in the levels of GSH, SOD, CAT, GPx, GST, G6Pase, total proteins, albumin and globulins of DF-exposed rats when compared with normal control. However, treatment of DF-exposed rats with 250 mg/kg CSE and NLE significantly ($p < 0.01$) reduced their serum ALT, AST, GGT, MDA, BUN and creatinine levels but significantly ($p < 0.01$) increased their levels of GSH, G6Pase, SOD, CAT, GPx, GST, total proteins, albumin and globulins when compared with DF control (Table 4).

Table 1 Radical scavenging effects of CSE and NLE on DPPH radicals

Concentration (mg/ml)	Percentage inhibition of DPPH radicals		
	% Inhibition by Ascorbic acid	% Inhibition by *C. spectabilis* extract	% Inhibition by *N. laevis* extract
5	85.0	49.40	51.32
3	90.01	57.83	72.26
2	90.41	66.90	72.45
1	91.33	66.57	59.05
0.5	91.32	66.87	55.09
0.1	90.47	21.08	22.07
0.05	90.19	0.60	21.69

Values are expressed as mean of duplicate readings

Discussion

Plant extracts have been used for treatment of several diseases, because they have therapeutic properties and are found to exert biological effects on body tissues [1–3]. The CSE and NLE used in this study contain phytochemicals such as alkaloids, tannins, phenolic compounds and flavonoids. These plant secondary metabolites may be responsible for the protective effects of the extracts against hepatorenal injuries in rats, as they are known to act as antioxidants which scavenge ROS [1, 16]. The findings of our in vitro study showed that CSE and NLE have radical scavenging effects on DPPH radicals, which agree with reports of previous studies that plant extracts have radical scavenging activities [15, 28]. Scientific evidence has shown that the medicinal values of plant extracts are due to the activities of their phytochemical constituents such as flavonoids, alkaloids, tannins and terpenoids [15, 16].

Drug-induced oxidative damage of hepatorenal tissues in animals is commonly caused by oxidative stress, which is as a result of the effect of excess ROS produced in the tissues [42]. The marked decrease in serum, liver and kidney SOD, CAT, GPx and GST activities after exposure of rats to DF may indicate oxidative stress, which lead to the reduction in antioxidant enzyme activities, may be attributed to the adverse effect of this drug. The drug was earlier found to generate reactive metabolites, 5-hydroxyl diclofenac and N, 5-dihydroxyl diclofenac, which may induce hepatorenal oxidative injuries in animals [6]. These findings are in agreement with earlier reports which showed that ROS produced by drugs may cause imbalance in the generation and elimination of ROS, leading to oxidative stress [42, 43]. However, the significant elevation in the activities of antioxidant enzymes; SOD, CAT, GPx and GST, after treatment of DF-exposed rats with CSE and NLE, may suggest that the plant extracts have protective effects against oxidative stress, thereby preventing excessive decrease in activities of antioxidant enzymes in rats. This may be attributed to phytochemicals in the plant extracts, which act as antioxidants and could be utilized in the antioxidant defense system, which consequently may have a sparing effect on the antioxidant enzymes of the animals [7]. These findings are in agreement with reports of previous studies which showed that plant extracts may improve the tissue antioxidant status of drug-exposed rats [4, 15].

The marked elevation in serum, liver and kidney levels of MDA but decrease in GSH levels, after the administration of DF to rats may indicate oxidative stress and lipid peroxidation, which may be attributed to the

Table 2 Effect of CSE and NLE on liver and kidney antioxidant enzymes activities in DF-exposed rats

Treatment groups	Levels of liver and kidney antioxidant enzymes in rats			
	Catalase (μmol/min/mg p)	SOD (μmol/min/mg p)	GPx (μmol/min/mg p)	GST (μmol/min/mg p)
Liver				
I. Normal saline	12.55 ± 0.03	14.27 ± 0.15	11.04 ± 0.05	43.07 ± 0.11
II. DF + Saline	3.72 ± 0.09[a]	4.42 ± 0.02[a]	1.81 ± 0.03[a]	18.11 ± 0.11[a]
III. CSE + DF	10.91 ± 0.01[b]	11.99 ± 0.06[b]	7.87 ± 0.02[b]	35.25 ± 0.41[b]
IV. NLE + DF	9.38 ± 0.02[b]	5.28 ± 0.02[b]	3.86 ± 0.02[b]	21.36 ± 0.21[b]
Kidney				
I. Normal saline	3.12 ± 0.01	11.24 ± 0.13	9.46 ± 0.05	16.91 ± 0.25
II. DF + Saline	0.89 ± 0.02[a]	3.16 ± 0.03[a]	1.28 ± 0.05[a]	4.13 ± 0.09[a]
III. CSE + DF	2.81 ± 0.05[b]	9.52 ± 0.12[b]	7.21 ± 0.05[b]	14.66 ± 0.14[b]
IV. NLE + DF	1.19 ± 0.02[b]	4.25 ± 0.02[b]	3.79 ± 0.01[b]	8.03 ± 0.03[b]

