The Journal of Basic and Applied Zoology

RESEARCH Open Access

Influence of hydroethanolic extract of *Cassia spectabilis* leaves on diclofenac-induced oxidative stress and hepatorenal damage in Wistar rats

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Abstract

Background: The medicinal potential of plant extracts for the management of liver and kidney disorders in humans has been harnessed for the past several centuries. However, the abundant plant resources have not been fully investigated, thus this study was initiated to evaluate the influence of *Cassia spectabilis* leaves extract (CSE) on diclofenac-induced oxidative stress and hepatorenal damage in Wistar rats. The rats in the 1st group were injected with normal saline, and rats in the 2nd group were injected with diclofenac sodium (DF) by intramuscular route. Rats in the 3rd to 5th groups were treated with graded doses of CSE by oral gavages, and injected with DF. The serum markers of oxidative stress and hepatorenal damage in rats were estimated by biochemical assays. In addition, histological examinations of liver and kidney tissues were evaluated.

Results: There was significant ($p < 0.05$) increase in the levels of total bilirubin, ALT, AST, ALP, GGT, LDH, urea, creatinine, uric acid, potassium ions, and MDA of rats injected with DF when compared with normal control. The treatment of DF-injected rats with CSE significantly ($p < 0.05$) reduced the levels of these markers of hepatorenal damage in rats when compared with DF control. There was significant ($p < 0.05$) decrease in the levels of GSH, SOD, CAT, GPx, GST, sodium ions, proteins and G6Pase after injection of rats with DF when compared with normal control. However, treatment of DF-injected rats with CSE significantly ($p < 0.05$) increased the levels of these markers of antioxidant status and oxidative damage in hepatorenal tissues of rats when compared with DF control. The photomicrographs of hepatorenal tissues showed structural features which corroborated our biochemical findings in this study.

Conclusion: The findings of this study have shown that CSE may have protective effect against DF-induced oxidative stress and hepatorenal damage in Wistar rats. Thus, the medicinal potential of this plant leaves extract may be harnessed for the development of phytotherapeutic products.

Keywords: Antioxidants, *Cassia spectabilis* leaves extract, Hepatotoxicity, Lipid peroxidation, Nephrotoxicity, Oxidative stress

Background

Plant-derived products have been used by humans over the centuries for food, medicines and other benefits (Jothy et al., 2012). The extracts obtained from leaves, stems, roots and fruits of several plants have been utilized as medicines (Al-Attar et al., 2017; Imo et al., 2019; Rajakrishnan et al., 2017). Thus, there is a growing

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interest in the evaluation of several plant extracts for their medicinal values in the management of liver and kidney disorders (Iseghohi & Orhue, 2017; Konda et al., 2016). *Cassia spectabilis* (DC.) Irwin & Barn. - is a leguminous plant belonging to the family, Fabaceae, and the subfamily Caesalpinioideae (Jothy et al., 2012). It is widely grown as ornamental tree in the tropical regions of the earth. It is known by the trade name, "Golden cassia", while in Nigeria it is called "Okpehekwu", by the Idoma people of Benue state. The tree has a round shape, with a height of about 15–20 feet. It has green leaves, yellow flowers, gray and smooth bark, with a green to brown pod (Omotayo, 1999). The plant products are used as traditional medicines for treatment of flu, cold, laxative and purgative (Sangetha et al., 2008). Scientific studies have shown that the plant extract has radical scavenging (Jothy et al., 2012), antifungal and antimicrobial (Sangetha et al., 2008) activities.

The biotransformation of foreign compounds, drugs, environmental pollutants or toxins and the excretion of metabolic waste products by the liver and kidney of animals, make them susceptible to oxidative damage (Imafidon et al., 2019; Javed et al., 2018). Oxidative stress occurs when there is disruption of balance in the levels of tissue natural oxidants and free radicals such as nitric oxide, hydrogen peroxide, super oxide anion radicals, hydroxyl radicals, and the antioxidants which eliminate them such as the reduced glutathione and uric acid (Pham-Huy et al., 2008). The oxidative damage of animal tissues is commonly caused by oxidative stress, which may be induced by reactive oxygen species (ROS) that attack the polyunsaturated fatty acids (PUFAs) of cell membrane, resulting in lipid peroxidation (Ozbek, 2012). Oxidative stress may also trigger the production of inflammatory cells, as reactive oxygen and nitrogen species (RONS) act with pro-inflammatory cytokines to cause tissue degenerative changes via the apoptotic and necrotic processes, resulting in cell death (Bechmann et al., 2010).

Liver and kidney diseases are major public health problems globally, and may be caused by several factors such as viral infections, environmental pollutants, toxins and therapeutic drugs used by humans (Al-Attar et al., 2017; Mosa & Khalil, 2015). Diclofenac sodium (DF) is one of the drugs found to cause adverse effects in liver and kidney tissues of humans and animals (Owumi & Dim, 2019). It is a phenylacetic acid derivative, which acts as a non-steroidal anti-inflammatory drug (NSAID), used for the treatment of inflammation, pain and musculoskeletal disorders in humans and animals (Adeyemi & Olayaki, 2018). The toxic effects of DF was attributed to the production of reactive metabolites, 5-hydroxydiclofenac and N, 5-dihydroxydiclofenac, which could disrupt the natural balance and promote excess reactive oxygen and nitrogen species (RONS) in the tissues. This may then induce oxidative stress and hepatorenal tissues damage in rats (Bort et al., 1999).

As a result of the fact that there seems to be paucity of scientific reports on the protective effect of *C. spectabilis* leaves extract (CSE) against drug-induced oxidative stress and hepatorenal damage in rats, coupled with the claims by traditional medicine practitioners that the plant extract is effective in the management of liver and kidney diseases in humans, there is need for scientific studies to validate this claim. Therefore, this study was initiated to evaluate the protective effect of CSE against DF-induced oxidative stress and hepatorenal damage in rats.

Methods

Chemicals and preparation of diclofenac sodium

Diclofenac sodium (DF) is an injectable liquid purchased from the North China Pharmaceutical Co. Ltd, 115 Hainan Road, Shijiazhuang, Hebei, China. The reagent kits for biochemical assays were purchased from Randox Laboratories Ltd, United Kingdom. All the reagents used are of analytical grade. Each 3 ml ampoule contains 75 mg of DF. A single dose of the drug was suspended in 0.2 ml of normal saline (0.9 g/dL NaCl) solution.

