

## Assessment of the Hepato-protective Effects of Combined Ethanolic Extract of *Psidium guajava* and *Carica papaya* Leaves in Rats Challenged with Cadmium

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*Date Received:* 02-11-2022

*Date Accepted:* 25-02-2023

*DOI:* <https://doi.org/10.48198/NJPAS/22.B14>

### ABSTRACT

It has been shown that herbal extracts can protect intoxicated hepatocytes. Therefore, this study examined the hepato-protective effects of pooled ethanolic extract of *Psidium guajava* and *Carica papaya* leaves in rats challenged with Cadmium (Cd). Exactly 200g of the grounded leaves were soaked in ethanol for 48 hours and was concentrated after filtration using a rotary evaporator. Fifteen adult female rats (150±10g) were randomly assigned into three groups: Group 1 (control: received feed and water alone), Group 2 (Cd group; received single dose of Cd, 30mg/kg b wt, Group 3 (Cd + extract: received Cd as in group 2 and leaf extract 200mg/kg bwt daily for 2 weeks). Rats were slaughtered and samples were taken for biochemical examination at the conclusion of the study period. When compared to controls, exposure to Cd alone resulted in a significant increase ( $p < 0.05$ ) in the activities of the alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH), as well as an increase in the level of total protein (TP). Hepatocytes exposed to Cd underwent histological changes as well. As opposed to rats exposed to Cd alone, there was a significant decrease in the values of ALT, AST, ALP, LDH, and TP when Cd-challenged rats were given the combination ethanolic extract of *P. guajava* and *C. papaya*. The outcomes suggest that the biherbal combination of *P. guajava* and *C. papaya* can shield liver cells against Cd damage.

**Keywords:** *Psidium guajava*, *Carica papaya*, Liver, Cadmium.

### Introduction

The liver, an extremely important organ of the body, is involved in the break down and build of proteins, carbohydrates, vitamins, and lipids. It is also involved in the detoxification and excretion of drugs and other xenobiotics (Rane *et al.*, 2016; Arige *et al.*, 2017). Since liver illness is one of the leading causes of death worldwide, an increase in cases of liver disease caused by consumption of pharmaceuticals, heavy metals, and other environmental toxins has been recorded

everywhere (Maximas *et al.*, 2013). So, maintaining a healthy liver is essential for good living.

Liver injury is displayed via rise in oxidative stress, increased peroxidation of lipids, cellular necrosis, depletion of protective antioxidant systems such as glutathione, and elevation in the levels of alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine aminotransferase (AST), and other liver biomarkers in the serum (Arige *et al.*, 2017).

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One environmental pollutant that has been revealed to cause severe liver damage is Cadmium (Cd), a non-essential toxic heavy metal that is ubiquitous in the environment due to its numerous applications in industrial processes, extended biological half-life and ability to bio-accumulate in tissues (Odewumi *et al.*, 2018; Orororo *et al.*, 2018; Genchi *et al.*, 2020; Orororo *et al.*, 2022). Cd induces oxidative stress in the liver, interferes with the activities of lactate dehydrogenase (LDH), AST, ALT, and ALP, and inhibits synthesis of proteins and glycogen metabolism in hepatocytes (Rahimzadeh *et al.*, 2017; Orororo *et al.*, 2017; Branca *et al.*, 2018;).

Plant extracts have been reported to possess hepatoprotective prowess and as such, the screening of different plant extracts and products for liver-healing abilities is an active and ongoing area of research (Uboh *et al.*, 2010, Udi *et al.*, 2018; Ekakitie *et al.*, 2021; Udi *et al.*, 2022). *Carica papaya* L. (pawpaw) and *Psidium guajava* (guava) are examples of such plants. Their leaves contain adequate phytochemicals (lycopene, quercetin chlorogenic acid, carotenoids, alkaloids, apigenin, caffeic acid, guaijaverin, phenolics, gallic acid, protocatechuic acid, catechin etc) and have been reported to have antioxidant, anti-cancer, antimicrobial, antimalarial, antifungal, hypolipidemic and hepatoprotective abilities (Uboh *et al.*, 2010; Manikandaselvi *et al.*, 2012; Porwal *et al.*, 2012; Ezekwe *et al.*, 2017; Vijayakumar *et al.*, 2017).

Polyherbal therapy (herb-herb combinations), employed in traditional medicine practice for a long time, is beginning to receive the attention of researchers in other to provide scientific backing for their observed therapeutic effects (Che *et al.*, 2013; Karole *et al.*, 2019). Working synergistically, polyherbal preparations provide certain helpful effects that supersedes what can be provided by single plant extracts (Ramaiah *et al.*, 2013; Abraham and Paridhavi, 2013; Parasuraman *et al.*, 2014; Karole *et al.*, 2019). In this regard,

Arshad *et al.* (2021) showed the restorative ability of a bi-herbal preparation of *Psidium Guajava* and *Carica Papaya* in diabetes induced by streptozotocin in male wistar rats. Okoro (2020) also displayed the ability of *Carica papaya* and *Allium sativum* hydroethanol extracts to guard against liver damage caused by carbon tetrachloride, in which the combined extract recorded the highest hepatoprotective and antioxidant potential than either plant used individually. Studies on the usefulness of polyherbal therapy in Cd-induced hepato-toxicity are few. Therefore, this study examined the hepatoprotective effects of combined ethanolic extract of *Psidium guajava* and *Carica papaya* leaves in rats challenged with Cd.

## Materials and Methods

### Chemicals/Reagents

Analytical grade chemicals were used in the experiment. Assessment of liver function was done using Randox assay kits.