Values are Mean ± SEM, $n = 6$; *P* Protein, *DF* Diclofenac sodium, [a]significantly different from normal control ($p < 0.01$), [b]significantly different from DF control ($p < 0.01$)

adverse effects of this drug. The ROS attack on cell membrane lipids or lipoproteins during oxidative stress starts lipid peroxidation, which is implicated in the development of several diseases including drug-induced liver and kidney damage in animals [5, 11, 44]. These findings are in agreement with Owumi and Dim [2] who reported that there were marked elevation in MDA levels and decrease in GSH levels after exposure of animals to DF. However, the marked decrease in levels of MDA and increase in GSH levels after treatment of DF-exposed rats with CSE and NLE may suggest that these plant extracts have protective effects against hepatorenal injuries, which may be attributed to the antioxidant properties of their phytochemicals, which consequently have a sparing effect on GSH, an important endogenous antioxidant. These findings are in accord with the reports by earlier researchers, who demonstrated the antioxidative activities of plant extracts [4, 15].

The marked increase in serum AST, ALT, GGT activities and marked decrease in the levels of total protein, albumin and globulin, after exposure of rats to DF may be indicative of hepatocellular and hepatobiliary injuries in rats, attributed to the adverse effects of the drug. These findings are in agreement with earlier studies which demonstrated that the alteration of these enzymes activities is a signal of an underlying pathological process, thus they are used as biomarkers of hepatic tissue injuries in animals [2, 4, 16]. The serum aminotransferases activities are elevated in all cases of liver diseases [2, 5]. The normal activities of these enzymes in blood are very low but when there is necrosis of liver cells, they leak out into the systemic circulation, thus their activities are increased in blood [2]. Albumin and total protein have earlier been reported to decrease as a result of damage to liver tissues, leading to reduced synthetic functions of liver, which was attributed to drug-induced hepatotoxicity [4].

Table 3 Effect of CSE and NLE on levels of Proteins, MDA, GSH and G6Pase in hepatorenal tissues of DF-exposed rats

Treatment groups	Levels of hepatorenal oxidative injuries markers in rats			
	MDA (nmol/mg p)	Protein (g/L)	GSH (μg/mg p)	G6Pase (U/mg p)
Liver				
I. Normal saline	1.99 ± 0.01	164.41 ± 0.29	64.65 ± 0.18	28.82 ± 0.25
II. DF + Saline	15.43 ± 0.19[a]	121.07 ± 0.06[a]	30.30 ± 0.23[a]	14.62 ± 0.02[a]
III. CSE + DF	7.11 ± 0.01[b]	148.16 ± 0.10[b]	52.48 ± 0.25[b]	21.03 ± 0.03[b]
IV. NLE + DF	11.10 ± 0.06[b]	135.40 ± 0.16[b]	39.15 ± 0.08[b]	14.04 ± 0.04[a]
Kidney				
I. Normal saline	1.75 ± 0.03	163.11 ± 0.30	12.46 ± 0.2	23.55 ± 0.43
II. DF + Saline	8.68 ± 0.20[a]	82.73 ± 0.47[a]	4.76 ± 0.03[a]	9.59 ± 0.12[a]
III. CSE + DF	3.23 ± 0.10[b]	144.52 ± 0.29[b]	9.04 ± 0.04[b]	17.28 ± 0.22[b]
IV. NLE + DF	6.12 ± 0.01[b]	132.14 ± 0.21[b]	5.34 ± 0.03[b]	11.01 ± 0.02[b]

Values are Mean ± SEM, $n = 6$; *DF* Diclofenac sodium, [a]significantly different from normal control ($p < 0.01$), [b]significantly different from diclofenac control ($p < 0.01$)

Table 4 Effect of CSE and NLE on serum markers of hepatorenal oxidative damage in DF-exposed rats

