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PROTECTIVE EFFECTS OF HEXANE FRACTION OF *COSTUS AFER* LEAVES AGAINST SODIUM ARSENITE-INDUCED HEPATOTOXICITY AND NEPHROTOXICITY IN MALE ALBINO WISTAR RATS

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Arsenite is a toxic metallic pollutant known to cause hepatotoxic and nephrotoxic injuries. Costus afer Ker Gawl is an indigenous medicinal plant used as therapy for numerous tissue disorders. Thus, this study investigated the protective potential of C. afer hexane leaf fraction (CALHF) on sodium arsenite-induced hepatic and renal injuries in albino rats. Twenty-five male albino rats were randomly divided into five groups of five rats each. Group 1: rats administered orally with 0.5 ml of 0.9% saline; Group 2: untreated rats with induced toxicity by 5 mg/kg body weight (b.w.) sodium arsenite (i.p.); Group 3: rats with sodium arsenite-induced toxicity and treated with 10 mg/kg b.w. silymarin (hepatoprotective drug); Group 4 and 5: rats with sodium arsenite-induced toxicity and treated with 100 and 200 mg/kg b.w. CALHF, respectively. CALHF was orally administered daily, while sodium arsenite was administered every 48 hours for 14 days. Thereafter, rats were sacrificed, blood was collected to estimate hepatic and nephrotic functions. Hepatic and renal function tests showed that 100 and 200 mg/kg CALHF and 10 mg/kg silymarin treated animals had significantly reduced ($P < 0.05$) plasma alanine aminotransferase, aspartate aminotransferase, creatinine and urea levels, when compared with those of untreated animals. C. afer hexane leaf fraction exhibited hepatoprotective and nephroprotective effects against sodium arsenite induced toxicity in rats.

K e y w o r d s: Costus afer, hepatotoxicity, nephrotoxicity, sodium arsenite, liver enzymes.

Arsenic is a naturally occurring metalloid element that is widely found in both organic and inorganic forms in the environment [1]. Inorganic arsenic has been projected to be more toxic than organic arsenic. Sodium arsenite is a toxic metallic pollutant of public health concern [2]. Arsenic can be emitted to the atmosphere from both natural and anthropogenic sources but can also be transported in the environment by runoff water [3]. There are epidemiological evidence associating arsenic inhalation with cancer risk [4].

The common denominator for arsenic pathogenesis has been demonstrated to involve reactive oxygen species (ROS)-induced oxidative damage [3]. The exact mechanism of action of arsenic toxicity is still unclear, however, exposure of humans to arsenic, increased the formation of ROS/reactive nitrogen species (RNS), including peroxyl radicals ($ROO^{\bullet}$), the superoxide radical, singlet oxygen, hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide, the dimethylarsenic radical, the dimethylarsenic peroxyl radical and/or oxidant-induced DNA damage. Arsenic induces the formation of oxidized lipids which in turn generate several bioactive molecules (ROS, peroxides and isoprostanes), malondialdehyde (MDA) and 4-hydroxy-nonenal (HNE) as the major end products [5].

Arsenic intoxication in experimental animals has also been associated with hepatic tumors [6]. The primary targets of sodium arsenite induced toxi-

cities are liver and kidneys [7]. Arsenic has an affinity to thiol group of proteins and leads to inhibition of cellular respiration, impaired glycolysis, oxidative process, and finally death of cells [8]. The most applied therapy against arsenite toxicity has been metal chelation therapy which forms metal complexes with the attendant removal of excess arsenite from the body system. This type of therapy has been associated with adverse effects to the biochemical system [9]. However, the use of plant extracts as a therapy against arsenite toxicity with minimal or no adverse effect could also be considered and scientifically validated.

The plant product such as silymarin has been widely used in the treatment of different liver disorders. Silymarin is an extract from the seeds or fruits of *Silybum marianum* or milk thistle plant and mainly consist of seven flavolignans such as silibilin, isosilibilin, silybin, silychristin, isosilychristin, silydianin and taxifolin. Studies have shown that silybin is most prevalent with important biological effects [10, 11]. Several studies have shown that silymarin is used medicinally to treat acute viral hepatitis, metal-induced hepatitis, cirrhosis, hepatocellular carcinoma and alcoholic liver diseases [12]. Silymarin has been demonstrated to protect and restore liver function against a number of toxic agents including acetaminophen, thioacetamide, carbon tetrachloride and galactosamine. Its mechanism of action includes prevention of hepatotoxin binding to receptor sites on the hepatocyte membrane; reduction of glutathione oxidation to enhance its level in the liver and intestine; antioxidant activity; and stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration [13]. Scientific evidence has also demonstrated that the tissue-protective mechanisms of action of silymarin is through silybin interacting with various tissues. Silybin acts through the turning-off of pro-inflammatory signals including nuclear factor-κB activation and induction of cytokines such as tumor necrosis factor α, interleukin-1, IL-6, and granulocyte-macrophage colony stimulating factor [14].

