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BIOACTIVITY-SAFETY EVALUATION AND ANTIHYPERTENSIVE EFFECT OF HYDRO- ETHANOL LEAVES EXTRACT OF ACALYPHA GODSEFFIANA ON DOCA-SALT INDUCED HYPERTENSION IN UNILATERAL NEPHRECTOMIZED WISTAR ALBINO RATS

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ABSTRACT

Patients are often unaware of the important similarities and differences between medicinal herbs and approved medication, and some fail to understand that herbs are composed of bioactive chemicals some which may be toxic. Hypertension constitutes a major risk factor for several cardiovascular diseases such as heart failure, renal insufficiency and stroke. This research was aimed to evaluate the bioactivity-safety and antihypertensive effect of hydro- ethanol leaves extract of *Acalypha godseffiana* on DOCA-salt induced hypertension in unilateral nephrectomized albino rats. Healthy wistar albino rats of both sexes between eight to twelve weeks of age weighing 150 -200g were used for the study. They were divided into five groups of five rats each. Group 1 and 2 served as sham control and negative control, while group 3 to 5 served as test groups treated with 100, 200 and 300mg/kg b.w. of the extract respectively. Uninephrectomy was achieved through the left flank incision and hypertension was induced using DOCA-salt and was measured weekly by non-invasive method. Treatment was by oral administration using gavage and lasted for 4 weeks. At the end of 4 weeks rats were sacrificed by cervical dislocation and blood was collected through ocular into heparin bottles. All the parameters were analyzed using standard analytical methods. The result showed that the significant ($p<0.05$) decrease in creatinine, urea, protein and albumin concentrations in group administered 400 and 200mg/kg b.w. of *Acalypha godseffiana* is an indication of

nephroprotective potential of the plant. The results obtained also showed a dose dependent significant ($p<0.05$) decrease in serum AST, ALP and ALT levels at doses of 100, 200 and 400 mg/kg compared to the negative control group, suggesting hepatocellular protection at the doses administered. The result showed that hydro-ethanol leaves extract of *Acalypha godseffiana* at doses of 200, 400 and mostly at 800mg/kg body weight reduced blood pressure in DOCA-salt hypertensive rats in time-dependent manner. However, the antihypertensive activity of hydro-ethanol leaves extract of *Acalypha godseffiana* may be due to 5-HT_{2B} and ET-1 receptors antagonism. Hydro- ethanol leaves extract of *Acalypha godseffiana* was found to be safe to the renal and hepatic cellular functions and also possesses antihypertensive activity on DOCA-salt induced hypertension in unilateral nephrectomized Wistar albino rats.

KEYWORDS: Deoxycorticosterone acetate, *Acalypha godseffiana***,** hypertension, hydro-ethanol.

INTRODUCTION

The incidence of renal disease is increasing dramatically and has become a significant public health problem both economically and medically (Salako, 2005). Several studies (Bwititi *et al*., 2000; Ijeh and Agbo, 2006) have indicated the possibility that the use of plant extracts in high doses could lead to toxic injury to the kidneys which interfere with renal tubular functions and induce acute renal failure. Folkloric history has indicated attempts made by inhabitants using plant-derived recipes in parts of Nigeria to treat what they described as ‘‘fever of crisis’’, shifting joint pains, exacerbations especially during rainy seasons and ‘‘constant abnormality of the blood’’ though relatively few have been validated scientifically (Egunyomi *et al.*, 2009). This calls for a need to further investigate safer concentration of ethnomedicinal preparations in view of the increasing reports of acute renal failure (Ijeh and Ukweni, 2007).

The liver plays a significant role in the body as the organ responsible for metabolism of toxic substances that enter the body (Alisi *et al.*, 2008). The major functions of the liver can be detrimentally altered by liver injury resulting from acute or chronic exposure to toxicants or by situations affecting both ß-oxidation and the respiratory chain enzymes (Alisi *et al*., 2008). Serum enzyme activities are used as indicators of chemically-induced liver damage (Drotman and Lawhorn, 1978). Hepatotoxicity has been viewed as liver injury associated with impaired liver function caused by exposure to drug or other noninfectious agents (Navarro, 2006).