Preparation of the hydro-ethanolic extract of *Cassia spectabilis* leaves

The *C. spectabilis* leaves were harvested from a forest at Obu, Otukpa, Ogbadibo local government area of Benue state, Nigeria. The plant was identified and authenticated by Mr. Mark Uleh, a Lecturer/Plant Taxonomist in the Department of Forestry and Forest Products, Federal University of Agriculture, Makurdi, Nigeria. A voucher specimen was deposited in the College of Forestry herbarium, with the voucher number given as FH/0258. The leaves were dried at room temperature for at least 3 weeks, pulverized to fine particles with mortar and pestle, and sieved with a porcelain sieve. The aqueous ethanol extract of the plant was prepared by a modified form of the method earlier described (Abotsi et al., 2010). Briefly, the solvent mixture was prepared by adding 800 ml of distilled water to 200 ml of absolute ethanol and mixed. Then, 100 g of the pulverized sample was macerated in 1000 ml of aqueous ethanol mixture and allowed to stand for 72 h. The mixture was sieved with a white piece of cloth and the liquid obtained was filtered with Whatman no. 1 filter papers. The filtrate was concentrated on a water bath at 45 °C, and the extract obtained was dried to a constant weight in a desiccator. The weight of the extract was determined and the percentage yield calculated by the expression:

$$\% \text{ Yield} = \frac{\text{Weight of extract}(g)}{\text{Weight of dry sample}(g)} \times 100$$

Experimental animals and management

Thirty adult albino Wistar rats (*Rattus norvegicus*) of either sex but equal numbers, having body weights of 200 - –250 g, were used for this study. They were purchased as litters at the age of 6 weeks, from the Animal house, College of Health sciences, Benue State University, Makurdi, Nigeria. Then they were kept in plastic cages to grow unto maturity, and acclimatized for about 4 weeks in the Department of Veterinary Physiology and Biochemistry research laboratory, Federal University of Agriculture, Makurdi, Nigeria. They were kept 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 handled with care according to International guidelines for the use of laboratory animals.

Animal groups and treatments

The thirty rats were randomly separated into 5 groups, consisting of 6 rats per group, but were separated into male and female cages, in order to avoid mating, and well labelled for proper identification. The co-treatment with plant extract and DF daily were separated by 5 h.

Group 1 rats were given 0.2 ml of normal saline daily, by intramuscular (i.m.) route for 5 days.
Group 2 rats were administered 10 mg/kg body weight (b. wt.) DF daily by i.m. route for 5 days (Adeyemi & Olayaki, 2018).
Groups 3 rats were injected with DF as in group 2 for 5 days and concurrently treated with 100 mg/kg b. wt. CSE by daily oral gavages for 7 days (Imafidon et al., 2019).
Group 4 rats were injected with DF as in group 2 for 5 days and concurrently treated with 300 mg/kg b. wt. CSE by daily oral gavages for 7 days.
Group 5 rats were injected with DF as in group 2 for 5 days and concurrently treated with 500 mg/kg b. wt. CSE by daily oral gavages for 7 days.

Collection and preparation of serum and tissue samples

A day after the end of treatment, blood was collected from the rats, under the influence of an anesthetic drug, ketamine, by intra-cardiac puncture. The blood was allowed to clot for at least an 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 with sharp blades, their liver and kidney were immediately excised, rinsed in normal saline, dried and placed in a bottle of 10% formal saline until they were processed.

Biochemical analyses

Biochemical assays of the biomarkers of hepatorenal injuries were determined by standard methods earlier described, and according to the manual procedures in the relevant reagent kits manufactured by Randox Laboratories Ltd, United Kingdom. The following methods were applied for serum aspartate aminotransferase and alanine aminotransferase (Reitman & Frankel, 1957), alkaline phosphatase (Kind & King, 1954), gamma glutamyl transferase (Szasz, 1969), glucose 6-phosphatase (Alegre et al., 1988), total proteins (Gornall et al., 1949), albumin (Doumas et al., 1971), creatinine (Bartels et al., 1972), uric acid (Fossati et al., 1980), and urea (Fawcett & Scott, 1960). The serum bilirubin, LDH, GSH, potassium, sodium, bicarbonate, and chloride ions were determined by standard procedures as outlined in the specific reagent kits' manuals. The samples were incubated at recommended temperatures, and absorbance of each biochemical parameter was determined with UV–Vis spectrophotometer at the appropriate time and wavelengths. The Globulin level was determined by taking the difference between total protein and albumin values of a sample (He et al., 2017).

Estimation of the levels of some oxidative stress markers

Estimation of lipid peroxidation product concentration

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation according to the method by Satoh (1978). Briefly, an aliquot of 0.4 ml sample was mixed with 1.6 ml Tris-KCl buffer, to which 0.5 ml 30% TCA was added. Then, 0.5 ml of 0.75% thiobarbituric acid (TBA) was added and placed in a water bath for 45 min at 80 °C. This was cooled in ice and centrifuged for 15 min at 3000 rpm. The absorbance of the resultant clear pink solution was measured at a wavelength of 532 nm against a reference blank of distilled water. The amount of malon dialdehyde (MDA) was calculated. Lipid peroxidation product level is expressed as n moles of MDA per mg protein.

Determination of superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined according to the method by Misra and Fridovich (1972). Briefly, 1 ml sample was diluted in 9 ml distilled water to make 1 in 10 dilution. An aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M bicarbonate buffer (pH 10.2) to equilibrate in the spectrophotometer; and

the reaction started by addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture, which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml substrate (adrenaline) and 0.2 ml of water. The absorbance at 480 nm was monitored every 30 s for 150 s. One unit of SOD activity is defined as the amount of enzyme required to produce a 50% inhibition of adrenaline oxidation.

Determination of catalase activity

The catalase (CAT) activity was determined according to the method by Sinha (1972). Briefly, different concentrations of H_2O_2 ranging from 10 to 100 µmoles were placed in small test tubes and 2 ml dichromate/acetic acid was added to each. Addition of the reagent produced an unstable blue precipitate of per chromic acid. Subsequent heating for 10 min in a boiling water bath changed the colour of the solution to a stable green, due to the formation of chromic acetate. After cooling at room temperature, the volume of the mixture was made up to 3 ml and the absorbance measured with a spectrophotometer at 570 nm. The concentrations of standard (H_2O_2) were plotted against absorbance. Furthermore, 1 ml of sample was mixed with 49 ml of distilled water to give a 1 in 50 dilution of the sample. The assay mixture contained 4 ml of 800 µmoles H_2O_2 solution and 5 ml phosphate buffer in a flask. Then, 1 ml of properly diluted enzyme preparation was rapidly mixed with the reaction mixture by gentle swirling. The reaction was done at room temperature. Thereafter, 1 ml portion of the reaction mixture was blown into 2 ml of dichromate/acetic acid reagent at 60 s intervals. The H_2O_2 contents of the samples were determined by the method described above. The velocity constant for the decomposition of H_2O_2 by catalase was determined by using the equation for a first order reaction. The results were expressed as unit per mg protein. One unit of catalase activity is defined as the amount of enzyme which breaks down 1 µmole H_2O_2/min/mg protein.

Determination of glutathione peroxidase activity

Glutathione peroxidase (GPx) activity was determined according to the method described by Tappel (1978). To a mixture of 500 µl phosphate buffer, 100 µl NaN_3, 200 µl GSH, 100 µl H_2O_2, and 600 µl distilled water in a test tube, 500 µl sample was added and mixed. The whole reaction mixture was incubated at 37 ^{0}C for 3 min, after which 0.5 ml TCA was added and centrifuged at 3000 rpm for 5 min. To 1 ml each of the supernatant, 2 ml K_2HPO_4 and 1 ml 2, 2-dithio-5, 5'-nitrodibenzoic acid (DTNB) were added. Then the absorbance was read at 412 nm against the blank. Glutathione peroxidase activity was determined by plotting a standard curve and the concentration of the remaining reduced glutathione (GSH) was extrapolated from the curve. GSH consumed = 245.34—GSH remaining.

