RESTORATION POTENTIAL OF *CELOSIA ARGENTEA* LEAF ON ACETAMINOPHEN-INDUCED LIVER TOXICITY

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ABSTRACT

This work was aimed at determining the protective effect of Celosia argentea aqueous leaf extract on acetaminophen induced liver attack in male albino rats. A total of 42 rats were divided into seven groups of six rats with the leaf extract of C. argentea administered for eight days at 200 and 400 mg/kg in groups 4, 5, 6 and 7. Acetaminophen (3 g/kg) was administered to rats in group 2, 3, 6 and 7 on the eight day. Forty-eight (48) hours after Acetaminophen was administered, the rats were sacrificed and blood collected for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP) and serum albumin (SA) as well as histopathological analysis. The results showed that acetaminophen at 3 g/kg altered liver function, synthetic and pathological state whereas C. argentea leaf restored liver function toward normalcy thus making revealing hepatotoxic nature of acetaminophen and the hepatoprotective potential of C. argentea leaf.

Keywords: Acetaminophen, *Celosia argentea*, Aqueous leaf extract, Liver, Toxicity, Rat

INTRODUCTION

Acetaminophen commonly called paracetamol is an over the counter analgesic and antipyretic drugs which is very safe at therapeutic dose but can cause serious adverse effects including organs toxicity if taken at overdose or extreme chronic administration (Jaeschke *et al.,* 2012; McGil *et al.,* 2012; Gum and Cho, 2013). Acetaminophen hepatotoxicity is known to be due to formation of N-acetyl-p-benzoquinoneimine (NAPQI) by the action of cytochrome P4502E1 (Mazaleuskaya *et al.,* 2015). In acetaminophen overdose, sulfate and glutathione supply get exhausted, thus more NAPQI is generated via CYP450 metabolism. This electrophilic intermediary then binds with available cellular proteins and initiate lipid peroxidation, mediated reactive oxygen species (ROS) and other free radical formation, thereby inducing oxidative stress and inflicting hepatic and renal tissue damage (Isik *et al.,* 2006; Ahmad *et al.,* 2012).

Celosia argentea commonly found in West Africa, Ethiopia, Kenya, Somalia and other parts of East and Central Africa, as well as Mexico is an annual herbaceous plant, with ridged stem and branches with alternate leaves linear to lanceolate, entirely simple and without stipules (Tang *et al.,* 2016). *C. argentea* is being used traditionally to treat/cure various diseases and ailments such as piles, mouth sores, blood diseases, bleeding nose, disinfectant, inflammation, hematological and gynecologic disorders and as an aphrodisiac (Varadharaj and Muniyappan, 2017). *C. argentea*, a leafy vegetable commonly known as quail grass, feather cockscomb, Lagos spinach and locally called by the Nigerian Yorubas as "*Shokoyokoto*" meaning "make husbands fat"

(Tobih *et al.,* 2011; Hamzah *et al.,* 2018) have been reported for its anti-inflammatory, immune-stimulating, anti-oxidant, anti-diabetic, anti-microbial and anti-bacterial activities (Ramesh *et al*., 2013; Usunobun *et al*., 2019) as well as been a source of phytochemicals such as saponins and flavonoids, and minerals such as calcium, phosphorus, potassium, sodium, magnesium, iron, zinc, manganese (Usunobun and Ekpemupolo, 2016). *C. argentea* have also been reported to contain numerous kinds of amino acids with a total amino acid content of 131.87 mg/g, with essential amino acid contents being 42.85 % of the total amino acids (Lin *et al*., 2002). Compounds such as Lutein and β-carotene (Bélanger *et al*., 2010) have also been reported to be isolated from *C. argentea.*

This study is aimed at studying the hepatoprotective activity of *C. argentea* aqueous leaf extract in acetaminophen-induced liver attack, injury and toxicity.

MATERIALS AND METHODS

Preparation and Extraction of *Celosia argentea* Aqueous Leaf Extract: Fresh mature leaves of *C. argentea* were collected from Ugbor village in Oredo Local Government Area of Edo State, Nigeria and thereafter identified and authenticated by a plant taxonomist in the Department of Basic Sciences, Benson Idahosa University, Benin City, Edo State. The collected *C. argentea* leaves were separated from the stalk, washed and air-dried under shade at room temperature for a period of 4 weeks and then pulverized, crushed into fine powder using an electric blender and weighed.

