PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 26 (4) 571-576 April 2022

Protective Activity of Chloroform Extract of *Gomphrena celosioides* Leaves (Amaranthaceae) on Some Biochemical Indices in Aspirin-induced Wistar Rats'

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ABSTRACT: The protective activities of *Gomphrena celosioides* on Aspirin-induced alterations in biochemical markers were investigated in the kidney and liver of Wistar rats. Serum creatinine and blood urea nitrogen concentrations were assessed to determine biochemical alteration in the kidney, while total bilirubin and direct bilirubin concentrations were assessed to determine biochemical alteration in the liver. Four groups of rats were used. In group 1, the control rats were treated with 1% Gum Acacia solution. Group 2 was treated with Aspirin at 200 mg/kg b.w alone. Group 3 was treated with Aspirin at 200 mg/kg b.w and *G. celosioides* at 500 mg/kg b.w while Group 4 received *G. celosioides* at 500 mg/kg b.w. All the administration and treatment lasted for 7 days. A significant increase ($P < 0.05$) in the concentrations of serum creatinine (69.60 ± 4.16 mg/dl), total bilirubin (38.36 ± 5.33 mg/dl), direct bilirubin (15.17 ± 2.22 mg/dl) and blood urea nitrogen (27.05 ± 4.85 mg/dl) was observed in the rats treated with 200 mg/kg b.w aspirin. There was significant decrease ($P < 0.05$) in the concentrations of creatinine, total bilirubin, direct bilirubin, and blood urea nitrogen in the rats in group 3 (59.58 ± 2.972 mg/dl, 31.41 ± 6.22 mg/dl, 11.09 ± 1.40 mg/dl, 13.12 ± 0.67 mg/dl) and in rats in group 4 (53.2 ± 1.16 mg/dl, 32.19 ± 3.36 mg/dl, 13.49 ± 4.06 mg/dl, 10.63 ± 3.16 mg/dl) when compared with rats in group 2. While there was no significant ($P < 0.05$) difference when compared with group 1 (control) (52.20 ± 3.25 mg/dl, 30.75 ± 1.23 mg/dl, 12.46 ± 1.12 mg/dl, 7.29 ± 2.07 mg/dl). The results reveal that *G. celosioides* can provide significant protection against biochemical alterations in aspirin-treated rats.

DOI: https://dx.doi.org/10.4314/jasem.v26i4.3

Impact factor: http://sjifactor.com/passport.php?id=21082

Google Analytics: https://www.ajol.info/stats/bdf07303d34706088ffffbc8a92c9c1491b12470

Dates: Received: 30 March 2021; Revised: 13 March 2022; Accepted: 07 April 2022

Keywords: *Gomphrena celosioides*, Aspirin, Nephroprotective, Biochemical alteration, Hepatoprotective.

Different classes of drugs by immunological mechanisms or direct toxicity initiate certain stereotyped biochemical responses which result in damage to many tissues such as the liver and kidney (Xiaolin and Xiangmei, 2020). These drugs include antibiotics (aminoglycosides, tetracycline, e.t.c.), chemotherapeutic, and immune-suppressants (cisplatin, methotrexate, mytomycin, cyclosporine, e.t.c.) radiocontrast agents and nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin. Aspirin causes a variety of adverse effects, such as stomach ulcers, stomach bleeding, coagulation disorders, anaphylaxis cerebral microbleeds, liver and kidney damage (Chakrapani *et al*., 2016). However, the liver and the kidney are the most susceptible organ to damage by toxins and drugs because they are the site for toxin filtration and metabolic breakdown (Gaikwad *et al*., 2012). Prostaglandins protect the lining of the stomach and intestines from the damaging effects of acid, promote blood clotting by activating platelets, and also improve kidney function (Marcella *et al*., 2017). They are also mediators of inflammation and pain (Emanuela and FitzGerald, 2011). Vasodilatory PGs (PGE_2 and PGI_2) increase renal blood flow and glomerular filtration rate (GFR). PGE2 is involved in the regulation of sodium and water reabsorption and PGI2 increases potassium secretion mainly by stimulating the secretion of renin (Marcella *et al*., 2017). NSAIDs are cyclooxygenase inhibitors; they block the cyclooxygenase (COX), involved in the production of prostaglandin, reducing the production of prostaglandins (Enma *et al*., 2012).