Glutathione peroxidase activity = x µmol GSH oxidized/min/mg protein. One unit of GPx activity is defined as the amount of enzyme that oxidizes 1 µmol GSH/min/mg protein.

Estimation of reduced glutathione concentration

The level of reduced glutathione (GSH) in serum was estimated according to the method by Ellman (1959). A 0.1 ml of test sample was diluted with 0.9 ml of distilled water to give 1 in 10 dilution. Then, 3 ml 4% sulphosalicylic acid (SSA) solution was added to the diluted sample to deproteinize it. The mixture was centrifuged at 3000 rpm for 10 min. Thereafter, 0.5 ml of the supernatant was added to 4 ml 0.1 M phosphate buffer and 4.5 ml of Ellman's reagent was added. Ellman's reagent is a solution containing 4.96 mg of 2, 2-dithio-5, 5′-nitrodibenzoic acid (DTNB) in 250 ml phosphate buffer (0.1 M; pH 6.5). A blank was prepared with the reaction mixture of 4 ml 0.1 M phosphate buffer, 0.5 ml of the diluted SSA solution (by addition of 3 ml SSA solution and 2 ml of distilled water), and 4.5 ml Ellman's reagent. All readings were taken within 5 min at 412 nm; as the colour formed is not stable, after the addition of Ellman's reagent. The concentration of GSH is proportional to the absorbance of solution at 412 nm.

Histological examinations of liver and kidney tissues

Histological examinations of hepatic and renal tissues were conducted according to a method previously described (Drury & Wallington, 1980). The liver and kidney tissues were fixed in 10% formalin, dehydrated with graded alcohol, and cleared with xylene. Then, they were embedded with paraffin wax, sectioned into appropriate shapes, and stained with haemotoxylin and eosin (H&E) stains. The stained tissue sections were mounted on glass slides and viewed under a camera fitted light microscope, using the × 10 and × 40 objective lenses.

Statistical analyses

All the data were analyzed by Statistical Package for Social Sciences (SPSS) version 21 software, produced by IBM Corp. Ltd, USA. The data were expressed as Mean ± Standard Error of mean (SEM). They were analyzed by one way analysis of variance (ANOVA), and the level of significance in differences between groups was determined by the Fischer's least significant difference (LSD) in a Post Hoc test. The differences between mean values of experimental groups were considered significant at $p < 0.05$.

Results

Effect of hydroethanolic extract of *Cassia spectabilis* leaves on the levels of hepatorenal injury markers in DF-treated rats

There was a significant ($p < 0.05$) increase in the levels of serum ALT, AST, ALP, GGT, LDH and bilirubin, and significant ($p < 0.05$) decrease in the levels of total proteins, globulins, albumin and A/G ratio of rats administered 10 mg/kg b. wt. DF when compared with normal control. The treatment of DF-injected rats with CSE significantly ($p < 0.05$) reduced the levels of ALT, AST, ALP, GGT, LDH and bilirubin, and significantly ($p < 0.05$) increased total protein, albumin and globulin levels when compared with DF group (Tables 1, 2).

There was a significant ($p < 0.05$) increase in the serum levels of creatinine, urea, uric acid, potassium ions, and significant ($p < 0.05$) decrease in the levels of sodium, bicarbonate and chloride ions of rats treated with DF when compared with normal control group. The treatment of rats with DF and CSE significantly ($p < 0.05$) reduced dose-dependently the levels of creatinine, uric acid, urea, and potassium ions but significantly ($p < 0.05$) elevated the level of sodium, bicarbonate and chloride ions when compared with DF control group (Tables 3, 4).

There was a significant ($p < 0.05$) decrease in the serum SOD, CAT, GPx and GST (antioxidant enzymes) activities of the rats administered DF when compared with normal control group. However, treatment of rats with DF and CSE significantly ($p < 0.05$) increased the antioxidant enzyme activities dose-dependently when compared with the DF group (Table 5).

There was a significant ($p < 0.05$) decrease in G6Pase activities, GSH levels and increase in MDA levels of the rats administered DF when compared with the normal control group. However, the treatment of rats with DF and graded doses of CSE significantly ($p < 0.05$) increased the G6Pase activities, GSH levels and decreased MDA levels dose-dependently when compared with DF control group (Table 6).

Table 1 Effect of hydroethanolic extract of *Cassia spectabilis* leaves on the serum enzyme activities in DF-treated rats

Treatment groups	Serum enzyme activities as markers of liver injuries in rats				
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)
1. Normal control	11.7 ± 1.0	15.5 ± 0.8	117.8 ± 1.4	26.6 ± 0.9	13.5 ± 2.7
2. DF control	32.3 ± 1.1[a]	47.3 ± 0.8[a]	384.7 ± 1.7[a]	95.5 ± 1.4[a]	40.5 ± 4.2[a]
3. DF + 100 mg CSE	24.4 ± 0.4[ab]	32.9 ± 0.7[ab]	267.8 ± 1.0[ab]	75.2 ± 0.9[ab]	37.7 ± 1.7[a]
4. DF + 300 mg CSE	28.2 ± 0.4[ab]	37.7 ± 0.6[ab]	277.6 ± 0.6[ab]	81.9 ± 0.6[ab]	40.5 ± 2.9[a]
5. DF + 500 mg CSE	15.2 ± 0.4[ab]	20.7 ± 0.6[ab]	234.9 ± 1.2[ab]	57.5 ± 0.6[ab]	29.7 ± 1.7[ab]

Values are Mean ± SEM, n = 6

DF diclofenac sodium, *CSE* hydroethanolic extract of *Cassia spectabilis* leaf (mg/kg body weight), *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma glutamyl transferase, *LDH* lactate dehydrogenase

[a] Significantly different from the normal control ($p < 0.05$)

[b] Significantly different from the DF control ($p < 0.05$)

Table 2 Effect of hydroethanolic extract of *Cassia spectabilis* leaves on the levels of serum proteins and bilirubin in DF-treated rats

Treatment groups	Serum proteins and bilirubin levels as markers of liver injuries in rats				
	T-proteins (g/L)	Albumin (g/L)	Globulins (g/L)	T-bilirubin (μmol/L)	D-bilirubin (μmol/L)
1. Normal control	77.2 ± 1.09	38.7 ± 0.44	38.5 ± 0.85	9.8 ± 0.46	3.5 ± 0.19
2. DF control	56.6 ± 0.68[a]	23.1 ± 0.62[a]	33.6 ± 1.30[a]	38.0 ± 0.74[a]	24.2 ± 1.06[a]
3. DF + 100 CSE	63.8 ± 0.32[ab]	27.7 ± 0.26[ab]	36.1 ± 0.28[ab]	28.1 ± 0.74[ab]	14.4 ± 0.50[ab]
4. DF + 300 CSE	60.6 ± 0.32[ab]	24.8 ± 0.17[ab]	35.7 ± 0.38[a]	32.9 ± 0.13[ab]	17.8 ± 0.21[ab]
5. DF + 500 CSE	72.1 ± 0.58[ab]	34.7 ± 0.50[ab]	37.4 ± 0.84[b]	18.7 ± 0.45[ab]	6.8 ± 0.57[ab]