Aqueous extracts of the powdered *C. argentea* leaves were prepared by soaking 466 g of dry powdered plant materials in 9000 ml of distilled water and then kept at room temperature for 48 hours with occasional shaking. At the end of the 48 hours, the extract was filtered first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The extracts were then concentrated using an oven at 40 – 50^{o} C to get crude extract. The dried residue (crude extract) was then stored at 4^{o}C in a refrigerator. Aliquot portions of the crude solid *C. argentea* extract residue was weighed and dissolved in distilled water for use on each day of the experiments. The dosage of 200 and 400 mg/kg were used based on previous non-toxic nature of *C. argentea* reported by Hamzah *et al.* (2018) even at 5000 mg/kg.

Animals and Experimental design: The study was conducted on healthy forty two (42) Wistar male albino rats weighing 160 ± 8 g, randomly assigned to seven treatment groups, replicated twice with each replicate having three albino rats. The animals were maintained under standard conditions, provided pelleted grower's mash (containing 18 % crude protein and 2600 Kcal/kg metabolizable energy, Guinea Feed, Nigeria PLC) and drinking water *ad libitum*. A daily cycle of 12 hours of light and 12 hours of darkness was also provided for the animals. Prior to the start of the experiment, the rats were acclimatized in the laboratory conditions for a period of one week. This study was carried out in accordance with the guidelines for ethical conduct in the care and use of nonhuman animals in research (APA, 2012).

Group 1 rats served as normal control and received only distilled water. Group 2 rats received a single oral dose of Acetaminophen (3 g/kg) on the eighth day while group 3, 4, 5, 6 and 7 rats orally received Vitamin C (100 mg/kg), *C. argentea* (200 mg/kg), *C. argentea* (400 mg/kg), *C. argentea* (200 mg/kg), and *C. argentea* (400 mg/kg) respectively daily for eight days. In addition, a single oral dose of Acetaminophen (3 g/kg) was administered to rats in group 3, 6 and 7 on the eighth day.

At the end of the experiment (48 hours after acetaminophen administration), the animals were placed under chloroform anesthesia. Blood from all the animals were collected using heart puncture technique without the use of anticoagulant and after being allowed to clot centrifuged at 3000 rpm for 15 minutes and the serum harvested in clean sterile glass test tubes for immediate analysis. The liver was removed and washed with normal saline and weighed. A small portion of the liver tissues were fixed in buffered 10 % formalin for histopathological examinations.

Biochemical Assays: Alanine aminotransferase (ALT) was determined according to the method of Reitman and Frankel (1957) by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine, while aspartate aminotransferase (AST) was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine also according to the method of Reitman and Frankel (1957). Alkaline Phosphatase (ALP) assay was done by monitoring change in absorbance at 405 nm due to liberation of para-nitrophenol. Total protein was determined according to the method of Lowry *et al.* (1951) based on the fact that peptide linkages in amino acids of protein react with copper in an alkaline solution to form a colour complex, violet which is read at 540 nm. Albumin was determined according to the method of Doumas *et al.* (1971) based on its quantitative binding to the indicator 3,3`,5,5`-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). All assays were done using their specific test kits obtained from Randox Laboratories, United Kingdom.

Histopathological Analysis: Using the method of Bancroft and Gamble (2002), the harvested liver were fixed in 10 % buffered formol saline and dehydrated in ascending grades of ethanol, after which the liver tissues were cleared in chloroform overnight, infiltrated and embedded in molten paraffin wax. The blocks were then trimmed and sectioned at 5 microns. The sections were deparaffinized in xylene, mounted on clean slides, stained with Haematoxylin and Eosin (H and E) and examined under Olympus/3H light microscope. Photomicrographs of the liver were captured using a Moticam Images Plus 2.0 digital fitted to the light microscope.

Statistical Analysis: The data obtained from this study were subjected to analysis of variance (ANOVA) and the results expressed as Mean ± SE. Statistical significance of difference in values between groups was analyzed using Dunnett's multiple comparison tests. The level of significance was $p<0.05$. All statistical analyses were dome using GraphPad Prism 6.05.

