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Modulation of rat serum lipid profile and nephrotic indices following oral exposure to the extracts of chilli pepper

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Abstract

Background: The increasing application of plants for medicinal purposes necessitates safety/toxicity profiling.

Objective: In the present study, we evaluated the toxicological effects of the ethanolic extracts of the leaves (CAL), root (CAR) and stem (CAS) of *Capsicum annum* in rats.

Methods: Male Wistar rats were randomly assigned groups and given oral administration of the extracts or distilled water for 28 days.

Results: Data showed that administration of ethanolic extracts of CAL, CAR and CAS did alter the liver function indices but not in a clear-cut manner to suggest hepatotoxicity. The CAR and CAS extracts decreased ($p < 0.05$) the rat serum albumin levels compared with the control. In contrast, CAL extracts raised ($p < 0.05$) the rat serum albumin level relative to the control. The plant extract administration raised rat serum bilirubin level compared with the control. Further, the extracts caused reduction ($p < 0.05$) in rat serum TAG levels compared with the control. The CAL, CAR and CAS extracts did not significantly affect the rat serum creatinine level, but caused significant elevation of rat serum urea compared with the control.

Conclusion: Taken together, findings do not only support the cardio-protective potential of *C. annum* extracts, but implicate the nephrotoxic tendency of the plant extracts.

Keywords: herbs, medicinal biochemistry, Phytoconstituents, spices, toxicity

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INTRODUCTION

Capsicum annum known as sweet pepper, bell pepper, cherry pepper and/or green pepper is an annual shrub with many angular branches (Messiaen 1992). *C. annum* belongs in the family of *Solanaceae* and the plant is cultivated throughout the year in both tropical and temperate regions. It is one of the oldest domesticated crops in the western hemisphere (Aguilar-Melendez et al. 2009, Kim et al. 2014). Varieties of pepper range from 30 to 90 cm tall. Worldwide, the production of 18 and 32 degrees (Pathirana 2013)

pepper has reached 21.3 million tonnes from an estimated area of 1.6 million hectares with China being the largest producer. Nigeria accounts for more than 50% of the 1 million tonnes believed to be produced in Africa (FAO 2018). In Nigeria, the local names for *C. annum* are Ata wewe (Yoruba), Ose (Ibo) and Tatasha (Hausa).

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C. annuum is an extremely valuable medicinal herb. Investigations have demonstrated the analgesic, antiangiogenic, antiparasitic, antiplatelet, anti-arthritis, antioxidant, antiviral, antifungal, antineoplastic, hypoglycemic, gastroprotective, and larvicidal potential of *C. annuum* (Verma and Singh 2008). Due to its rich nutritional value, the consumption of the *C. annuum* continues to grow. The *C. annuum* is a rich source of vitamins C and E as well as provitamin A and carotenoids, compounds with well-known anti-oxidant properties (Nadeem et al. 2011). *C. annuum* leaves and fruits are used for abortion and to correct menstrual disorders by some tribal communities in India (Sharma and Sridevi 2016). The fruits are useful in cephalgia, gout, arthritis, selatica, anorexia, dyspepsia, flatulence, cough, malaria and intermittent fevers, cholera, indolent fevers and other vitiated conditions of kapha (Jin et al. 2009, Turkyilmaz and Islek 2015).

It is an established fact that herbal remedies or medicinal plant preparations have served the human society in the treatment of diverse disease conditions from time immemorial, with about 80% of the world's population currently relying almost exclusively on traditional medicines for their primary form of health care (Adeyemi et al. 2012, Medicine 2018). For example, in Nigeria, there has been upsurge in demand for herbal remedies as alternative medicines but in spite of the growing patronage of these medicinal plants as either herbals or food supplements, little is known about toxicities that may result from repeated exposure. Medicinal plants may have recognizable therapeutic effects but may also have toxic side-effect. More so, evaluation of medicinal plants and/or herbal remedies for toxicity and safety profiling is a necessity that could aid integration of traditional medicines with conventional therapies. Therefore, in the present study, we investigated the *in vivo* toxicity profile of the ethanolic extracts of the *C. annuum* leaves, stems and roots.