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Therefore, inflammation, pain, fever, blood clotting as well as the protection produced on the stomach by prostaglandins are reduced by COX inhibitors (Emanuela and FitzGerald, 2011). Thus, NSAIDs can cause ulcers in the stomach and intestines, and increase the risk of bleeding. Aspirin also known as acetylsalicylic acid is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication has been discovered to cause ulcer (Chakrapani *et al*., 2016). This is as a result of its inhibitory effect on the production of thromboxane which result in excessive bleeding in the blood vessel and ulceration of the stomach walls. It has also been shown to be toxic to the kidneys by inhibiting renal PG-synthesis at usual therapeutic dosage (Chakrapani *et al*., 2016). Aspirin also results in a special form of hepatotoxicity known as Reye Syndrome; associated with development of lactic acidosis, microvesicular fat and hepatic dysfunction that involves encephalopathy and coma (Whitten *et. al*., 2013). Long-term administration have been shown to cause meta-bolomic changes in blood and liver injury indicated by increased level of alanine transaminase (ALT) and aspartate aminotransferase (AST)(Congcong *et al*., 2018). Chronic ingestion of aspirin causes toxicity due to an increase in its serum concentration. When metabolized, it causes uncoupling of oxidative phosphorylation in the mitochondria which results in increase in anaerobic metabolism and subsequent increase in lactic acid (Congcong *et al*., 2018). The increased lactic acid along with a slight contribution from the salicylate metabolites result in metabolic acidosis and eventually hemodynamic instability and end-organ damage (Patel, 2013). The search for pharmacological agents has made man turn to alternative medicine. It is a well-documented fact that a number of medicinal plants show beneficial effects in renal disorders (Gaikwad *et al*., 2012). Studies also suggested that the pharmacological activities of the plants are due to their antioxidant potential (Tijani, 2019). Currently, much interest is paid to medicinal plants for the prevention and treatment of many ailments (Upadhyaya and Saikia, 2011).

Gomphrena celosioides (GC) also commonly called Soft khaki weed or White-eye is a short-lived perennial plant (Abalaka, 2013, Tijani, 2019). It belongs to the Amaranthaceae family, and over 120 species of the family are found in America, Australia, Brazil, East and West of Africa (Abalaka, 2013). *Gomphrena celosioides* is used in ethnomedicinal practice in Nigeria for treatment of various skin diseases, diarrhea, malaria fever, bronchial infection, and diarrhea (Tijani, 2019). Bioactive compounds such as saponins, steroids, amino acids, non-reducing sugars, phenols and flavonoids have been isolated from the methanol extract of *G. celosioides* (Dosumu *et al*., 2005). Adeoti *et al*., (2016) also reported the presence of polyphenols, flavonoids, saponins, sterols and triterpenes, tannins and alkaloids in the ethanol extract of the plant. Anti-inflammatory and antioxidant properties have also been observed in the ethanol extract of *G. celosioides* plant (Adeoti *et al.*, 2016). However, there are limited studies on the efficacy of the chloroform extract of *G. celosioides on* renal toxicity. Considering the health benefits and the antioxidant activity possessed by this plant and the fact that aspirin induces alterations in many biochemical parameters resulting in damage to organs; hence, the aim of this study was to the reveal the protective activities of *Gomphrena celosioides* on aspirin-induced biochemical markers alteration in the kidney and liver of rats.