Values are Mean ± SEM, n = 6

DF diclofenac sodium, *T* total, *D* direct, *CSE* hydroethanolic extract of *Cassia spectabilis* leaf (mg/kg body weight)

[a] Significantly different from the normal control ($p < 0.05$)

[b] Significantly different from the diclofenac control ($p < 0.05$)

Table 3 Effect of hydroethanolic extract of *Cassia spectabilis* leaves on the serum levels of renal injury markers in DF-treated rats

Treatment groups	Serum levels of the markers of renal injuries in rats		
	Creatinine (µmol/L)	Urea (mmol/L)	Uric acid (µmol/L)
1. Normal control	78.08 ± 0.80	5.64 ± 0.25	205.82 ± 1.96
2. DF control	457.73 ± 8.25[a]	28.34 ± 0.77[a]	489.91 ± 3.07[a]
3. DF + 100 mg/kg CSE	215.03 ± 0.89[ab]	22.51 = 0.76[ab]	441.97 ± 0.69[ab]
4. DF + 300 mg/kg CSE	224.66 ± 0.59[ab]	26.53 ± 0.51[ab]	449.27 ± 0.37[ab]
5. DF + 500 mg/kg CSE	172.48 ± 1.96[ab]	13.77 ± 0.44[ab]	425.83 ± 4.39[ab]

Values are Mean ± SEM, n = 6

DF diclofenac sodium, *CSE Cassia spectabilis* leaf extract

[a] Significantly different from the normal control ($p < 0.05$)

[b] Significantly different from the diclofenac control ($p < 0.05$)

Table 4 Effect of hydroethanolic extract of *Cassia spectabilis* leaves on the levels of serum electrolytes in DF-treated rats

Treatment groups	Levels of electrolytes as markers of acid base imbalance in rats			
	Sodium ions (mmol/L)	Potassium ions (mmol/L)	Chloride ions (mmol/L)	Bicarbonate ions (mmol/L)
1. Normal control	142.17 ± 0.75	3.75 ± 0.14	111.67 ± 0.80	24.83 ± 0.48
2. DF control	130.50 ± 0.50[a]	6.05 ± 0.06[a]	99.0 ± 1.18[a]	12.0 ± 0.37[a]
3. DF + 100 mg CSE	134.50 ± 0.22[ab]	5.63 ± 0.02[ab]	102.67 ± 0.80[ab]	18.83 ± 0.31[ab]
4. DF + 300 mg CSE	133.0 ± 0.00[ab]	5.73 ± 0.02[ab]	100.0 ± 0.37[a]	14.83 ± 0.40[ab]
5. DF + 500 mg CSE	138.17 ± 0.40[ab]	4.73 ± 0.04[ab]	108.0 ± 0.37[ab]	18.50 ± 1.78[ab]

Values are Mean ± SEM, n = 6

DF diclofenac sodium, *CSE Cassia spectabilis* leaf extract

[a] Significantly different from the normal control ($p < 0.05$)

[b] Significantly different from the diclofenac control ($p < 0.05$)

Table 5 Effect of hydroethanolic extract of *Cassia spectabilis* leaves on the serum antioxidant enzyme activities in DF-treated rats

Treatment groups	Serum antioxidant enzyme activities in rats			
	SOD (U/mg p)	CAT (U/mg p)	GPx (U/mg p)	GST (U/mg p)
1. Normal control	11.36 ± 0.43	10.43 ± 0.29	10.25 ± 0.37	41.15 ± 1.06
2. DF control	4.16 ± 0.27[a]	2.24 ± 0.19[a]	2.83 ± 0.19[a]	15.18 ± 0.53[a]
3. DF + 100 mg CSE	4.66 ± 0.19[a]	5.27 ± 0.32[ab]	5.38 ± 0.28[ab]	22.29 ± 0.68[ab]
4. DF + 300 mg CSE	6.64 ± 0.19[ab]	6.60 ± 0.21[ab]	4.46 ± 0.18[ab]	19.35 ± 0.41[ab]
5. DF + 500 mg CSE	9.17 ± 0.36[b]	6.52 ± 0.25[ab]	5.49 ± 0.41[ab]	32.21 ± 0.74[ab]

Values are Mean ± SEM, n = 6

DF diclofenac sodium, *CSE Cassia spectabilis* leaf extract, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GST* glutathione S-transferase, *P* protein

[a] Significantly different from the normal control ($p < 0.05$)

[b] Significantly different from the diclofenac control ($p < 0.05$)

Effect of hydroethanolic extract of *Cassia spectabilis* leaves on histological changes of liver and kidney tissues in DF-treated rats

The photomicrograph of liver section of rat injected with only normal saline appeared normal, without the signs of degenerative changes or tissues necrosis. The hepatocytes and liver sinusoids were intact, and appeared normal. The liver section of rat injected with 10 mg/kg b. wt. DF showed degenerative changes and coagulative necrosis of hepatocytes, with severe congestion of liver sinusoids. Some hepatocyte nuclei were completely lost while some were partially damaged. However, the liver section of

Table 6 Effect of hydroethanolic extract of *Cassia spectabilis* leaves on the levels of serum MDA, GSH and G6Pase in DF-treated rats

Treatment groups	Levels of hepatorenal oxidative damage markers in rats		
	G6Pase (ng/μL)	MDA (nmol/mg p)	GSH (μg/mL)
1. Normal control	33.89 ± 0.91	3.46 ± 0.20	64.44 ± 1.05
2. DF control	14.71 ± 0.44[a]	14.44 ± 0.73[a]	30.13 ± 0.86[a]
3. DF + 100 mg/kg CSE	20.98 ± 0.71[ab]	7.49 = 0.44[ab]	37.13 ± 0.53[ab]
4. DF + 300 mg/kg CSE	17.01 ± 0.59[ab]	7.25 ± 0.24[ab]	36.05 ± 0.80[ab]
5. DF + 500 mg/kg CSE	27.26 ± 0.80[ab]	4.74 ± 0.18[b]	47.54 ± 0.74[ab]

Values are Mean ± SEM, n = 6

DF diclofenac sodium, *CSE Cassia spectabilis* leaf extract, *G6Pase* glucose 6-phosphatase, *MDA* malon dialdehyde, *GSH* reduced glutathione

[a] Significantly different from the normal control ($p < 0.05$)

[b] Significantly different from the diclofenac control ($p < 0.05$)

rat injected with DF and treated with 100 mg/kg b. wt. CSE showed normal liver tissues with intact hepatocytes, without any evidence of degenerative changes. The liver section of rat injected with DF and treated with 300 mg/kg b. wt. CSE showed normal hepatocyte nuclei with their intact cytoplasm, but some of them had mild pyknotic changes. The hepatic tissue degenerative changes are less severe compared with the changes observed in DF control rat. The liver section of rat injected with 10 mg/kg b. wt. DF and treated with 500 mg/kg b. wt. CSE showed normal hepatocyte nuclei surrounded by intact cytoplasm. There were mild degenerative changes in some hepatocytes, while some have lost their cellular integrity. However, the tissue degenerative changes are less severe compared with those observed in DF control rat (Fig. 1).