Hypertension constitutes a major risk factor for several cardiovascular diseases such as atherosclerosis, heart failure, renal insufficiency, coronary artery disease and stroke. The risk factor increases with the age in both sexes. According to World Health Organization (WHO), about one-third of the world's population suffers from hypertension and the incidence has been increasing at a rapid rate due to life style modification (WHO, 2011).

Conventional antihypertensives are usually associated with many side effects. About 75 to 80% of the world population use herbal medicines, mainly in developing countries, for primary health care because of their better acceptability with human body and lesser side effects. In the last three decades, a lot of concerted efforts have been channelled into researching the local plants with hypotensive and antihypertensive therapeutic values. The antihypertensive effects of some of these medicinal plants have been validated and others disproved. However, ayurvedic knowledge needs to be coupled with modern medicine and more scientific research needs to be done to verify the effectiveness, and elucidate the safety profile of such herbal remedies for their antihypertensive potential (Nahida and Feroz, 2011).

Many synthetic drugs have been developed for the treatment of hypertension because of the severity and prevalence of the disease. Most of these drugs have demonstrated better efficacy but possess a number of side effects. Herbal medicines therefore, have been regaining importance because of their ease of availability, less side effects and cost effective (Kalian, 2005).

Early detection and commencement of chemotherapy are essential in preventing or delaying these complications and enhancing survival of the afflicted patients (Meredith, 2007). To treat hypertension coupled with associated complications, use has been made of drugs derived from plants such as digitoxin from *Digitalis purpurea* (foxglove), reserpine from *Rauwolfia serpentina* (snakeroot), aspirin from *Salix alba* (willow bark), tetramethylpyrazine, also known as Ligustrazine from *Jatropha podagrica,* and tetrandrine from *Stephenia tetradra* (Ojewole and Odeyebi, 1980). These plant-derived pharmaceuticals have scientifically been proven to elicit antihypertensive activity via multiple mechanisms. These mechanisms are elicited to counteract the effect of hypertension and associated risk factors such as hypercholesterolemia, hypertriglyceridaemia, and oxidative stress on blood vessel walls.

Acalypha godseffiana has been reported to have medicinal properties for the treatment of malaria, dermatological and gastrointestinal disorders respectively, antihypertension properties, and for its antimicrobial activities (Adesina *et al*., 2000). *Acalypha godseffiana* (Copperleaf, Jacob's coat, fire dragon) which belongs to the family Euphorbiaceae is an ornamental plant commonly planted in the gardens or surroundings in Southern Nigeria, although it can also be grown indoors as a container plant. According to Akinde and Odeyemi, (1986), the expressed juice or boiled concoction of this plant are used for the treatment of gastrointestinal disorders and fungal skin infections such as *Pityriasis versicolor, Impetigo ontagiosa, Candida intetrigo, Tinea versicolor, Tinea corporis*, and *Tinea pedis.*

MATERIALS AND METHOD

Collection of plant material

Acalypha godseffiana leaves were collected by hand-plucking from parent plants at different locations within Federal Polytechnic Nekede, Owerri, Imo State, Nigeria. They were identified by a botanist, Dr. Duru C. M. of the department of Biological Sciences, Federal University of Technology Owerri, Imo State, Nigeria.

Preparation of the plant extracts

The leaves of *Acalypha godseffiana* were chopped into small pieces and were air dried at room temperature for 21 days. The dry leaves were ground into fine powder using an electric grinder. The powdered plant material (1 Kg) was sequentially extracted by cold extraction method with 1 L of hydro-ethanol mixture (80/20, v/v) for 8 h. This was left at room temperature for 72 hours. The extract was filtered through Whatman No. 1 filter paper and was concentrated with a rotary evaporator at 40 ^{0}C to dryness. The dried extract was transferred to sample bottle and was placed in a dessicator containing anhydrous sodium sulphate to remove any traces of water that could have been present. The dry extract was kept in tightly stoppered bottles in a refrigerator for further analysis.

Experimental animals

Thirty healthy wistar albino rats of both sexes between eight to twelve weeks of age weighing 150 -200g were used for the study. They were purchased from the Laboratory Animal House of College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria. The rats were acclimatised in different cages of six per cage (standard laboratory metal animal cage). The animals were maintained under good laboratory practice (12 hour light and 12 hour dark cycle at uniform temperature of 28-33^{o}C). All

animals had free access to food (vital feed grower, Ibadan) and water. All the investigations involving the experimental animals were conducted in accordance with the accepted principles for laboratory animal use and care.