Costus afer Ker Gawl (Family: Costaceae) is a medicinal plant which is among 150 species of stout, perennial and rhizomatous herbs with diverse range of pharmacological profile [15]. It can be located in the forest belt of Senegal, South Africa, Guinea, Niger, Sierra Leone, Ghana, Cameroon and Nigeria. The plant is commonly known as gingerlily or bush cane. It is known as “okpete” or “okpoto” in Igbo-land, “kakizawa” in Hausa “tete-egun” in Yoruba and “mbritem” in Efik all in Nigeria [16]. Anglophone Cameroon calls it “monkey sugar cane”. It bears white and yellow flowers. The stem, seeds, leaves and rhizomes are harvested from the wild plant and they contain several bioactive metabolites [17, 18]. In ethnomedical practice, *C. afer* leaf is used as therapy in the management of hepatic disorders among others. Previous study had shown that *C. afer* possesses antioxidant, hepatocurative and anti-inflammatory properties [19, 20].

The following suspected anti-inflammatory chemical constituents viz. naphthalene 2,3 dimethyl (0.15%); naphthalene 1,6 dimethyl (0.36%), phenol 2,4-bis (1,1-dimethylethyl) (0.76%); phytol (4.79%), 2(4H)-benzofuranone 5,6,7,7a-tetrahydro 4,4,7a-trimethyl (0.84%); pentadecanoic acid (0.28%); hexadecanoic acid methylester (7.76%); n-hexadecanoic acid; linoleic acid (1.77%), α-linolenic acid (3.93%) and cis-vaccenic (8.64%) were identified in 0.2 g/ml hexane fraction of *C. afer* leaves using gas chromatography/mass spectrometry analytical method [21, 22]. Therefore, this study was designed to evaluate the protective effects of hexane fraction of *C. afer* leaves on sodium-arsenite induced hepatotoxicity and nephrotoxicity in rats.

Materials and Methods

*Plant material. Costus afe*r plant were obtained from a farmland in Araromi, Ogun State. The plant was identified, authenticated and assigned a voucher specimen number as FHI/110398 at the Forestry Herbarium Ibadan, Oyo State.

Preparation of plant extracts. C. afer leaves were washed thoroughly to remove debris and oven-dried at 35°C. Dried plant leaves were pulverized using a mechanical blender. Pulverized *C. afer* leaves were soaked in 70% methanol with intermittent shaking and allowed to stay for 48 h at room temperature. Subsequently, the suspension was filtered using Whatman No. 1 filter paper. The obtained filtrate was concentrated using a rotary evaporator (RE52-3 model, LIDA Instrument, Minervation Ltd., Oxford, United Kingdom) at 40°C. The obtained methanol extract was reconstituted in distilled water (ratio 1:2) and subjected to solvent partitioning to obtain hexane fraction. The hexane fraction was then concentrated using the rotary evaporator at 40°C and immediately stored at 4°C until further use.

Experimental animals. Twenty five male albino rats (Wistar strain) weighing between 100–200 g

were purchased from the Animal Facility, Department of Physiology, Babcock University, Ogun State. They were acclimatized for two weeks in aerated plastic cages under natural light condition at room temperature and fed with rat pellets and water *ad libitum*. Animals were humanely handled and maintained following the National Institute of Health Animal Care and Use Guidelines. Institutional ethical approval was obtained with certificate number BUHREC/383/18.

Experimental design. The experimental animals were randomly distributed into five groups of five animals each, using 0.9% NaCl as vehicle for oral administration, following the scheme below: group 1 (normal): rats orally administered with 0.5 ml 0.9% saline; group 2 (control): untreated rats induced with toxicity using 5 mg/kg body weight (b.w.) sodium arsenite (i.p.) and orally administered with 0.5 ml 0.9% saline; group 3 (standard): rats induced with sodium arsenite and treated orally with 10 mg/kg b.w. silymarin (Sigma-Aldrich, St. Louis, MO) a hepatoprotective drug; group 4 (test 1): rats induced with sodium arsenite and treated with 100 mg/kg b.w. hexane fraction of *C. afer* leaves; group 5 (test 2): rats induced with sodium arsenite and treated with 200 mg/kg b.w. hexane fraction of *C. afer* leaves.