Biochemical parameters	Treatment groups			
	I. Normal control	II. 10 mg/kg DF + Saline	III. 250 mg/kg CSE + DF	IV. 250 mg/kg NLE + DF
ALT (U/L)	8.92 ± 0.05	80.94 ± 0.74[a]	15.72 ± 0.18[b]	27.2 ± 0.40[b]
AST (U/L)	14.06 ± 0.05	124.91 ± 0.53[a]	20.66 ± 0.19[b]	36.6 ± 0.20[b]
GGT (U/L)	39.97 ± 0.10	125.39 ± 0.22[a]	47.73 ± 0.18[b]	66.7 ± 0.16[b]
G6Pase (U/L)	33.35 ± 0.46	14.07 ± 0.10[a]	27.06 ± 0.28[b]	18.0 ± 0.18[b]
Protein (g/dl)×10^{-2}	16.0 ± 0.78	7.0 ± 0.44[a]	13.0 ± 2.35[b]	10.0 ± 0.29[b]
Albumin (g/dl)×10^{-2}	4.0 ± 0.24	2.0 ± 0.34[a]	4.0 ± 0.26[b]	3.0 ± 0.18[b]
Globulin (g/dl)×10^{-2}	12.0 ± 0.91	5.0 ± 0.19[a]	9.0 ± 2.35[b]	7.0 ± 0.29[b]
BUN (mmol/L)	5.03 ± 0.04	34.23 ± 0.81[a]	6.47 ± 0.10[b]	20.8 ± 0.24[b]
Creatinine (μmol/L)	100.6 ± 0.43	481.2 ± 5.64[a]	123.6 ± 0.23[b]	341.1 ± 0.32[b]
MDA (nmol/mg p)	2.96 ± 0.03	12.51 ± 0.22[a]	4.66 ± 0.05[b]	8.01 ± 0.08[b]
GSH (μg/ml)	60.49 ± 0.53	30.67 ± 0.27[a]	48.15 ± 0.27[b]	36.3 ± 0.21[b]
SOD (U/mg p)	11.29 ± 0.11	3.60 ± 0.07[a]	8.99 ± 0.08[b]	6.10 ± 0.02[b]
CAT (U/mg p)	8.99 ± 0.08	2.02 ± 0.05[a]	6.10 ± 0.03[b]	6.17 ± 0.03[b]
GPx (U/mg p)	9.13 ± 0.25	2.39 ± 0.02[a]	6.24 ± 0.04[b]	4.46 ± 0.25[b]
GST (U/mg p)	41.05 ± 0.59	15.17 ± 0.19[a]	31.99 ± 0.68[b]	19.14 ± 0.24[b]

Values are Mean ± SEM, $n = 6$; *DF* Diclofenac sodium, [a]significantly different from normal control ($p < 0.01$), [b]significantly different from diclofenac control ($p < 0.01$)

The marked elevation in the levels of serum creatinine and BUN after the exposure of rats to DF may be indication of renal tissue injuries, which can be attributed to the adverse effects of this drug. The marked reduction in serum, liver and kidney G6Pase activities after exposure of rats to DF may suggest hepatorenal tissue damage, which consequently lead to a decrease in the enzyme activity; may be attributed to the adverse effects of reactive metabolites produced by the drug. These findings are in agreement with Owumi and Dim [2] who reported that DF is a powerful drug which causes nephrotoxicity, and this was demonstrated by the increases of serum creatinine and urea levels in rats. Konda et al. [15] also found that there were elevations in serum creatinine and BUN levels of rats after they were exposed to a drug. The reduced G6Pase activity is in agreement with Kumashiro et al. [45] who found that accumulation of ROS reduced the expression level of G6Pase in hepatocytes of animals after they were exposed to a toxin.

The marked decrease in serum AST, ALT and GGT activities by treatment of DF-exposed rats with CSE and NLE may suggest that these plant extracts have protective effects against DF-induced hepatocellular and hepatobiliary injuries in rats. The reports of previous studies have shown that plant extracts have protective effects against drug-induced liver damage in animals while others may reverse the elevation of serum enzymes to near normal values [4, 16]. The marked reduction in the levels of serum creatinine and BUN after treatment of DF-exposed rats with CSE and NLE may suggest that the plant extracts have protective effects against renal tissue damage in rats and may promote the rapid healing of the tissue injuries. The marked increase in serum, liver and kidney G6Pase activities after treatment of DF-exposed rats with both plant extracts may suggest that the extracts have protective effects against hepatorenal tissue damage in rats, which may prevent sharp decrease in the activity of this enzyme, which plays important roles in glucose metabolism. These findings are in agreement with several studies which showed that plant extracts may have protective effects against drug-induced hepatorenal tissue injuries in rats [1, 3, 4, 15].

Conclusion

This study has shown that CSE and NLE may have protective effects against DF-induced acute hepatorenal oxidative damage in rats, however CSE has greater bioactivity than NLE. The activities of these plant extracts may be attributed to their phytochemicals, which act as potent 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 traditional medicine, for the management of liver and kidney diseases in humans. However, there is need to conduct bioassay-guided fractionation and characterization of bioactive compounds responsible for the medicinal properties of these plant extracts.

Abbreviations

CSE: *Cassia spectabilis* leaf extract; NLE: *Newbouldia laevis* leaf extract; DF: Diclofenac sodium; GFR: Glomerular filtration rate; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; DPPH-2: 2-diphenyl-1-picryl hydrazyl

radicals; LSD: Least significant difference; NSAID: Non steroidal anti-inflammatory drug

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Authors' contributions
RJO and GIA designed the experiment while GIA and CDL supervised it. RJO conducted the biochemical, histologicaldelete and, statistical analyses, and interpreted the results. RJO wrote the first draft of this manuscript while GIA and CDL revised the manuscript for intellectual content. All authors read and approved the final draft of the manuscript.

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Availability of data and materials
The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The protocols of this study were approved by the Animal Welfare and Ethics Committee of the College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria. The ethical approval was given with a clearance certificate reference number: FUAM/CVM/002.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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