Induction of DOCA salt-hypertension

Animals were anesthetized by using ketamin (75 mg/kg; i.p) and xylazine (7.5 mg/kg; i.p). Hypertensive group underwent uninephrectomy through the left flank incision. Then, the wounds were closed with silk suture (Ethicon). All operated rats received an injection of ampicillin (10 mg/kg, i.m.) daily for 5 days. Neosporin powder (polymyxin B sulfate BP, Zinc bacitracin BP, and neomycin sulfate IP) were applied locally to prevent infection. A week after unilateral nephrectomy, DOCA (25 mg/kg, once a week for 4 weeks) dispersed in cotton seed oil was injected to uninephrectomized rats. 1% saline and 0.2% KCl ad libitum was given throughout the experiment instead of drinking water (Seyle and Bois, 1957).

Experimental protocol for administration *acalypha godseffiana*

The unilateral nephrectomized rats were divided into 5 groups of 5 rats each.

Group I: Sham control, unilateral nephrectomized animals received daily injection of 0.1 ml of sterilized cotton seed oil subcutaneously for 4 weeks and 0.2% KCl ad libitum as drinking water.

Group II: negative control, unilateral nephrectomized animals received DOCA injection (25 mg/kg/week, s.c.) for 4 weeks, dissolved in sterilized cottonseed oil subcutaneously and 1% saline and 0.2% KCl ad libitum as drinking water.

Group III: Unilateral nephrectomized animals received DOCA injection (25 mg/kg/week), *Acalypha godseffiana* (200 mg/kg/day) for 4 weeks and 1% saline and 0.2% KCl ad libitum as drinking water.

Group IV: Unilateral nephrectomized animals received DOCA injection (25 mg/kg/week), *Acalypha godseffiana* (400 mg/kg/day) for 4 weeks and 1% saline and 0.2% KCl ad libitum as drinking water.

Group V: Unilateral nephrectomized animals received DOCA injection (25 mg/kg/week), *Acalypha godseffiana* (800 mg/kg/day, p.o.) for 4 weeks and 1% saline and 0.2% KCl ad libitum as drinking water.

Measurement of blood pressure (Wolfgand, 1997).

Measurement of blood pressure by non-invasive (indirect) method.

The rats were trained for one week until the blood pressure is steadily recorded with minimal stress and restrain. The first cardiovascular parameters were discarded and mean of five subsequent measurements was recorded. Systolic blood pressure was measured weekly for 4 weeks by an indirect non-invasive tail-cuff method using power lab.

Collection of blood sample for analysis

At the end of the experimental period, the animals were fasted overnight and sacrificed by cervical dislocation. The blood sample collection was through the ocular into a set of heparin and Ethylene Diamine Tetra Acetic acid (EDTA) bottles (for hematology), then centrifuged at 2500rpm for 10 minutes to obtain the blood serum for analysis.

Determination of renal function tests

Determination of Protein Concentration

The Randox kit was used for the determination of total protein in serum.

Principle:

Coptic ions, in an alkaline medium, interacts with proteins peptide bonds resulting in the formation of a colored complex.

Method:

The reagent blank, standard and sample tubes were set up in duplicates and 0.02 ml of distilled water, 0.02 ml of standard (containing protein) and 0.02 ml of serum were added to reagent blank, standard and sample tubes respectively. 1.0 ml of Reagent (containing sodium hydroxide, sodium tartare, potassium iodide and cupric sulphate) was pipetted to the three test tubes.