The hexane extract was orally administered daily to the respective groups, while sodium arsenite was administered intraperitoneally every 48 h for 14 days.

Blood sample collection for biochemical analysis. Twenty four hours after the last day of treatments, all the animals were euthanized by cervical dislocation and sacrificed. Blood samples were collected using hypothermal syringe into heparin bottles and centrifuged at 3000 g for 15 min to obtain plasma for biochemical analysis. The plasma was stored at 4°C until further analysis.

Liver function test. Determination of plasma alanine aminotransferase and aspartate amino transferase activities. Effects of hexane fraction of *C. afer* leaves on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were carried out using the procedures provided by the Randox diagnostic kit manufacturer (Randox, United Kingdom).

Kidney function test. Effects of hexane fraction of *C. afer* leaves on plasma creatinine and urea concentrations were performed using colorimetric procedure as described by Randox kit manufacturer (Randox, United Kingdom).

Statistical analysis. This was done with the aid of GraphPad Prism® 7.0 to determine difference between means using one way Analysis of Variance (ANOVA). Data was reported as mean ± standard deviation and the significance level was set at $P < 0.05$.

Results and Discussion

Data in Fig. 1 shows that untreated control animals (55.60 ± 1.60 U/l) induced with toxicity using sodium arsenite had a significantly elevated ($P < 0.05$) plasma ALT activity when compared with the normal animals (39.02 ± 1.17 U/l). However, animals treated with 10 mg/kg b.w. silymarin (36.20 ± 2.54 U/l), 100 mg/kg (47.92 ± 2.03 U/l) and 200 mg/kg (38.46 ± 2.89 U/l) b.w. *C. afer* leaves hexane fraction (CALHF) had significantly reduced ($P < 0.05$) plasma ALT activities when compared with untreated control animals. More so, there was no significant difference between 10 mg/kg silymarin and 200 mg/kg CALHF treated animals. Data in Fig. 2 showed untreated control animals (61.24 ± 2.05 U/l) induced with toxicity using sodium arsenite had a significantly elevated ($P < 0.05$) plasma AST activity when compared with the normal animals (40.36 ± 2.62 U/l). However, animals treated with 10 mg/kg b.w. silymarin (47.24 ± 2.45 U/l), 100 mg/kg b.w. (54.76 ± 1.05 U/l) and 200 mg/kg b.w. (52.18 ± 1.74 U/l) CALHF had significantly reduced ($P < 0.05$) plasma AST activities when compared with untreated control animals. Arsenite ingestion has been demonstrated to cause hepatotoxicity and nephrotoxicity [23-25]. The findings from this study have shown that *C. afer* leaves hexane fractions reduced the plasma AST and ALT activities in rats induced with toxicity using sodium arsenite when compared with untreated control animals. This suggested that the test samples possess hepatoprotective activity against sodium arsenite-induced toxicity in rats. Several reports have shown that plant fractions could suppress the metal-induced leakage of liver cytoplasmic AST and ALT into the plasma thereby eliciting hepatoprotective effects [26, 27].

It could be deduced that the bioactive compounds such as benzofuran 2,3 dihydro, hexadecaboic acid, cis-vaccenic acid and oleic acid present in the *C. afer* leaves hexane fraction might be responsible for the hepatoprotective effect [21]. Molecularly, arsenite is known to induce tissue toxicity through generation of oxidative stressors and cellular signals that result into the binding of arsenite to thiol groups of macromolecules with the subsequent

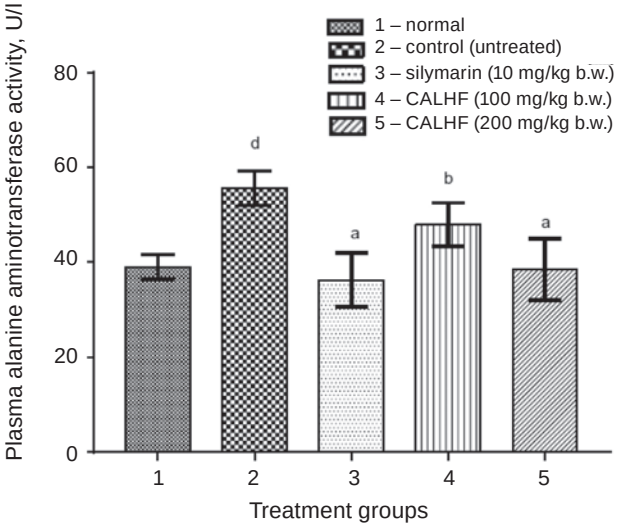

Fig. 1. Effects of 100 and 200 mg/kg of C. afer leaves hexane fraction (CALHF) on plasma alanine aminotransferase activity in animals with sodium arsenite-induced toxicity. Normal – rats administered orally with 0.5 ml 0.9% saline; control (untreated) – group 2 rats with induced toxicity by 5 mg/kg b.w. sodium arsenite (i.p.)