MATERIALS AND METHODS

Materials

Assay kits for the determination of creatinine, triglycerides, cholesterol, bilirubin, urea, albumin, alanine transferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were products of Randox Laboratories Limited, UK.

Capsicum annuum

Fresh whole plant part (leave, stem and root) of *C. annuum* were collected from flower garden Ilorin, Kwara state, Nigeria. Identification was done by Mr Bolu of the Herbarium Unit, Department of Plant Biology, University of Ilorin, Ilorin, Kwara state, where voucher specimens were deposited. The voucher numbers for *C. annuum* is UILH/112/532.

Preparation of the Ethanolic Extracts of *C. annuum*

One kilogram of the plant was weighed, the root, stem and leaves were separated and was thoroughly washed and air dried to constant weight. The dried leaves, roots and stems were pulverized with a blender. The pulverized leaves, root and stem of *C. annuum* were then separately extracted with ethanol (1:5 w/v) over 72 h. The extract mixtures were filtered using a muslin cloth and concentrated on a rotary evaporator (R110E – Buchi, USA). The percentage yield for the extracts of leaves, roots and stems respectively was 6.56, 5.88 and 9.95 % [L-8.38, R-4.11, S-7.42 g].

Experimental Animals

Twelve (12) apparently healthy male Wistar rats weighing between 140 – 150 g were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were housed in well ventilated plastics with sawdust as beddings, fed on standard rodent feed and allowed free access to water. The animals were acclimated for two weeks before the commencement of experiment. Handling of animals was consistent with relevant guidelines on the care and use of laboratory animals (National Research Council 2011) as described by (Adeyemi and Akanji 2010a, Akanji et al. 2009, Sulaiman and Adeyemi 2010).

Animal Grouping and Treatments

All rats were maintained under standard laboratory conditions at 25±2°C with alternate 12 h light/dark cycle. The animals were then randomly distributed into four (4) groups of three rats each. The extract administration was done orally using oral gavage. Rat body weight was monitored daily. The experimental treatment lasted for twenty-eight days. Further details are;

- Distilled water only – Control
- Ethanolic extract of *C. annuum* leaves (200 mg/kg) – CAL
- Ethanolic extract of *C. annuum* root (200 mg/kg) – CAR
- Ethanolic extract of *C. annuum* stem (200 mg/kg) – CAS

Blood Collection and Tissue Homogenates

After twenty-eight days of administration of the plant extracts, the animals were anaesthetized (24 h after last treatment) with diethyl ether and sacrificed by simply incising the jugular vein, the blood samples were collected into sterile sample tubes. Blood samples for serum were allowed to stand at room temperature for 30 min, after which they were centrifuged for 10 min using a Uniscope Centrifuge (Model SM800B, Surgifriend Medicals, England, U.K.). The supernatant (serum) was collected using a Pasteur's pipette and stored frozen until required for further analysis.

Organs of interest (liver, heart and kidney) were collected and cleaned with cotton wool to remove blood

Table 1. Average weight (g) of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Weeks	Control	CAL	CAR	CAS
One	152.03±1.07 ^a	162.03±2.58 ^a	147.64±2.9 ^a	165.16±2.11 ^a
Two	156.90±2.11 ^b	164.99±0.93 ^b	155.53±6.09 ^b	169.26±1.77 ^b
Three	166.71±3.41 ^c	167.28±2.33 ^c	171.04±2.39 ^c	163.71±1.04 ^a
Four	179.11±3.89 ^d	173.10±1.43 ^d	176.29±3.86 ^d	168.56±3.93 ^b

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w.); CAR – *Capsicum annuum* roots (200 mg/kg b.w.); CAS – *Capsicum annuum* stems (200 mg/kg b.w.). Mean values with different superscripts in a row are significantly different at $p < 0.05$