They were mixed and incubated for 30 minutes at 25^0C. The absorbance of the sample and the standard was read against the reagent blank at 540nm. And the total protein was calculated as follows:

$$\text{Protein concentration (mg/dl)} = \frac{\text{Absorbance of sample} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

Determination of Urea concentration

Principle:

Urea is hydrolyzed in the presence of water n urea produce ammonia and carbon dioxide. The ammonia produced in the first reaction combines with a - oxoglutarate and NADH in the presence of glutamate dehydrogenase to yield glutamate and NAD+.

$$\text{Urea} + H_2O \longrightarrow 2NH_3 + CO_2$$

$$2\text{ a - oxoglutarate} + 2\ NH_4^+ + 2\ NADH_2 \longrightarrow \text{L- glutamate} + 2NAD^+ + 2H_2O$$

Method:

The reagent blank, standard and sample tubes were set up in duplicates. 10ul of standard (urea), and 10ul of sample were pipetted to the tubes labelled standard and sample. 1000ul of reagent (containing grease, GLDH, NADG, Adenosine- 5- diphosphate) were pipetted to tubes labelled reagent blank, standard and sample. After which they were mixed and the initial absorbance was read against reagent blank at 340nm after 30 seconds and after 1, 2 and 3 minutes. The Urea concentration was calculated as follows:

$$\text{Urea concentration (mg/dl)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

Determination of creatinine concentration

Principle:

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Method:

Standard and sample cuvettes were set up in duplicates and 2 ml of working reagent containing picric acid and sodium hydroxide was pipetted to the tubes. 0.2 ml standard solution (creatinine) was pipetted to the tubes labelled standard, while 0.2 ml of sample was pipetted to the cuvette labelled sample. They were mixed and absorbance of the standard and sample was read after 30 seconds against air. The absorbance of the standard and sample was also read after 2 minutes at 500 nm. The concentration of Creatinine was calculated as follows:

$$\text{Creatinine concentration (mg/dl)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

Determination of Albumin concentration

Principle:

The measurement of serum albumin is based on its quantitative binding to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-

BCG-complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Method:

Reagent, standard and sample tubes were set up in duplicates and 0.01 ml of distilled water, 0.01 ml of standard (albumin) and 0.01 ml of serum were pipetted into the different tubes respectively. 3.0 ml of reagent containing bromocresol green was added to all the tubes. The tubes were mixed and incubated for 5 minutes at 25^0C. The absorbance of the sample and the standard were measured against the reagent blank at 630nm. Albumin concentration was calculated as follows:

$$\text{Albumin concentration (mg/dl)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

Assay of alanine amino transferase (ALT) activity

Serum ALT activity was estimated by the method of Reitman and Frankel (1957).

Principle: this method is based on the production of pruvate by the transamination activity of alanine amino transferase. Pruvate reacts with 2,4 dinitrophenylhydrazone (DNPH) to give a brown coloured hydrazine that is measured colorimetrically at 546 nm.

$$\text{a-oxoglutarate + L- alanine} \xleftrightarrow{\text{ALT}} \text{L- glutamate + pyruvate}$$

Reagent composition

- R_1 is a reagent containing Phosphate buffer (100 mmol/L, Ph 7.4), L- alanine (200mmol/L), a-oxoglutarate (2 mmol/L).
- R_2 is a reagent containing 2, 4- dinitrophenyl hydrazine (2 mmol/L).

Procedure: In two separate test tubes, a volume of 0.1 ml of serum and water were mixed with 0.5 ml of R_1 as the test and blank respectively. The solutions were mixed and incubated respectively for 30 minutes at 37^0C. Subsequently, 0.5 ml of R_2 was added to both test tubes and was incubated for 20 minutes at 25^0C, followed by addition of 5 ml of sodium hydroxide (NaOH) solution. The resultant solutions were mixed and the absorbance of test sample against reagent blank was read after 5 minutes at 546 nm.

Assay of serum aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) activity was determined according to the method of Reitman and Frankel (1957).

Principle: Oxaloacetate reacts with AST and is decarboxylated spontaneously to pyruvate. The pyruvate is measured by hydrazone formation after pyruvate reacts with 2,4-dinitrophenylhydrazine (DNPH) to give a brown coloured hydrazine which can be measured at 546 nm.

a-oxoglutamate + L- aspartate ⟷ AST ⟷ L- glutamate + oxaloacetate

- R_1 is a reagent containing Phosphate buffer (100 mmol/L, Ph 7.4), L-Aspartate (100mmol/L), a-oxoglutarate (2 mmol/L).
- R_2 is a reagent containing 2,4- dinitrophenyl hydrazine (2 mmol/L).