RESULTS

Acetaminophen treated rats had significantly elevated liver function enzymes (AST, ALT, ALP) in blood compared to control and *C. argentea* leaf extract treated rats. However in animals pre-treated with Vitamin C and *C. argentea* leaf (200 and 400 mg/kg) for eight days and administered 3 g/kg acetaminophen on the 8th day, there was significant decrease in liver function enzymes (Table 1).

The effect of *C. argentea* treatment on total protein and albumin concentrations of rats showed significantly elevated activity compared to the significant decrease in total protein and albumin in animal group that received acetaminophen only (Table 2).

The liver histology of normal control rats given distilled water only showed portal vein with well fenestrated sinusoids and hepatocytes with distinct nucleus (Figure 1). The liver of rats that received Acetaminophen alone revealed necrosis, sinusoidal congestion with focal damage around the central vein, feathery degeneration, loss of lobular architecture and damaged cellular outlines (Figure 2). Liver of rats given Vitamin C and Acetaminophen (Figure 3) showed mild sinusoidal congestion around the central vein, slightly reduced feathery degeneration. Liver of rats given *C. argentea* alone at a dose of 200 mg/kg revealed distinct centriole and well feneserated sinusoidal space (Figure 4) while Liver of rats given *C. argentea* alone at a dose of 400mg/kg revealed visible centriole that appear thickened surrounded by mild inflammatory cells as well as hepatocytes with distinct with visible nucleus (Figure 5). The liver of rats given *C. argentea* (200 mg/kg) and Acetaminophen (3 g/kg) showed distinct centrioles with radiating hepatocytes with visible nucleus as well as mild fatty changes and mild focal inflammatory cell (Figure 6), while the liver of rats given *C. argentea* (400 mg/kg) and Acetaminophen (3 g/kg) revealed distinct centrioles surrounded by mild focal inflammtory cell, distinct visible nucleus and well feneserated sinusoids and reduced fatty changes (Figure 7).

Table 1: Effect of graded doses of *Celosia argentea* leaf extract on aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activity in acetaminophen-induced liver injury in rat

Parameter	AST (U/l)	ALT (U/l)	ALP (U/l)
Control	21.75 ± 1.70[a]	15.75 ± 2.75[a]	25.76 ± 0.99[a]
Acetaminophen (3 g/kg)	115.00 ± 2.92[c]	80.40 ± 5.41[c]	136.67 ± 5.16[d]
Vitamin C (100 mg/kg) + Acetaminophen (3 g/kg)	53.60 ± 3.65[b]	41.20 ± 2.39[b]	61.79 ± 2.90[b]
***Celosia argentea* (200 mg/kg)**	22.00 ± 1.83[a]	15.50 ± 1.73[a]	25.82 ± 2.20[a]
***Celosia argentea* (400 mg/kg)**	21.60 ± 2.07[a]	15.75 ± 1.71[a]	25.68 ± 0.90[a]
***Celosia argentea* (200 mg/kg) + Acetaminophen (3 g/kg)**	58.20 ± 3.64[b]	54.40 ± 2.79[b]	83.43 ± 2.83[c]
***Celosia argentea* (400 mg/kg) + Acetaminophen (3 g/kg)**	55.20 ± 4.32[b]	42.20 ± 2.77[b]	64.25 ± 3.92[b]

Values are mean ± SE. Mean values in each column (between groups) having different superscript are significantly different (p<0.05)

Table 2: Effect of graded doses of *Celosia argentea* extract on total protein (TP) and albumin (ALB) concentrations in acetaminophen-induced liver injury in rat

Parameter	TP (mg/dl)	ALB (mg/dl)
Control	8.44 ± 0.18[c]	4.17 ± 0.09[c]
Acetaminophen (3 g/kg)	4.33 ± 0.14[a]	2.01 ± 0.11[a]
Vitamin C (100 mg/kg) + Acetaminophen (3 g/kg)	6.95 ± 0.03[b]	3.55 ± 0.14[b]
***Celosia argentea* (200 mg/kg)**	8.41 ± 0.38[c]	4.21 ± 0.13[c]
***Celosia argentea* (400 mg/kg)**	8.56 ± 0.24[c]	4.23 ± 0.11[c]
***Celosia argentea* (200 mg/kg) + Acetaminophen (3 g/kg)**	6.78 ± 0.73[b]	3.40 ± 0.43[b]
***Celosia argentea* (400 mg/kg) + Acetaminophen (3 g/kg)**	6.79 ± 0.12[b]	3.42 ± 0.22[b]