Table 2. Average weight of liver, heart and kidney of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Groups	Liver	Kidney	Heart
Control	4.14±1.18 ^a	0.89±0.13 ^a	0.50±0.16 ^a
CAL	3.46±1.26 ^a	0.68±0.29 ^b	0.29±0.12 ^b
CAR	3.67±0.35 ^a	0.81±0.02 ^a	0.38±0.05 ^b
CAS	3.74±0.80 ^a	0.85±0.01 ^a	0.36±0.04 ^b

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w.); CAR – *Capsicum annuum* roots (200 mg/kg b.w.); CAS – *Capsicum annuum* stems (200 mg/kg b.w.). Mean values with different superscripts in a row are significantly different at $p < 0.05$

Table 3. Percentage organ-body ratios of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Groups	Liver	Kidney	Heart
Control	2.20±0.35 ^a	0.48±0.03 ^a	0.26±0.55 ^a
CAL	2.89±1.03 ^a	0.65±0.33 ^a	0.29±0.16 ^a
CAR	2.00±0.05 ^a	0.44±0.04 ^a	0.20±0.06 ^a
CAS	2.16±0.15 ^a	0.37±0.19 ^a	0.24±0.08 ^a

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w.); CAR – *Capsicum annuum* roots (200 mg/kg b.w.); CAS – *Capsicum annuum* stems (200 mg/kg b.w.). Mean values with different superscripts in a row are significantly different at $p < 0.05$

stains, weighed and placed in dispensing bags and immediately stored in ice. A known weight of the liver, kidney and the heart was cut with a clean blade, and then homogenized in ice-cold 0.25 M sucrose solution (1:5, w/v). The homogenates were stored in the freezer until required for further analysis.

Biochemical Assays

The levels of rat serum and tissue total protein (TP), albumin, aspartate aminotransferase (AST – EC: 2.6.1.1), alanine aminotransferase (ALT – EC: 2.6.1.2), alkaline phosphatase (ALP – EC: 3.1.3.1), bilirubin, urea, creatinine and glucose were determined using Randox assay kits (Crumlin, UK).

Data Analysis

Data were analyzed using the analysis of variance (ANOVA) and Duncan multiple range test (GraphPad Prism version 5.0). The data were presented as mean ± standard error of mean. Group mean value at 5% level of confidence ($p < 0.05$) was considered significant.

RESULTS

Oral administration of the ethanolic extracts of CAL, CAR and CAS showed no significant effect on average body weight of experimental rats compared with the control (Table 1), as animals in the various treatment

Table 4. Alanine transferase activity (IU) in the liver, kidney, heart and serum of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Groups	Liver	Kidney	Heart	Serum
Control	96.65±12.38 ^a	62.67±19.96 ^a	89.27±17.67 ^a	57.12±2.41 ^a
CAL	100.78±7.42 ^a	86.96±6.84 ^b	122.50±9.05 ^b	12.49±0.08 ^c
CAR	102.38±6.06 ^a	84.06±9.45 ^b	124.22±2.63 ^b	11.29±0.67 ^c
CAS	100.24±6.71 ^a	90.69±7.91 ^c	129.92±13.09 ^b	21.14±0.53 ^b

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w.); CAR – *Capsicum annuum* roots (200 mg/kg b.w.); CAS – *Capsicum annuum* stems (200 mg/kg b.w.). Mean values with different superscripts in a row are significantly different at $p < 0.05$

Table 5. Alkaline phosphatase activity (IU) in the liver, kidney, heart and serum of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Groups	Liver	Kidney	Heart	Serum
Control	4620.37±952.63 ^a	658.68 ± 13.10 ^a	1477.09±313.02 ^a	1321.67±17.51 ^a
CAL	1240.77±128.22 ^c	590.07±203.68 ^a	1182.76±405.29 ^{ab}	2453.65±404.11 ^b
CAR	2655.06±204.13 ^b	1351.01±215.39 ^c	277.78±186.85 ^c	5320.59±230.83 ^c
CAS	1508.20±964.18 ^c	393.55±54.35 ^c	3190.62±275.28 ^b	2419.66 ± 62.71 ^b