The photomicrograph of kidney section of rat injected with only normal saline appeared normal, without the signs of degenerative changes or tissues necrosis. The glomeruli, Bowman's capsules and renal tubules were intact, and appeared normal in shapes and sizes. The kidney section of rat which was injected with 10 mg/kg b. wt. DF showed some signs of degenerative changes. There was haemorrhage in the renal tubules, indicating the presence of red blood cells, severe congestion of collecting tubules and infiltration of the interstitial areas of the kidneys by inflammatory cells. However, the light micrograph of kidney section of rat injected with DF and treated with 100 mg/kg b. wt. CSE showed normal kidney tissues with intact glomeruli, Bowman's capsule with normal capsular space, and clear renal tubules. The kidney section of rat injected with DF and treated with 300 mg/kg b. wt. CSE showed intact glomeruli, surrounded by the Bowman's capsule, and clear renal tubules but reduced capsular space. The kidney section of rat which was injected with 10 mg/kg b. wt. DF and treated with 500 mg/kg b. wt. CSE showed intact glomeruli with their normal Bowman's capsules, but few deformed glomeruli with reduced capsular space and mild renal tubular congestion (Fig. 2).

Discussion

This study evaluated the influence of hydroethanolic extract of *Cassia spectabilis* leaves on DF-induced oxidative stress and hepato-renal tissues damage in Wistar rats. The injuries inflicted on hepato-renal tissues in animals are commonly induced by the toxic effects of therapeutic drug overdose, and diclofenac sodium (DF) is one of such drugs. It may accumulate in human tissues, as a result of its frequent use in the treatment of musculoskeletal disorders and long duration of intake (Owumi & Dim, 2019). The mechanism of DF-induced hepatotoxicity has been partially attributed to the generation of reactive oxygen species (ROS), which may cause oxidative stress (Adeyemi & Olayaki, 2018), the alteration of protein integrity, immune-mediated processes, and mitochondrial damage (Masubuchi et al., 2002). The MDA level is a marker of lipid peroxidation in animal tissues (Rajakrishnan et al., 2017). Thus, the marked increase in serum MDA levels in rats treated with DF, indicates lipid peroxidation in the animal tissues, which may be attributed to the adverse effect of the drug. It was earlier shown that DF may generate highly reactive metabolites in animal tissues after treatment with the drug (Bort et al., 1999). These metabolites may cause imbalance in the antioxidant defense system of animals, which triggers oxidative stress and lipid peroxidation, ultimately resulting in cell death and tissue damage (Pham-Huy et al., 2008). However, the dose-dependent marked decrease in MDA levels, by treatment of DF-injected rats with 100, 300 and 500 mg/kg b. wt. CSE may suggest that the plant extract has protective effect against lipid peroxidation, which can cause hepato-renal tissue damage in the animals. This may be attributed to phytochemical compounds such as flavonoids, phenols and alkaloids previously found in the plant extract, which can protect cells against lipid peroxidation because they act as potent antioxidants that scavenge excess ROS, which are capable of damaging cell membrane lipids in the animal tissues (Ogbe et al., 2020).

The reduced glutathione (GSH) act as endogenous antioxidant to protect cells from oxidative damage, thereby protecting the vital organs of the animal body. They act in combination with other antioxidants to bind free radicals or oxidants and neutralize their effects (Raghuvanshi

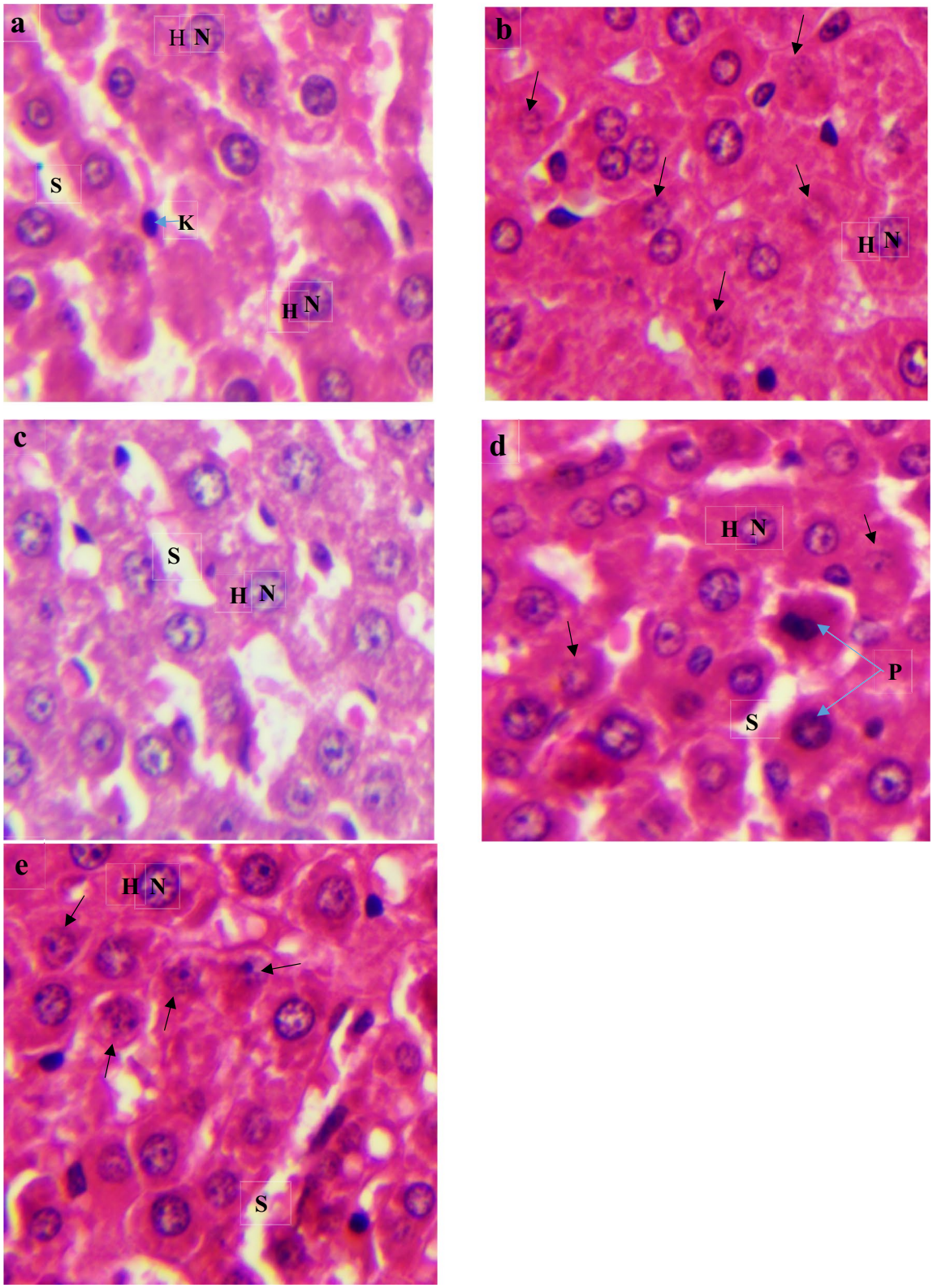

Fig. 1 Light photomicrographs of the liver sections of rats (H&E X400). **a** Control, **b** DF group, **c** DF + 100 mg/kg b. wt. CSE, **d** DF + 300 mg/kg b. wt. CSE and **e** DF + 500 mg/kg b. wt. CSE. K: Kupffer cell, N: Nucleus, H: Hepatocyte cytoplasm, Thin black arrows: Hepatocytes with degenerative changes (necrosis), some have lost their nuclei and cytoplasm, S: Hepatic sinosoid, P: Pyknotic hepatocytes