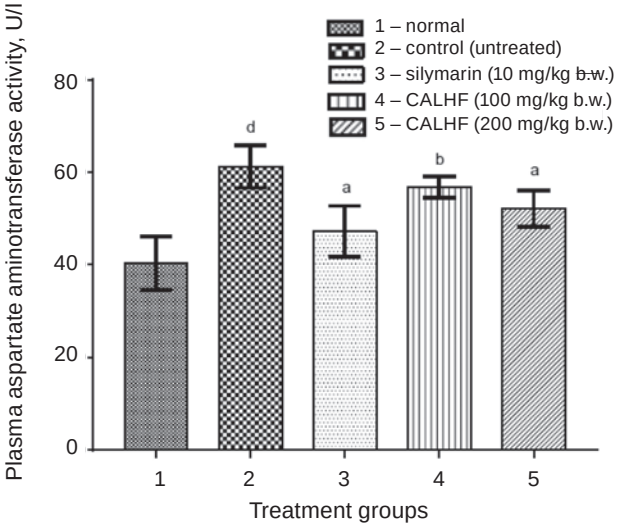

Fig. 2. Effects of 100 and 200 mg/kg of C. afer leaves hexane fraction (CALHF) on plasma aspartate aminotransferase activity in animals with sodium arsenite-induced toxicity. Normal – rats administered orally with 0.5 ml 0.9% saline; control (untreated) – group 2 rats with induced toxicity by 5 mg/kg b.w. sodium arsenite (i.p.)

propagation of deleterious chemical species and apoptosis [28, 29]. *C. afer* hexane leaf fraction has been shown to possess antioxidant and anti-inflammatory property against reactive oxygen species (ROS) and pro-inflammatory mediators [19]. Hence, the capacity of CALHF to attenuate the arsenite-induced chain reaction may have contributed to elicit the hepatoprotective effect.

Data in Fig. 3 shows that plasma urea concentration of untreated animals (54 ± 1.95 mg/dl) was significantly ($P < 0.05$) elevated when compared with normal animals (39 ± 3.41 mg/dl). However, 10 mg/kg silymarin (38.80 ± 1.32 mg/dl), 100 mg/kg (36.98 ± 1.53 mg/dl) and 200 mg/kg (33.78 ± 2.05 mg/dl) CALHF treated animals had significantly ($P < 0.05$) reduced plasma urea concentrations when compared with untreated control animals. In addition, there were no significant difference ($P > 0.05$) when comparing the normal animals with those of treated animals. Data in Fig. 4 showed that plasma creatinine concentration of untreated animal (1.36 ± 0.02 mg/dl) was significantly elevated ($P< 0.05$) compared with normal animals (0.62 ± 0.01 mg/dl). However, 10 mg/kg silymarin (0.65 ± 0.01 mg/dl), 100 mg/kg (0.86 ± 0.02 mg/dl) and 300 mg/kg (0.75 ± 0.03 mg/dl) CALHF treated animals had significantly ($P < 0.05$) reduced plasma creatinine concentrations, when compared with untreated control animals. This observation suggested that the hexane leaves fraction may possess nephroprotective activity. This might have been through interference with the mechanism of reabsorption and inhibition of urea and creatinine in nephrons. Previous researches had shown that *C. afer* extracts

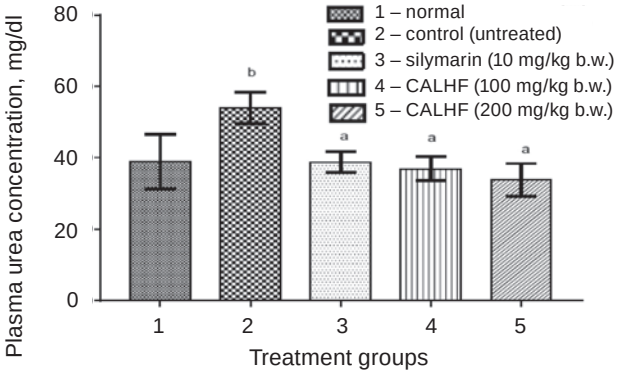

Fig. 3. Effects of 100 and 200 mg/kg of C. afer leaves hexane fraction (CALHF) on plasma urea concentration in animals induced with toxicity using sodium arsenite. Normal – rats administered orally with 0.5 ml 0.9% saline; control (untreated) – group 2 rats with induced toxicity by 5 mg/kg b.w. sodium arsenite (i.p.)