MATERIALS AND METHODS

All the chemicals used (such as Sulphanilic acid, Hydrochloric acid, Sodium, Nitrite, Sodium benzoate, Sodium benzoate Tartrate etc) were obtained from FBC Industries Inc, while Aspirin was purchased from Glaxosmithkline; a licensed Pharmaceutical store in Nigeria were of analytical grade. The kits (Bilirubin, Cholesterol, Creatinine and Urea) were obtained from Sigma Aldrich**.**

Plant Material: Gomphrena celosioides (*G. celosioides*) leaves were obtained from Iwo, Osun state, identified and authenticated at the Botany Department of Bowen University, Iwo, Nigeria with the voucher specimen (BUH-097) deposited at the University herbarium.

Preparation of G. celosioides Leaf Extract: The leaves were plucked, air-dried and then pulverized. 1 kg of powdered plant was weighed and soaked in chloroform for 76 h with intermittent shaking. This was filtered and filtrate was concentrated into semi-solid using rotary evaporator at 40°C. The extract was then dried and stored at −4°C until ready to use.

Animal Protocol: Healthy adult male Wistar rats weighing about 100–150 g were obtained from the veterinary Anatomy Department, University of Ibadan, and housed in the animal house of the Department of Biochemistry, Bowen University, Iwo, Osun State, Nigeria. They were kept in wire meshed cages, fed with commercial rat pellets, and had access to water ad libitum. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and

use of animals (US Department of Health and Human Services) and as approved by the Research Ethical Committee, Bowen University Iwo, Osun State, Nigeria. The "Principle of Laboratory Animal Care" (NIH publication No. 85-23) guidelines and procedures were considered in this study (NIH publication revised, 1985) (World Medical Association). The rats were deprived of food for 24 hours but had free access to clean water prior to the commencement of the experiment.

Experimental Protocol: Twenty male Wistar rats were used for the study and animals were divided into four groups of five animals in each group. Group 1 (Control): Received 1% Gum Acacia solution for 7 days. Group 2: Received aspirin only at the dose of 200 mg/kg b.w dissolved in 1% Gum Acacia solution for 7 days. Group 3: Received aspirin at the dose of 200 mg/kg b.w dissolved in 1% Gum Acacia solution and *G. celosioides* extract at a dose of 500 mg/kg b.w for 7 days. Group 4: Received *G.celosioides* only at the concentration of 500 mg/kg b.w for 7 days.

Sample Collection: Animals were sacrificed after 7 days of the study by cervical dislocation. Blood were collected into plain tubes and these were centrifuged at 3000 rpm for 10minutes to produce the serum

Parameters Assessed: Body weight; the weight of the animals (in grams) was noted on Day 1 and last day of the study and the differences in body weights was noted. Serum creatinine level was estimated using Bones *et al.*, 1945 method by measuring the concentration picric acid in the reaction mixture and absorbance was read at 520 nm.

Blood urea Nitrogen (BUN) level in the serum was estimated using Randox Kit (UR2821).

Bilirubin (Total and Direct) was estimated using colorimetric assay kit (ab235627) which utilizes the Jendrassik-Grof principle to detect bilirubin. Total bilirubin (unconjugated + conjugated) concentration was determined in the presence of catalyst, where bilirubin reacts with diazo-salt to form azobilirubin and absorbance was read at 600 nm. Direct bilirubin (conjugated) was determined in the absence of catalyst and absorbance was read at 550 nm).

Statistical analysis: Results are expressed as mean ± standard deviation. The data were analyzed by one-way analysis of variance. Means values were compared using Duncan test. The SPSS statistical package by IBM was used, and the value of $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Effect of chloroform extract of G. celosioides on body weight: Figure1 shows the effect of aspirin on body weights. A significant ($p< 0.05$) weight loss was observed in rats administered 200 mg/kg b.w aspirin (1.0±1.99 g) as compared to the control group (1.4±2.08 g) while *G. celosioides* chloroform extract treatment significantly ($p< 0.05$) attenuated aspirin-induced changes in body weight (1.8±3.75 g).