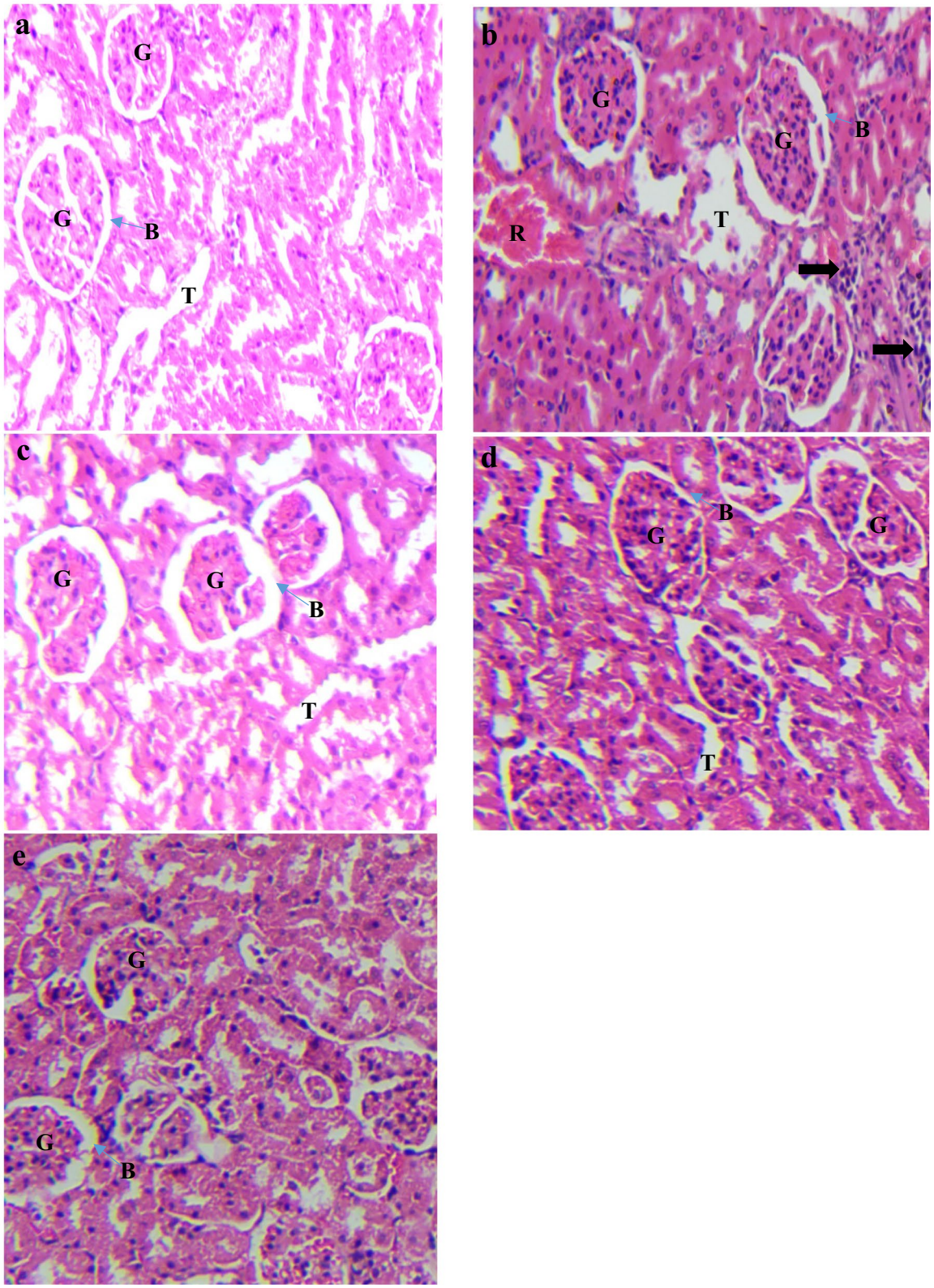

Fig. 2 Light photomicrographs of the kidney sections of rats (H&E X100). **a** Control, **b** DF group, **c** DF + 100 mg/kg b. wt. CSE, **d** DF + 300 mg/kg b. wt. CSE and **e** DF + 500 mg/kg b. wt. CSE. Thick black arrows: Inflammatory cells, R: Haemorrhage containing red blood cells, T: Renal tubule, B: Bowman's capsule, G: Glomerulus

et al., 2020). The antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST), play important roles in the endogenous antioxidant defense system, to protect cells from oxidative damage induced by reactive oxygen and nitrogen species (RONS) generated in animal tissues (Owumi & Dim, 2019). Thus, the marked decreases in the serum SOD, CAT, GPx, and GST activities, and GSH levels in rats, after they were administered 10 mg/kg b. wt. DF, may indicate down regulation of the antioxidant system in animal tissues, which might be attributed to adverse effects of the drug. It was earlier shown that the oxidative stress induced by DF (a pro-oxidative agent) in animal tissues, can cause the down regulation of their antioxidant system, consequently resulting in decreased antioxidant enzyme activities and GSH levels in the hepato-renal tissues of animals (Adeyemi & Olayaki, 2018; Owumi & Dim, 2019). However, the markedly elevated antioxidant enzymes activities and GSH levels, by treatment of DF-injected rats with 100, 300, and 500 mg/kg b. wt. CSE, may suggest that the plant extract has protective effect against the oxidative stress, depletion in tissue GSH pool, and inactivation of antioxidant enzymes in the tissues of animals. These findings may be attributed to the activities of certain health-promoting phytochemicals in the plant extract, which can act as potent exogenous antioxidants, and contribute to the tissue antioxidant pool. They can be utilized by the antioxidant system of the animals, thereby preventing over-utilization of endogenous GSH, oxidative stress, and its inhibitory effect on the antioxidant enzyme activities in the animal tissues (Ogbe et al., 2020; Raghuvanshi et al., 2020).