Values are mean ± SE. Mean values in each column (between groups) having different superscript are significantly different (p<0.05)

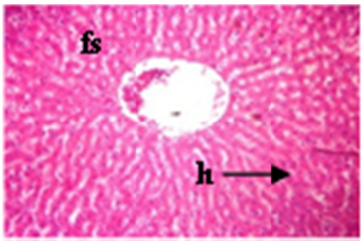

Figure 1: Photomicrograph of liver of control rat given showing portal vein with well fenestrated sinusoids (fs) and hepatocytes (h) with distinct nucleus, H&E, Mag. x400

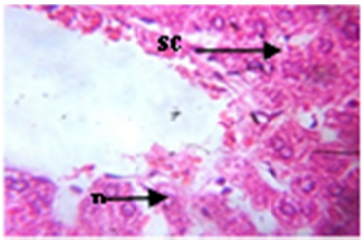

Figure 2: Photomicrograph of liver of rats given Acetaminophen only at day 8 showing necrosis (n), sinusoidal congestion (sc) with focal damage around the central vein, feathery degeneration, loss of lobular architecture and damaged cellular outlines H&E, Mag. x400

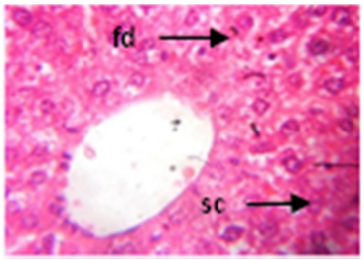

Figure 3: Photomicrograph of liver of rats given Vitamin C for 8 days and Acetaminophen on day 8 revealing mild sinusoidal congestion (sc) around the central vein, slightly reduced feathery degeneration (fd), H&E, Mag. x400

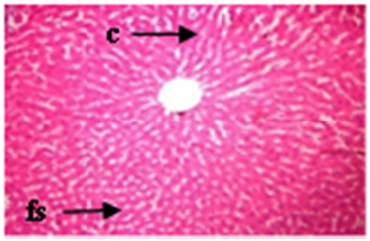

Figure 4: Photomicrograph of liver of rats given *C. argentea* (200 mg/kg for 8 days) revealing distinct centriole (c) and well feneserated sinusoidal space (fs), H&E, Mag. x400

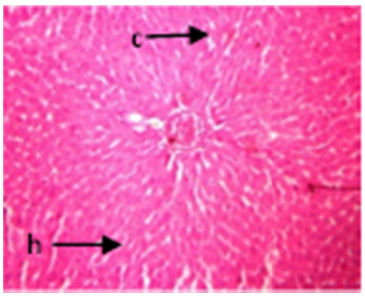

Figure 5: Photomicrograph of liver of rats given *C. argentea* (400 mg/kg for 8 days) revealing visible centriole (c) and hepatocytes (h) with distinct with visible nucleus, H&E, Mag. x400

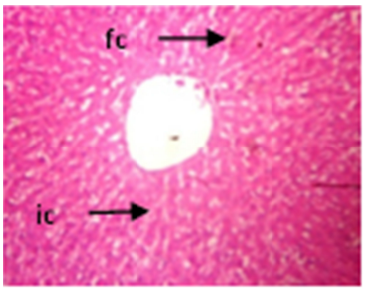

Figure 6: Photomicrograph of liver of rats given *C. argentea* (200 mg/kg for 8 days) and Acetaminophen (3 g/kg) on day 8 revealing clear and distinct centrioles with mild inflammatory cells (ic) with visible nucleus as well as mild fatty changes (fc), H&E, Mag. x400