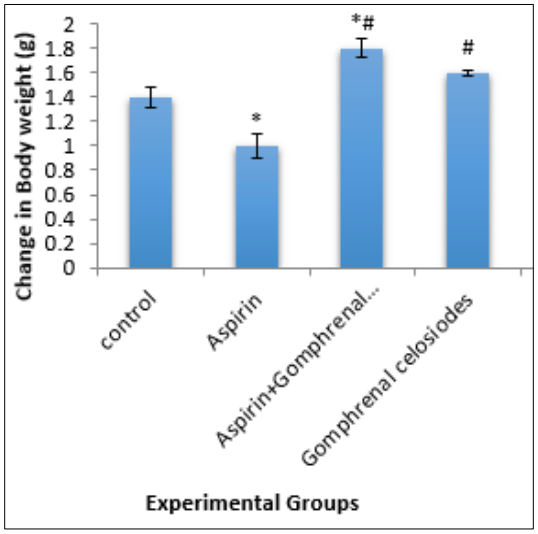

Fig 1: Effect of *Gomphrena celosioides* chloroform extract on change in body weight. All value represent mean ± SEM (n=5)*P=0.05 significant when compared with the control group #P < 0.05; significant when compared with aspirin treated group

Effect of chloroform extract of G. celosioides on Serum Creatinine, Total bilirubin, Direct bilirubin and Blood Urea Nitrogen (BUN): Figures 2 to 5 show the levels of serum creatinine, total bilirubin, direct bilirubin and blood urea nitrogen increased significantly ($p< 0.05$) in animals that received 200 mg/kg b.w aspirin (69.60 ± 4.16 mg/dl, 38.36 ± 5.33 mg/dl, 15.17 ± 2.22 mg/dl, 27.05 ± 4.85 mg/dl) when compared to normal animals. The level serum creatinine, total bilirubin, direct bilirubin and blood urea nitrogen was reduced significantly ($p<0.05$) by co-administration of aspirin with *G. celosioides* chloroform extract (500 mg/ kg) (59.58 ± 2.972 mg/dl, 31.41 ± 6.22 mg/dl, 11.09 ± 1.40 mg/dl, 13.12 ± 0.67 mg/dl). The levels of serum creatinine, total bilirubin, direct bilirubin and blood urea nitrogen for animals administered *G. celosioides* only was not significantly ($p< 0.05$) different (53.2 ± 1.16 mg/dl, 32.19 ± 3.36 mg/dl, 13.49 ± 4.06 mg/dl, 10.63 ± 3.16 mg/dl) from those co-administered with *G. celosioides*. Pharmaco-chemical studies of *G. celosioides* has revealed the presence of saponins, steroids, amino acids and non-reducing sugars in all the plant parts, phenols and flavonoids in leaves, inflorescence and stem.

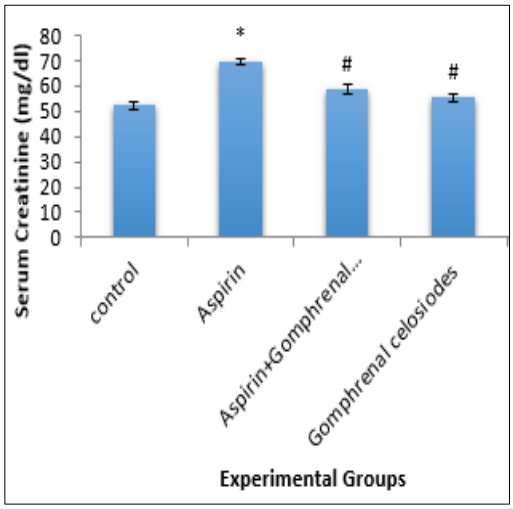

Fig 2: Effect of *Gomphrena celosioides* chloroform extract on serum. All value represent mean ± SEM (n=5),*P=0.05 significant when compared with the control group, #P < 0.05; significant when compared with Aspirin treated group