Procedure: 0.1 ml of serum and blank in different test tubes were mixed with 0.5 ml of R1. The solution was incubated for 30 minutes at 37 ^{0}C. 0.5 ml of R2 was added to both test tubes and allowed to stand for 20 minutes at 25 ^{0}C. 5 ml of sodium hydroxide (NaOH) was added, the solution was mixed, the absorbance of sample was read against blank after 5 minutes at 546 nm.

Statistical analysis

Data generated from the study was presented as mean ± SEM. Statistical analysis was done by One way analysis of variance using the SPSS version 21.0. The mean difference at $P<0.05$ was considered statistically significant.

RESULTS

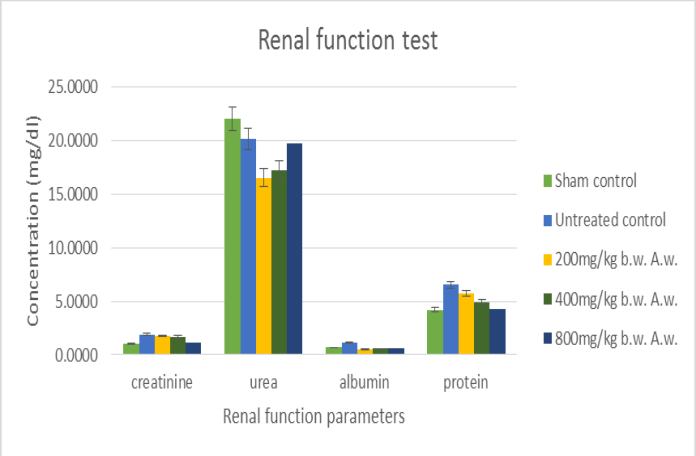

Figure 1: Effect of hydro- ethanol leaves extract of *Acalypha godseffiana* on DOCA-salt induced hypertension in kidney function parameters of unilateral nephrectomized albino rats.

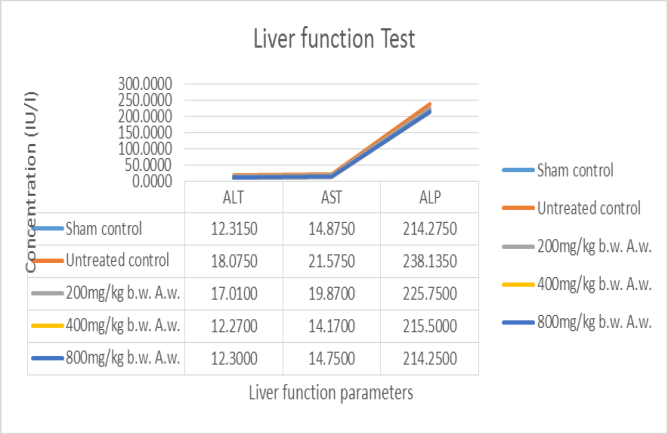

Figure 2: Effect of hydro- ethanol leaves extract of *Acalypha godseffiana* on DOCA-salt induced hypertension in liver function parameters of unilateral nephrectomized albino rats.

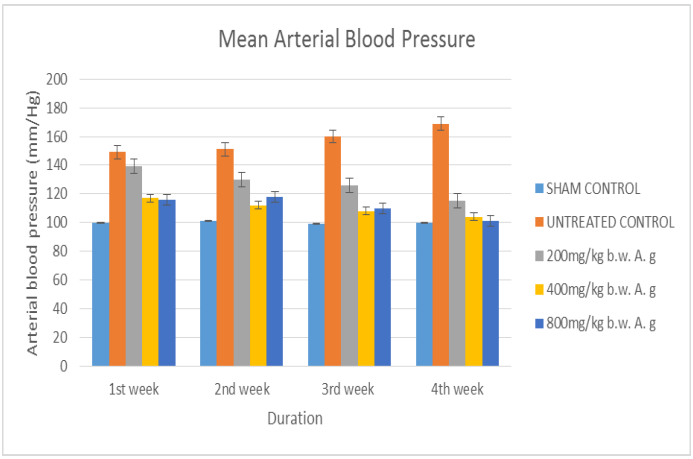

Figure 3: Effect of hydro- ethanol leaves extract of *Acalypha godseffiana* on DOCA-salt induced hypertension in arterial blood pressure of unilateral nephrectomized albino rats.