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w.); CAR – *Capsicum annuum* roots (200 mg/kg b.w.); CAS – *Capsicum annuum* stems (200 mg/kg b.w.). Mean values with different superscripts in a row are significantly different at $p < 0.05$

Table 6. AST activity (IU) in the liver, kidney, heart and serum of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Group	Liver	Kidney	Heart	Serum
Control	225.59±7.30 ^a	83.97±4.71 ^a	77.83±5.94 ^a	130.45±32.23 ^a
CAL	165.29±10.15 ^b	90.26±3.43 ^b	112.52±14.43 ^b	215.57±34.38 ^c
CAR	126.81±13.71 ^c	116.95±1.74 ^c	111.96±4.73 ^b	254.60±6.36 ^c
CAS	117.72±13.71 ^c	94.70±0.70 ^b	86.49±12.01 ^c	180.15±4.30 ^b

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w.); CAR – *Capsicum annuum* roots (200 mg/kg b.w.); CAS – *Capsicum annuum* stems (200 mg/kg b.w.). Mean values with different superscripts in a row are significantly different at $p < 0.05$

groups recorded weight gain over the course of the experiments. In like manner, there was no significant difference in the average weight of rat liver and kidney of the *C. annuum*-treated groups compared with the control (Table 2). In contrast, *C. annuum* extracts caused significant reduction in average weight of rat heart compared with the control. Nevertheless, the organ-to-body weight ratio showed no significant difference among the various treatment groups (Table 3).

In order to evaluate for rat liver function, we determined the level of ALT, ALP and AST in rat tissues and serum. Administration of extracts of CAL, CAR and CAS inconsistently altered rat kidney and heart ALT activity compared with the control (Table 4). However, *C. annuum* extracts caused reduction ($p < 0.05$) in rat serum ALT activity. In contrast, *C. annuum* extracts caused elevation in rat serum ALP activity compared with the control (Table 5). Similarly, the extracts caused significant alterations in the rat tissue ALP activity compared with the control. Additionally, extracts of *C. annuum* caused significant alterations to rat tissue and serum AST activity compared with the control (Table 6). Taken together, the alteration in rat liver function indices following exposure to extracts of *C. annuum* did not follow a definite pattern and might indicate adaptive

Table 7. Albumin concentration in the serum of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Groups	Serum albumin (g/dL)
Control	3.46±0.35 ^a
CAL	3.83±0.14 ^c
CAR	2.97±0.00 ^b
CAS	3.34±0.38 ^b

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w); CAR – *Capsicum annuum* roots (200 mg/kg b.w); CAS – *Capsicum annuum* stems (200 mg/kg b.w). Mean values with different superscripts in a row are significantly different at $p < 0.05$

Table 8. Bilirubin Concentration in the serum of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Groups	Serum bilirubin (µmol/L)
Control	0.15±0.04 ^a
CAL	0.27±0.15 ^b
CAR	0.76±0.17 ^c
CAS	3.34±0.38 ^d

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w); CAR – *Capsicum annuum* roots (200 mg/kg b.w); CAS – *Capsicum annuum* stems (200 mg/kg b.w). Mean values with different superscripts in a row are significantly different at $p < 0.05$

Table 9. Triglyceride concentration in the serum of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Groups	Serum triglyceride (mg/dL)
Control	101.89±6.16 ^a
CAL	66.82±26.57 ^b
CAR	43.81±8.13 ^c
CAS	63.50±14.68 ^b

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w); CAR – *Capsicum annuum* roots (200 mg/kg b.w); CAS – *Capsicum annuum* stems (200 mg/kg b.w). Mean values with different superscripts in a row are significantly different at $p < 0.05$

Table 10. Creatinine in the Serum of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Groups	Serum creatinine (µmol/L)
Control	0.14±0.11 ^a
CAL	0.23±0.15 ^a
CAR	0.24±0.06 ^a
CAS	0.30±0.23 ^a

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w); CAR – *Capsicum annuum* roots (200 mg/kg b.w); CAS – *Capsicum annuum* stems (200 mg/kg b.w). Mean values with different superscripts in a row are significantly different at $p < 0.05$

mechanism by the animals in order to cope with stress likely imposed by administration of the extracts.