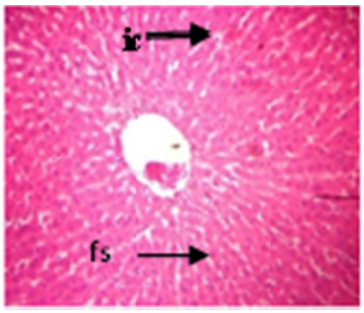

Figure 7: Photomicrograph of liver of rats given *C. argentea* (400 mg/kg) for 8 days and Acetaminophen (3 g/kg) on day 8 revealing distinct centriols surrounded by mild focal inflammtory cell (ic), distinct visible nucleus and well feneserated sinusoids (fs) and reduced fatty changes, H&E, Mag. x400

DISCUSSION

Acetaminophen, a commonly used analgesic drug has the potential to cause centrilobular hepatic necrosis in experimental animals and in humans (Hinson *et al.*, 2010). Damage to the liver or hepatotoxicity does not result from acetaminophen itself, but from one of its metabolites, N-acetyl-pbenzoquinoneimine (NAPQI) (Rumack, 2002). NAPQI is a highly reactive toxic and cytotoxic intermediate metabolite that is damaging to cell components if not detoxified by conjugation with glutathione (GSH). NAPQI can rapidly react with reduced GSH and lead to 90 % hepatic GSH depletion in the cells and mitochondria, which can result in hepatocellular death and mitochondrial dysfunction (Jaeschke and Baht, 2010).

AST, ALT and ALP are the most sensitive biochemical markers employed in the diagnosis of hepatic dysfunction (Nnodim *et al.*, 2010) as their high concentration in blood suggests a spill from the liver as a result of damage. In this study, there was significant increase in level of AST, ALT and ALP in blood of rats given acetaminophen only compared to other study groups indicating the hepatotoxic nature of acetaminophen. Acetaminophen administration induced hepatic attack and damage could have caused disturbance in transport function of hepatocytes, resulting in liver cell membrane leakage and subsequent leakage of AST, ALT and ALP into blood, indicative of loss of plasma membrane functional integrity. However, in group of rats given *C. argentea* or vitamin C for 8 days, there was protection of the liver cell membrane as seen in decrease in release of AST, ALT and ALP from liver into blood signifying the hepatoprotective nature of *C. argentea* and Vitamin C.

Total protein levels are rough measures of protein status but reflect major functional changes in liver functions (Pachathundikandi and Varghese, 2006). According to Iyanda *et al.* (2010), estimation of total protein level in serum is an important way to assess acetaminophen-induced hepatic damage. As seen in this study, there was a decrease in total protein and albumin in rats that received acetaminophen only compared to rats in other groups which suggest the inability of the liver to produce required amount of protein thereby suggesting damage. The increase in total protein and albumin in rats that received *C. argentea* or Vitamin C for 8 days compared to rats given only acetaminophen suggest hepatoprotective

nature of *C. argentea* and Vitamin C. A decrease in albumin level is usually the result of decreased protein synthesis by the liver or increased protein loss through the gut or the kidney (Orhue *et al.,* 2005; Akpan *et al.,* 2012).

The histological observations of necrosis, inflammation and congestion reported in rats given Acetaminophen as well as in amelioration and recovery following *C. argentea* administration to rats also supported the results obtained from serum assays. It has been previously reported that *C. argentea* leaf contains phytoconstituents such as saponins and flavonoids, and concentrations of calcium, phosphorus, potassium, sodium, magnesium, iron, zinc and manganese among others (Usunobun and Ekpemupolo, 2016). Previous researchers have isolated saponins, phenols and peptides among other phytochemicals from *C. argentea* leaf (Lin *et al.,* 2002; Molehin *et al.,* 2014; Tang *et al*., 2016). Thus, the hepatoprotective potential of *C. argentea* may be attributed to its bioactive constituents which scavenges free radicals and prevent oxidative stress, thus helping to prevent liver membrane peroxidation and stabilize the hepatocellular membrane (Molehin *et al.,* 2014). The results obtained in this study agreed with previous related studies on plant leaf protection against acetaminophen or other toxicants induced liver attack and damage (Shardul and Gangadhar, 2010; Khedr and Khedr, 2014; Bartimaeus and Waribo, 2018; Usunobun *et al*., 2019)

Conclusion: Acetaminophen liver-induced attack and injury was reduced by *C. argentea* leaf pre-treatment with reduction in blood levels of liver function enzymes, liver synthetic molecules and marked improvement in liver morphology improvement and restoration, thus giving further prove to hepatotoxic nature of acetaminophen and the hepatoprotective potential of *C. argentea* leaf.