The glucose 6-phosphatase (G6Pase) is a smooth endoplasmic reticulum (SER) membrane enzyme involved in glucose metabolism; particularly the gluconeogenesis, in the step which glucose 6-phosphate is converted to free glucose by the action of this enzyme. Thus, reduction in G6Pase activity directly reflects the degradation of SER membrane (Raghuvanshi et al., 2020). This membrane-bound enzyme is found mainly in the liver, with lower concentration in the kidney, so its activity in these tissues can be used as marker of liver and kidney damage in animals (Ogbe et al., 2019). Thus, the marked decrease in the serum G6Pase activity after the treatment of rats with10 mg/kg b. wt. DF may indicate inhibition of this enzyme, which might be attributed to the adverse effect of the drug. The state of oxidative stress created as a result of RONS generated by DF, may damage protein structures of enzymes and other vital biological molecules (Owumi & Dim, 2019). However, the dose-dependent markedly elevated G6Pase activity, by treatment of DF-injected rats with 100, 300, and 500 mg/kg b. wt. CSE, may suggest that the plant extract has protective effect against hepatorenal damage elicited by the deleterious actions of DF metabolites. This may be attributed to the phytochemical compounds such as flavonoids, alkaloids and phenols, which act as exogenous antioxidants that can scavenge RONS generated by DF in the animal tissues, thereby preventing oxidative stress, and its inhibitory effect on G6Pase activity in the tissues of rats (Ogbe et al., 2020). The findings of previous studies showed that treatment of drug-injected rats with plant extracts may protect their hepatorenal tissues against oxidative damage, demonstrated by dose-dependent significant increases in the G6Pase activities of animal tissues (Ogbe et al., 2019; Raghuvanshi et al., 2020).

The level of bilirubin and activities of several enzymes are used as markers of hepatic tissue damage (Adeyemi & Olayaki, 2018; Mosa & Khalil, 2015). Thus, the marked elevation in serum AST, ALT, ALP, GGT, LDH, and bilirubin levels, after the treatment of rats with DF may indicate hepatocellular and hepatobiliary impairments in the animal tissues, which might be attributed to the adverse effects of the drug. The highly reactive metabolites generated by DF can damage the hepatic cell membrane structures, and result in the leakage of liver marker enzymes (ALT, AST, and LDH) into the blood circulation, thereby increasing their activities in the serum of rats (Owumi & Dim, 2019). Hence, these enzymes are used as markers of hepatocellular damage in animals (Mosa & Khalil, 2015). The levels of serum bilirubin, ALP and GGT are commonly elevated as a result of hepatobiliary impairments, which result in reduced metabolic and excretory functions of the liver. The elevation in these liver injury markers may also be due to cholestasis or biliary duct obstruction. These findings are in agreement with Adeyemi and Olayaki (2018) who found significant increase in serum bilirubin, and Owumi and Dim (2019) who reported marked increase in the activities of GGT and ALP, after the administration of DF into rats. However, the dose-dependent marked reduction in the levels of serum bilirubin, ALT, AST, ALP, LDH, and GGT activities, by the oral treatment of DF-injected rats with 100, 300 and 500 mg/kg b. wt. CSE, may suggest that the plant extract has protective effect against hepatocellular and hepatobiliary impairments elicited by DF toxicity in rats. This may be attributed to the activities of phytochemical compounds in the extract, which can inhibit the deleterious effects of DF on hepatocellular membrane structures. Several studies have demonstrated the protective effects of plant extracts against drug-induced hepatic tissue damage in rats, by lowering the serum levels of liver injury markers in rats, which were earlier treated with drugs and plant extracts (Jothy et al., 2012; Mosa & Khalil, 2015; Ogbe et al., 2019).

The damage to hepatocytes usually result in reduced biosynthetic functions of the liver, which leads to decreased levels of serum proteins (Javed et al., 2018; Mosa & Khalil, 2015). Thus, the marked reduction in the levels of serum total protein, albumin and globulins, after the treatment of rats with 10 mg/kg b. wt. DF may be an indication of hepatocellular damage, which might be attributed to the hepatotoxic effect of the drug in animals. Albumin is a plasma protein synthesized by the liver, which involves in several physiological activities of the body. It binds and transports cations, bilirubin, drugs and other biomolecules (He et al., 2017). The present findings are in agreement with previous studies which reported significant reduction in serum total proteins, albumin, and globulin levels, as indicators of hepatocellular damage, as a result of drug-induced hepatotoxicities in animals (Konda et al., 2016; Ogbe et al., 2019). However, the notable increases in the levels of serum total protein, albumin, and globulin toward the normal levels, by treatment of DF-injected rats with 100, 300, and 500 mg/kg b. wt. CSE, may suggest that the plant extract has protective effect against drug-induced hepatocellular damage in rats. The reports of previous studies showed that the rats which were given both drugs and plant extracts had serum protein levels, which were closer to the normal values than the protein levels of drug-injected rats that were not treated with plant extracts (Konda et al., 2016; Mosa & Khalil, 2015; Ogbe et al., 2020).

The levels of serum creatinine, uric acid and urea are used as some of the clinically important markers of renal tissue damage in animals (Al-Attar et al., 2017; Rajakrishnan et al., 2017). Thus, the marked elevation of serum creatinine, uric acid, and urea levels, by treatment of rats with 10 mg/kg b. wt. DF may indicate acute renal damage, which might be attributed to the adverse effects of the drug. Creatinine is the excretory product of creatine metabolism, uric acid is the end product of purine degradation, and urea is the excretory product derived from protein catabolism (Rajakrishnan et al., 2017). As waste products of metabolism, they are readily removed from the blood stream to avoid accumulation, by the renal glomerular filtration, and are excreted by the kidney nephrons into the urine (Al-Attar et al., 2017). However, drug-induced nephrotoxicity may cause renal tissue damage, which manifests as impairment of renal functions, with marked reduction in glomerular filtration rate (GFR). Consequently, the reduced GFR may cause significant increase in the levels of these serum markers of renal tissue damage in animals (Imafidon et al., 2019; Konda et al., 2016). The markedly reduced levels of creatinine, urea, and uric acid, by treatment of DF-injected rats with 100, 300 and 500 mg/kg b. wt. CSE, may be attributed to the nephroprotective activity of the plant extract in animals. Thus, there may be phytochemical compounds in the CSE which can mitigate against the nephrotoxic effects of DF in animals (Ogbe et al., 2020). These findings are in agreement with Al-Attar et al. (2017) and Rajakrishnan et al. (2017), who reported on the nephroprotective effects of plant extracts against drug-induced nephrotoxicity, with evidences of lowered serum levels of creatinine, urea, and uric acid in rats.

Previous studies have shown that high doses of xenobiotic compounds, toxins or drugs could cause kidney damage, which may result in electrolytes imbalance (Al-Asmari et al., 2020; Rajakrishnan et al., 2017). The inhibition of renal prostaglandin synthesis by non-steroidal anti-inflammatory drugs (NSAIDs) causes various electrolyte and acid–base disturbances including sodium retention, hyponatremia (reduced blood sodium levels), hyperkalemia (elevated blood potassium levels), and decreased renal function (Kim & Joo, 2007). The marked decreases in the levels of sodium, chloride and bicarbonate ions, and marked increase in the level of potassium ions, by treatment of rats with DF may indicate disruption of electrolyte balance, which might be attributed to the adverse effects of this drug. The kidney performs the function of regulating electrolyte concentrations in the blood plasma of animals. Thus, when there is acute or chronic kidney damage, it may result in electrolyte imbalance, evident by alteration in the concentrations of electrolytes (sodium, potassium, chloride, and bicarbonate ions) in the blood of rats (Imo et al., 2019). Our findings of hyponatremia and hyperkalemia in DF-administered rats are in disagreement with Rajakrishnan et al. (2017) who reported elevated levels of sodium ions and decreased levels of potassium ions in the serum of drug-injected rats, but are in agreement with Kim and Joo (2007), who reported that NSAIDs may cause hyponatremia and hyperkalemia in susceptible patients.