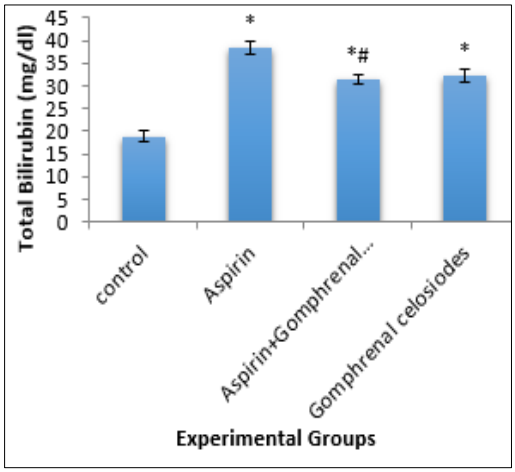

Fig 3: Effect of *Gomphrena celosioides* chloroform extract on total bilirubin. All value represent mean ± SEM (n=5), *P=0.05 significant when compared with the control group, #P < 0.05; significant when compared with Aspirin treated group

Betacyanins was found in the stem; reducing sugars in the inflorescence, while ketoses were found in the root and stem which contributes to its activity. (Sangare *et al.*, 2012, Abalaka, 2013, Nandini, 2018 and Tijani, 2019). In this study, the protective role of *G. celosioides* on biochemical parameters such as renal function indices and liver damage markers following administration of aspirin was investigated. Aspirin a salicylate is a nonsteroidal anti-inflammatory drug (NSAID). It has been implicated in damage to several organs of the body such the kidney, liver and the brain (Ge *et al*., 2011, Gaikwad *et al*., 2012). The kidney is the main excretory organ of the body and participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and regulation of blood pressure.

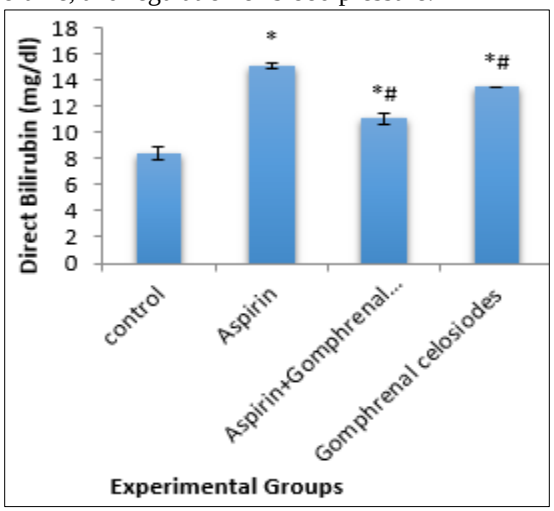

Fig 4: Effect of *Gomphrena celosioides* chloroform extract on direct bilirubin. All value represent mean ± SEM (n=5),*P=0.05 significant when compared with the control group, #P < 0.05; significant when compared with Aspirin treated group.

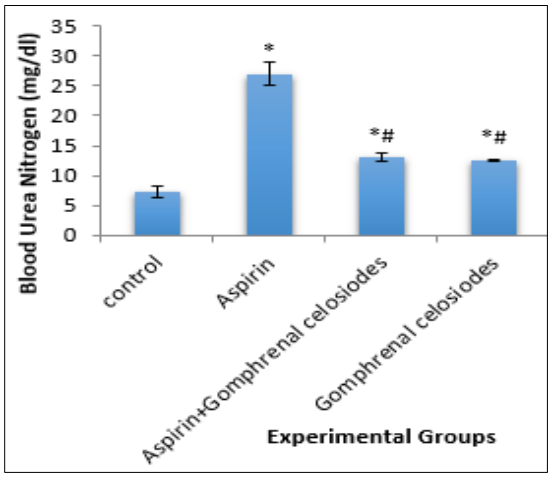

Fig 5: Effect of *G. celosioides* chloroform extract on blood urea nitrogen**.** All value represent mean ± SEM (n=5), *P=0.05 significant when compared with the control group, #P < 0.05; significant when compared with Aspirin treated group