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REFERENCES

AHMAD, S. T., ARJUMAND, W., NAFEES, S., SETH, A., ALI, N., RASHID, S. and SULTANA S. (2012). Hesperidin alleviates acetaminophen induced toxicity in wistar rats by abrogation of oxidative stress, apoptosis and inflammation. *Toxicology Letters,* 208: 149 – 161.

AKPAN, U. P., NNA, V. U., IKPI, D. E., OSIM E. E. and ANTAI, A. B. (2012). Effect of crude *Aloe vera* gel on serum enzymes, proteins and liver histology in alloxan – induced diabetic rats. *International Journal of Science and Research,* 3(6): 2319 – 7064.

APA (2012). *Guidelines for Ethical Conduct in the Care and Use of Non-Human Animals in Research*. American Psychological Association (APA), Washington DC, USA. Retrieved June 13, 2015 from https://www.apa.org/science/leadership/care/care-animal-guidelines.pdf

BANCROFT, J. D. and GAMBLE, M. (2002). *Theory and Practice of Histological Techniques*. Churchill Livingstone, Edinburgh.

BARTIMAEUS, E. S. and WARIBO, H. A. (2018). The prophylactic antioxidant and hepatoprotective potential of *Garcinia kola* pretreatment in acetaminophen induced toxicity in albino rats*. American Journal of Biomedical Science,* 10(2): 106 – 114.

BÉLANGER, J., BALAKRISHNA, M., LATHA, P., KATUMALLA, S. and JOHNS, T. (2010). Contribution of selected wild and cultivated leafy vegetables from South India to lutein and beta-carotene intake. *Asia Pacific Journal of Clinical Nutrition*, 19(3): 417 – 424.

DOUMAS, B. T., WATSON, W. A. and BIGGS, H. G. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. *Clinica Chimica Acta*, 31(1): 87 – 96.

GUM, S. I. and CHO, M. K. (2013). Recent updates on acetaminophen hepatotoxicity: the role of nrf2 in hepatoprotection. *Toxicological Research*, 29(3): 165 – 172.

HAMZAH, R. U., LAWAL, A. R., MADAKI, F. M. and ERUKAINURE, O. L. (2018). Methanolic extract of *Celosia argentea* var. crista leaves modulates glucose homeostasis and abates oxidative hepatic injury in diabetic rats. *Comparative Clinical Pathology*, 27(4): 1065 – 1071.

HINSON**,** J. A., ROBERTS, D. W. and JAMES, L. P. (2010). Mechanisms of acetaminophen-induced liver necrosis. *Handbook of Experimental Pharmacology*, 196: 369 – 405.

ISIK, B., BAYRAK, R., AKCAY, A. and SOGUT, S. (2006). Erdosteine against acetaminophen induced renal toxicity. *Molecular and Cellular Biochemistry*, 287(1-2): 185 – 191.

JAESCHKE, H. and BAHT, M. L. (2010). Mechanisms of acetaminophen hepatotoxicity. Pages 457 – 473. *In*: MCQUEEN, C. A. (Ed.). *Comprehensive Toxicology.* Second Edition, Elsevier B. V., USA.

JAESCHKE, H., MCGILL, M. R. and RAMACHANDRAN, A. (2012). Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. *Drug Metabolism Reviews*, 44(1): 88 – 106.

LIN, W. Q., CHEN, Z. and LIU, J. Q. (2002). The chemical constituents of *Perilla frutescens* (L.) Britt. Var. acuta (Thunb.) and *Celosia argentea* L. seeds grown in Fujian province. *The Chinese Academic Medical Magazine of Organisms*, 4(11): 57 – 59.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193: 265 – 275.

NNODIM, J., EMEJULU, A., AMAECHI, A., NWOSU, N. and CHUKWUNYERE, E. (2010). Alterations in biochemical parameters of Wister rats administered with sulfadoxine and pyrimethamine (Fansidar^{R}). *Al Ameen Journal of Medical Sc*ience, 3(4): 317 – 321.