The balance in blood electrolyte is a good indicator that the kidneys are functioning well. Sodium ion, as the major cation of the extracellular fluid, is regulated by the kidney, so the extremes in blood sodium ion levels may cause cells of the kidney to malfunction and could result in tissue damage (Imo et al., 2019). Though it may occur rarely, but NSAIDs can cause hyponatremia by decreasing the renal free water clearance (Kim & Joo, 2007). Chloride ion is important in the maintenance of cation/anion balance between the intra and extra-cellular fluids. It is essential for the regulation of proper hydration, osmotic pressure, and acid–base balance. Low serum chloride ion levels are found in nephritis and other conditions (Imo et al., 2019). Potassium ion is the major cation of the intracellular fluid but may also be an important constituents of the extracellular fluid. Hyperkalemia is often associated with renal failure, as the elevation in

blood potassium levels may occur to an extent that is enough to cause cardiac arrhythmias, and the renal function of animals may decline sufficiently to cause acute renal failure (Kim & Joo, 2007). The plasma bicarbonate ion concentration is an important indicator of electrolyte dispersion and anion deficit. Alteration of serum bicarbonate level is a characteristic of acid–base imbalance, which may be due to renal tubular acidosis, hyperkalemic acidosis, keto-acidosis or renal failure (Imo et al., 2019). The marked reduction in the levels of potassium ions, and marked elevation in the levels of sodium, chloride and bicarbonate ions, after treatment of drug-injected rats with graded doses of CSE, may suggest that the plant extract has potential to resist the disruption of electrolyte balance in animals. These findings are in agreement with previous studies which found that treatments of drug-injected rats with plant extracts mitigated against disruption in the levels of serum electrolytes in animals (Al-Asmari et al., 2020; Rajakrishnan et al., 2017).

The photomicrographs obtained from histological assessments of animal tissues may reveal the microstructural features of their organs, which can provide evidence to complement the biochemical findings of a particular study (Al-Asmari et al., 2020; Al-Attar et al., 2017). Therefore, the degenerative changes in hepatic tissues of DF-injected rats, depicted by focal necrosis of hepatocytes, vacuolation and loss of hepatocyte nuclei, and the congestion of hepatic sinusoids, may indicate liver damage in rats. The haemorrhage in renal tubules, congestion of collecting tubules and infiltration of interstitial areas by inflammatory cells, are evidence of renal tissue injuries in rats. These findings may be attributed to the toxic effects of DF, which was found to induce tissue damage by oxidative stress. This process may trigger the production of inflammatory cells and their infiltration of tissue spaces, induction of hepatocyte apoptosis, significant distortion of hepatocytes and renal tissues, which ultimately result in tissue necrosis (Adeyemi & Olayaki, 2018; Owumi & Dim, 2019). The reactive metabolites generated by DF have been found to cause hepatocytes apoptosis (Gómez-Lechón et al., 2003). Apoptotic and necrotic processes trigger pathological changes in animal tissues resulting in cell death, thus they play important roles in the pathogenesis of hepatorenal damage in animals (Bechmann et al., 2010). One of the consequences of oxidative stress is tissue inflammation, as it was found that the pathways that lead to production of inflammatory mediators are triggered by oxidative stress in animal tissues (Adeyemi & Olayaki, 2018).

It was earlier reported by Owumi and Dim (2019) that the DF act synergistically with pro-inflammatory cytokine, tumor necrosis factor alpha (TNF-α), to increase the levels of inducible nitric oxide (NO), and the production of NO, mediated by nuclear factor kappa-B (NF-κB) signaling, can cause cell death, resulting in tissue damage. Inflammation and oxidative stress are closely associated; as certain ROS may initiate intracellular signaling cascade that promotes the expression of pro-inflammatory gene, and inflammatory cells at the site of inflammation release free radicals, which may lead to elevated pro-oxidative response (Adeyemi & Olayaki, 2018). However, treatment of drug-injected rats with plant extracts may provide health benefits, which might result in early recovery from tissue injuries, prevent severe tissue damage and the death of animals (Iseghohi & Orhue, 2017). The markedly reduced degenerative changes in hepatorenal tissues, decrease in the number of inflammatory cells and other evidence of inflammation in the tissues, by co-treatment of rats with DF and graded doses of CSE may show protective effect of the plant extract against hepatorenal damage; which may be a confirmation of our biochemical findings. The present study has shown that the plant extract has potential to mitigate against DF-induced hepatorenal tissue injuries in rats, probably by inhibiting the action of reactive metabolites generated by DF and the production of inflammatory mediators. These findings are in agreement with some researchers who found that the treatment of drug-injected rats with plant extracts have protective effects against hepatic and renal tissue injuries, with evidence of reduced degenerative changes in the tissues of animals (Al-Asmari et al., 2020; Raghuvanshi et al., 2020; Rajakrishnan et al., 2017).

Conclusion

The present study has demonstrated that CSE may have protective effect against DF-induced oxidative stress and acute hepatorenal tissue damage in rats. The biological activity of this plant extract may be attributed to its phytochemical compounds which have anti-oxidative and anti-inflammatory activities, capable of mitigating against oxidative damage to animal tissues. These findings have given credence to the use of this plant extract in African traditional medicine for the management of liver and kidney diseases in humans. However, there is need for further research to elucidate the mechanism of action by which the phytochemical compounds mitigate against tissue oxidative damage in rats. It is also desirable to conduct bioassay-guided isolation and characterization of the bioactive compounds which may be responsible for the medicinal values of this plant extract.

Abbreviations

DF: Diclofenac sodium; CSE: Hydro-ethanolic extract of *Cassia spectabilis* leaves; RONS: Reactive oxygen and nitrogen species; TNF-α: Tumor necrosis factor alpha; NSAIDs: Non-steroidal anti-inflammatory drugs; NF-κB: Nuclear

factor kappa-B; NO: Nitric oxide; GFR: Glomerular filtration rate; GSH: Reduced glutathione; MDA: Malon dialdehyde; SEM: Standard error of mean; H & E: Haematoxylin and eosin stain; ANOVA: Analysis of variance; B. wt.: Body weight.

Acknowledgements
The authors wish to appreciate Mr. Vincent Upev, of the Department of Veterinary Physiology and Biochemistry, Federal University of Agriculture, Makurdi, Nigeria; for his technical assistance during the conduct of the experiments.

Authors' contributions
All the authors contributed to the conceptualization of the research and experimental design. RJO conducted the experiments; including the histological examinations, analyzed the results, and wrote the first draft of the manuscript. CDL and GIA interpreted the results and reviewed the manuscript for intellectual content. All the authors read and approved the final manuscript.

Funding
This work was partially funded by a grant from the Tertiary Education Trust Fund (TETFUND), through the staff training and development programme of the Federal University of Agriculture, Makurdi, Nigeria.

Availability of data and materials
The datasets generated for this study could be made available by the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
All animals and experimental protocols were in compliance with the international guidelines for the use of experimental animals. The ethical approval for the study was given by the Animal Welfare and Ethics Committee of the College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria.

Consent for publication
Not applicable.

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

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Received: 3 September 2020 Accepted: 19 February 2022
Published online: 07 March 2022

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