DISCUSSION

By removal of body wastes, the kidney maintains optimum chemical composition of the body fluids such as urea, uric acid, electrolytes and creatinine. These parameters have been reported to elevate in serum in a state of renal dysfunction (Burtis *et al*., 2012), and are employed as biomarkers of kidney function. Creatinine is produced by the metabolism of creatinine phosphate in the skeletal muscle but can also be obtained from dietary sources such as meat, while urea is a metabolic product of protein metabolism (Nankivell, 2001). The present study revealed that there was significant ($p<0.05$) increase in creatinine and urea concentrations in the untreated control when compared to groups treated with different doses of *Acalypha godseffiana*. The decrease in creatinine, urea, protein and albumin concentrations

in group administered 200 and 400mg/kg b.w. of *Acalypha godseffiana* is an indication of nephroprotective potential of the plant, and this may be due to the presence of phytochemicals in the plant such as flavonoid (Oyewole and Akingbala, 2011), this implies that there was restoration in glomerular function of the renal tubule of the animals. This result is in tandem with work done by Ndem *et al.,* (2020), who reported that leaves extract of *Jatropha tanjorensis* exhibits nephroprotective activity.

Liver is saddled with the responsibility of detoxifying chemicals and other xenobiotics by inactivating and metabolizing those substances (Izuanya *et al.,* 2010). ALT, AST and ALP are amino acids metabolizing enzymes localized within the liver and their levels in the serum are indicators of the liver status. Increased serum levels of these enzymes indicate hepatic injury because these enzymes leak into the serum when hepatic membrane damage occurs (Izuanya *et al.,* 2010). The results obtained also showed a dose dependent significant ($p<0.05$) decrease in serum aspartate amino transferase (AST), alkaline phosphatase (ALP) and alanine amino transferase (ALT) levels at doses of 100, 200 and 400 mg/kg compared to the negative control group, suggesting hepatocellular protection at the doses administered. This study is in agreement with work done by Ikewuchi *et al*., (2011), who evaluated the effect of subcutaneous administration of aqueous extract of *A. wilkesiana 'godseffiana'* on hepatoprotection, and his results showed that there was a decreased AST, ALT and ALP level in Albino rats administered 100mg/kg *A. wilkesiana 'godseffiana'* compared to control.

Blood pressure is influenced by cardiac output, systemic vascular resistance, blood volume and arterial stiffness, and varies depending on patient's situation, emotional state, activity and relative health or disease state. The result showed that hydro-ethanol leaves extract of *Acalypha godseffiana* at doses of 400, 200 and mostly at 800mg/kg body weight reduced blood pressure in DOCA-salt hypertensive rats in time-dependent manner according to figure 2. It has been well documented that DOCA-salt as a mineralocorticoid causes salt and water retention, and this results to the development of hypertension. 5-hydroxytryptamine ($5\text{-}HT_{2B}$), Endolethelin-1 (ET-1) (Matsumura *et al.,* 1999), and atrial natriuretic peptides (ANP) (Ogawa *et al.,* 1999) are involved in the pathogenesis of this type of hypertension. However, the antihypertensive activity of hydro-ethanol leaves extract of *Acalypha godseffiana* may be due to $5\text{-}HT_{2B}$ and ET-1 receptors antagonism. It has been assumed that in hypertension there is upregulation of $5\text{-}HT_{2B}$ receptor in order to maintain an elevated blood pressure in rats made hypertensive by DOCA (Banes and Watts, 2002).

Moreover, hydro- ethanol leaves extract of *Acalypha godseffiana* was found to be safe to the renal and hepatic cellular functions and also possesses antihypertensive activity on DOCA-salt induced hypertension in unilateral nephrectomized Wistar albino rats. Hence, this plant could be a promising drug agent in the treatment of hypertension and restoration of hepatic and renal activity.

CONCLUSION

Hydro-ethanol leaves extract of *Acalypha godseffiana* was found not to show any toxicity or increase in the serum biomarkers of kidney and liver. The result showed that hydro-ethanol leaves extract of *Acalypha godseffiana* at doses of 400, 200 and mostly at 800mg/kg body weight reduced blood pressure in DOCA-salt hypertensive rats in time-dependent manner.

Conflict of interest

The authors report no conflict of interest.

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