KHEDR, N. F. and KHEDR, E. G. (2014). Antioxidant and anti-inflammatory effects of curcumin on CCl_4 induced liver fibrosis in rats. *American Journal of Biomedical Science,* 6(3): 191 – 200.

IYANDA, A. A., ANETOR, J. I., ADENIYI, F. A. A. and IHEAKANWA, C. I. (2010). Effect of methionine on hepatic indices in male Wistar rat dosed to acetaminophen formulation. *African Journal of Pharmacy and Pharmacology*, 4(9): 653 – 657.

MAZALEUSKAYA, L. L., SANGKUHL, K., THORN, C. F., FITZGERALD, G. A., ALTMAN, R. B. and KLEIN, T. E. (2015). PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. *Pharmacogenetics and Genomics*, 25(8): 416 – 426.

MCGILL, M. R., WILLIAMS, C. D., XIE, Y., RAMACHANDRAN, A. and JAESCHKE, H. (2012). Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts, mitochondrial dysfunction, and oxidative stress in the mechanism of toxicity. *Toxicology and Applied Pharmacology*, 264(3): 387 – 394.

MOLEHIN, O. R., ADEFEGHA, S. A., OBOH, G., SALIU, J. A., ATHAYDE, M. L. and BOLIGON, A. A., (2014). Comparative study on the phenolic content, antioxidant properties and HPLC fingerprinting of three varieties of *Celosia* species. *Journal of Food Biochemistry,* 38: 575 – 583.

ORHUE, N. E. J., NWANZE, E. A. C. and OKAFOR, A. (2005). Serum total protein, albumin and globulin levels in *Trypanosoma brucei*-infected rabbits: effect of orally administered *Scoparia dulcis. African Journal of Biotechnology,* 4(10): 1152 – 1155.

PACHATHUNDIKANDI, S. K. and VARGHESE, E. T. (2006). Blood zinc protoporphyrin, serum total protein, and total cholesterol levels in automobile workshop workers in relation to lead toxicity: our experience. *Indian Journal*

of Clinical Biochemistry, 21(2): 114 – 117.

RAMESH, B. N., MAHALAKSHMI, A. M. and MALLAPPA, S. H. (2013). Towards a better understanding of an updated ethnopharmacology of *Celosia argentea* L. *International Journal of Pharmaceutical and Pharmacological Sciences*, 5(3): 54 – 59.

REITMAN, S. and FRANKEL, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28(1): 56 – 63.

RUMACK, B. H. (2002). Acetaminophen hepatotoxicity: the first 35 years. *Journal of Toxicology: Clinical Toxicology*, 40(1): 3 – 20.

SHARDUL, S. W. and GANGADHAR, B. S. (2010). Antioxidant and hepatoprotective activity of *Tridax procumbens* Linn, against paracetamol-induced hepatotoxicity in male albino rats. *Advanced Studies in Biology,* 2(3): 105 – 112.

TANG, Y., XIN, H. L. and GUO, M. L. (2016). Review on research of the phytochemistry and pharmacological activities of *Celosia argentea*. *Revista Brasileira de Farmacognosia*, 26(6): 787 – 796.

TOBIH, F. O., ADEGBITE, A. A. and ONONUJU, C. C. (2011). Evaluation of some plant materials as organic mulch for the control of root-knot nematode *Meloidogyne* sp. and its impact on the yield of *Celosia argentea* L. *International Journal of Agricultural Sci*ence, 1(14): 195 – 202.

USUNOBUN, U. and EKPEMUPOLO, I. S. (2016). Phytochemical analysis, mineral composition and *in vitro* antioxidant activities of *Celosia argentea* leaves. *International Journal of Scientific World,* 4(1): 19 – 22.

USUNOBUN, U., UGBENI, C. O. and AGU, K. C. (2019). Protective effect of *Celosia argentea* aqueous leaf extract on liver function in carbon-tetrachloride (CCl_4)-induced hepatotoxicity. *Malaysian Journal of Biochemistry and Molecular Biology,* 22(1): 93 – 96.

VARADHARAJ, V. and MUNIYAPPAN, J. (2017). Phytochemical and phytotherapeutic properties of *Celosia* species – a review. *International Journal of Pharmacognosy and Phytochemical Research*, 9(6): 820 – 825.