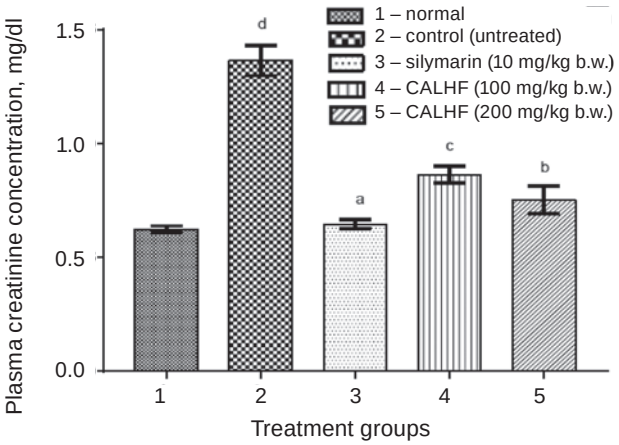

Fig. 4. Effects of 100 and 200 mg/kg of C. afer leaves hexane fraction (CALHF) on plasma creatinine concentration in animals with sodium arsenite-induced toxicity. Normal – rats administered orally with 0.5 ml 0.9% saline; control (untreated) – group 2 rats with induced toxicity by 5 mg/kg b.w. sodium arsenite (i.p.)

possess phytochemicals exhibiting nephroprotective effects against drug-induced kidney toxicity [30, 31]. It is also possible that the antioxidant and bioactive compounds in *C. afer* leaves hexane fraction could have counteracted the toxic metabolites of sodium arsenite in liver and kidney cells, thereby suppressing the deterioration in hepatic and nephrotic functions.

Conclusion. The findings from this study have shown that *C. afer* hexane leaf fraction may possess hepatoprotective and nephroprotective actions against sodium arsenite induced toxicity in rats. It is therefore recommended that the hexane fraction of *C. afer* leaves be explored further for lead drug candidate in drug discovery process.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ЗАХИСНИЙ ЕФЕКТ ГЕКСАНОВОЇ ФРАКЦІЇ З ЛИСТЯ *COSTUS AFER* ЗА ІНДУКОВАНОЇ АРСЕНІТОМ НАТРІЮ ГЕПАТОТОКСИЧНОСТІ ТА НЕФРОТОКСИЧНОСТІ В ЩУРІВ-АЛЬБІНОСІВ

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Арсеніт – токсична речовина, яка, як відомо, спричинює гепато- та нефротоксичні ушкодження. У дослідженні вивчали захисний потенціал гексанової фракції з листя лікарської рослини *Costus afer* Ker Gawl (*C. afer*) за індукованого арсенітом натрію ураження печінки та нирок у щурів-альбіносів. Двадцять п'ять щурів-альбіносів було розподілено на п'ять груп (n = 5): 1 – тваринам вводили перорально 0.5 мл фізіологічного розчину; 2 – тваринам індукували токсичне ураження введенням арсеніту натрію (в/ч, 5 мг/кг кожні 48 год протягом 14 днів); 3 – тварини з індукованим токсичним ураженням (ін'єкція арсеніту натрію) отримували гепатопротектор силімарин (перорально 10 мг/кг); 4 та 5 – тварини з індукованим токсичним ураженням (ін'єкція арсеніту натрію) отримували щоденно гексанову фракцію з листя *C. afer* (перорально 100 та 200 мг/кг відповідно). Після цього збирали кров для оцінки функції печінки та нирок. Виявили, що в тварин, які отримували 100 та 200 мг/кг гексанової фракції з листя *C. afer* та 10 мг/кг силімарину, значно знизились ($P < 0.05$) рівні аланінамінотрансферази, аспартатамінотрансферази, креатиніну та сечовини у порівнянні з тваринами, які не отримували ніякого лікування. Показано, що гексанова фракція з листя *C. afer* виявляла гепатопротекторну та нефропротекторну дію за індукованої арсенітом натрію токсичності в щурів.

Ключові слова: *Costus afer*, гепатотоксичність, нефротоксичність, арсеніт натрію, печінкові ензими.

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