In addition, extracts of CAR and CAS decreased ($p < 0.05$) the rat serum albumin levels compared with the control (**Table 7**) while CAL extracts raised ($p < 0.05$) rat serum albumin level relative to the control. The *C. annuum* extracts raised rat serum bilirubin level compared with the control (**Table 8**). Meanwhile, the *C. annuum* extracts caused reduction ($p < 0.05$) in levels of rat serum TAG compared with the control (**Table 9**). Furthermore, biochemical determinations to evaluate for kidney function revealed that CAL, CAR and CAS extracts did elevate rat serum creatinine level but not significantly (**Table 10**). In contrast, the extract treatments caused elevation ($p < 0.05$) in rat serum urea compared with the control (**Table 11**). This may indicate

Table 11. Urea concentration in the serum of experimental rats administered extracts of *Capsicum annuum* leaves, roots and stems

Groups	Serum urea (mg/dL)
Control	33.51±13.61 ^a
CAL	77.07±08.61 ^c
CAR	60.55±19.46 ^b
CAS	88.05±11.60 ^d

Values are expressed as mean ± standard error of mean (SEM) n=3. CAL – *Capsicum annuum* leaves (200 mg/kg b.w); CAR – *Capsicum annuum* roots (200 mg/kg b.w); CAS – *Capsicum annuum* stems (200 mg/kg b.w). Mean values with different superscripts in a row are significantly different at $p < 0.05$

perturbation of rat kidney function by oral exposure to the extracts of *C. annuum*.

DISCUSSION

Evaluation of medicinal plants and/or herbal remedies for toxicity and safety profiling is imperative to integration of traditional medicines with conventional therapies. Chili pepper (*Capsicum annuum* L) is a specie widely cultivated and it is said to be used since ancient times as flavouring, food and for human health (Ballina-Gomez et al. 2013). In the present study, we investigated the toxicity profiling of the ethanolic extracts of *C. annuum* in rats as well as determined the GC-MS fingerprints of the extracts.

The increase in average rat weight following oral exposure to *C. annuum* roots, stems and leaves over the course of the experimental treatments may attribute to increased food intake. Chemical compositions of *C. annuum* include vitamins such as A, C, B-complex, zinc and potassium, all of which can aid digestion and improve food intake. That rat weights were not affected adversely by extract treatments may also suggest no toxicity. To assess toxicity of oral exposure to the extracts, we assayed liver function indices. The results showed no clear-cut alterations of liver function parameters, suggesting adaptive mechanism by the animals so as to cope with stress likely imposed by the extract administration. This is plausible if we consider that adaptive mechanism is a biological instrument to offset cellular stress. More so, administration of plant extracts in rats has been shown to cause passing stress (Adeyemi and Akanji 2010b, Adeyemi and Orekoya 2014, Adeyemi et al. 2017). The reduced albumin level due to *C. annuum* administration may indicate impaired synthetic capacity of rat liver while the elevated bilirubin level suggests blockage of rat hepatobiliary. Taken together, our finding suggests mild perturbation of liver function but this might be far from hepatotoxicity (Adeyemi and Sulaiman 2012, 2014, Adeyemi et al. 2012). Conversely, daily administration of the extracts of *C. annuum* significantly elevated rat serum urea level in manners reminiscent of renal impairment. Together, the finding suggests that prolonged administration of the extracts of *C. annuum* might potentiate toxicity in rats. In addition, extract treatments dramatically reduced the level of triglyceride in manners that suggest lipid

modulating potential. This is consistent with the findings of Kim and Park (2014), which showed *C. annuum* extracts modulated serum lipids in mice. In conclusion, finding suggest duration-dependent nephrotoxicity of *C. annuum* extracts in rats. Additionally, results support the lipid modulating potential of *C. annuum* extracts and this

may be relevant for management of cardiovascular disorders.

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