The liver is the front line of defense, involved in the detoxification of many xenobiotics. (Whitten *et. al.*, 2013). Both the kidney and the liver are thus the main target organ of many drugs and therefore assessment of parameters that are markers of kidney and liver damage may of importance in assessing the effect of aspirin on biochemical parameters (Congcong *et al*., 2018). Damage to organs by aspirin is mediated through its inhibition of the electron transport chain. Kidney damage or dysfunction is reflected by increase in serum creatinine level and blood urea nitrogen (Cao, 2015). Creatinine is derived from creatine and creatine phosphate in muscle tissue and may be defined as a nitrogenous waste product. Creatinine is not reutilized

but excreted from the body in the urine via the kidney. It is produced and excreted at a constant rate which is proportional to the body muscle mass. As a consequence of the way creatinine is excreted by the kidney, creatinine measurement is used almost exclusively in the assessment of kidney function. Creatinine is regarded as the most useful endogenous marker in the diagnosis and treatment of kidney disease. Urea is mostly produced in the liver as the end product of amino acid metabolism and it is taken up by the kidney for excretion. Elevated urea in the blood and thus blood urea nitrogen is an indication of kidney disease or impairment. The result shows a significant ($p<0.05$) increase in serum creatinine concentration and blood urea nitrogen in aspirin group when compared with control. Co-administration of *G. celosioides* with aspirin significantly ($p<0.05$) decreased their values when compared with the Aspirin group. An increase in blood urea nitrogen and serum creatinine concentration in the aspirin group when compared with the control and its significant reduction by *G. celosioides* indicates a possible kidney dysfunction or impairment in the aspirin group that may be ameliorated by *G. celosioides.* This result support the findings of Konda *et al.,* 2015 that determined the nephroprotective effect of ethanolic extract of *Azima tetracantha* root in glycerol induced acute renal failure in Wistar albino rats. Cases of liver damage by aspirin have also been reported. One of such occurred in the treatment of juvenile rheumatoid arthritis which resulted in apoptosis and hepatocellular failure with elevated levels of aminotransferase and bilirubin (Chakrapani *et al*., 2016). Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. In the liver, bilirubin is conjugated with glucoronic acid to form a soluble compound (conjugated or direct bilirubin). This conjugated bilirubin passes down the bile duct and is excreted into the gastrointestinal tract. An unconjugated, albumin bound form is also present in the circulation. The result from this study also shows a significant increase in total and direct bilirubin concentration in the aspirin group and in groups co-administered with *G. celosioides* when compare with the control, indicating that there may be liver damage in these groups. However, amelioration by *G. celosioides* was still observed as total and direct bilirubin level were significantly ($p<0.05$) lowered in the *G. celosioides* co-administered compared to the aspirin group. This result confirm *G. celosioides* effectiveness in the functioning of liver cells as shown by Pal *et al*., (2006), based on bilirubin, with groups of rats treated by isoniazid . It also confirm the results obtained with by Sangare *et al.,* (2012) they revealed the presence of saponins, steroids, amino acids, nonreducing sugars, phenols and flavonoids in *G. celosioides*. Flavonoids are known for their hepatoprotective (Sangara *et al*., 2012). This hepatoprotective activities of the G. Celosioides may be due to the presence of flavonoids.

Conclusion: The results of the study reveals that chloroform extract of *G. celosioides* leaves showed nephroprotective and hepatoprotective effects against aspirin- induced toxicity.

Acknowledgement: The authors are grateful to the management and staff of the Department of Biochemistry and Botany Bowen University. We appreciate the relentless contributions of Dr. S. O Jeje the acting HOD of the Department of Physiology Federal University of Technology Akure, Ondo State, Nigeria; towards the success of this manuscript.

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