### Plant Material

Fresh pawpaw and guava leaves were obtained from Otu-Jeremi, Udu Local Government Area, Delta State. After identification by a botanist (ECU/FOS-03-22), the leaves were sorted to eliminate any dead matter and other unwanted particles. Thereafter, they were air dried for (2) weeks and ground into fine powder using an electric dry mill. The fine powders obtained were stored in air-tight containers until further use.

### Extraction

Exactly 200g of the grounded powder was immersed in 500ml of ethanol for 48 hours at room temperature. Thereafter, the concoction was filtered into 500ml conical container with whatmann filter paper. The filtrate was then dried using a rotary evaporator to yield a thick dark green viscous liquid used for the experiment.

### Experimental Design

A total number of 15 adult Wistar rats weighing  $150 \pm 10\text{g}$  were utilized for the investigation. The rats were acclimatized in the investigation house for a week prior to the commencement of the investigation. The rats were ascertained arbitrarily into three groups of five rats each and treated as shown in Table 1.

**Table 1:** Experimental Grouping and Treatment

Groups	Designation	Treatment
1	Control	Feed and water only
2	Cadmium	Single oral dose of Cd (30mg/kg body weight) at the start of the experiment plus feed and water
3	Cd + Extract	Cd (as in group 2), combined leaf extract (200mg/kg body weight daily) plus feed and water

All the animal experiments were conducted in accordance with standard guidelines for animal care.

### Sample Collection

The investigation lasted for 21 days. Thereafter, the rats were sacrificed by cervical dislocation. The animals were opened up and blood samples obtained by cardiac puncture. The blood samples were centrifuged and the serum allowed to clot. Serum obtained was stored frozen until used for analysis. Portions of the liver were obtained as well and processed for histological examination.

### Hepatic Function Assessment

Standard protocols were used in assessing the hepatic function of the animals in the different groups. The activities of AST, ALT, and LDH were determined following the methods of Reitman and Frankel (1957) in accordance with the procedures outlined in the Randox assay kits as reported by Ekakitie et al. (2021). ALP activity was determined by the method of Annino and Giese (1976) while the method of Henry (1979) was

used in the determination of LDH following instructions in TECO Diagnostics kit. The total protein of serum samples was determined by the biuret technique as described by Tietz (1995) also following the Randox assay kit protocol.

### Assessment of Liver Histology

Assessment of liver histology was performed following standard laboratory operating protocols and procedures (Zhang et al., 2017). Tissue samples previously fixed in formalin were sliced, processed (slicing, removal of paraffin and staining with hematoxylin) and inspected under the light microscopic (Brunel SP35 Digital Trinocular) connected to a computer.

### Analysis of Data

Standard descriptive statistics, the one – way analysis of variance (ANOVA) as well as least significance difference (LSD) test were employed in the analysis of the data obtained. P value was fixed at 0.05 and results were reported as mean  $\pm$  SD of three determinations.

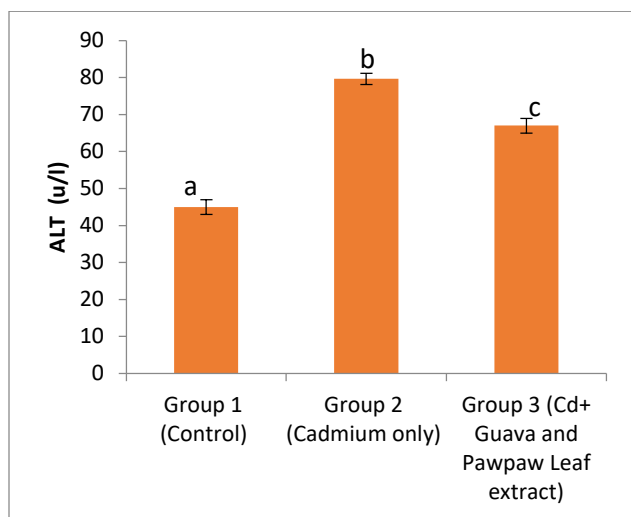
### Results

#### *The influence of combined ethanolic extract of *Psidium guajava* and *Carica papaya* leaves on selected hepatic function parameters in rats challenged with Cd*

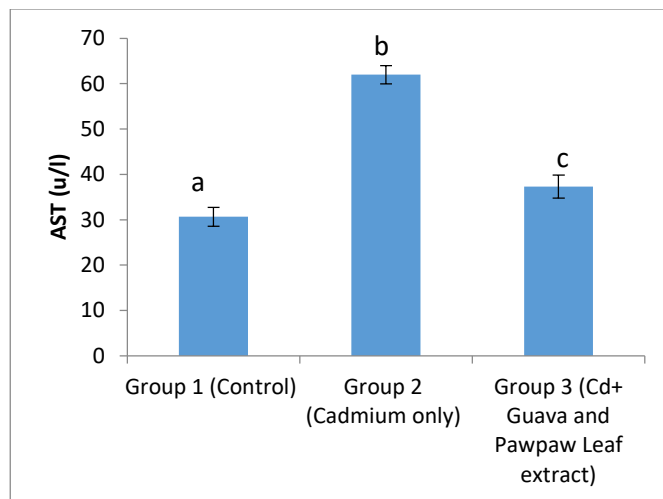
The influence of combined ethanolic extract of *P. guajava* and *C. papaya* leaves on selected hepatic function parameters in rats challenged with Cd is shown in Figures 1-5. Exposure to Cd alone (Group 2) brought about a significant rise ( $p < 0.05$ ) in ALT and AST activities in the serum compared to rats in the control group (Group 1). Serum ALT activity in Cd-challenged rats treated with the extract (Group 3) was notably lower than that recorded for rats exposed to Cd alone (Group 2). This was also observed in AST activity, demonstrating that Cd toxicity induced significant boost in the activities of ALT and AST in the serum, but was ameliorated by the ingestion of the

combined ethanolic extract of *P. guajava* and *C. papaya* leaves.

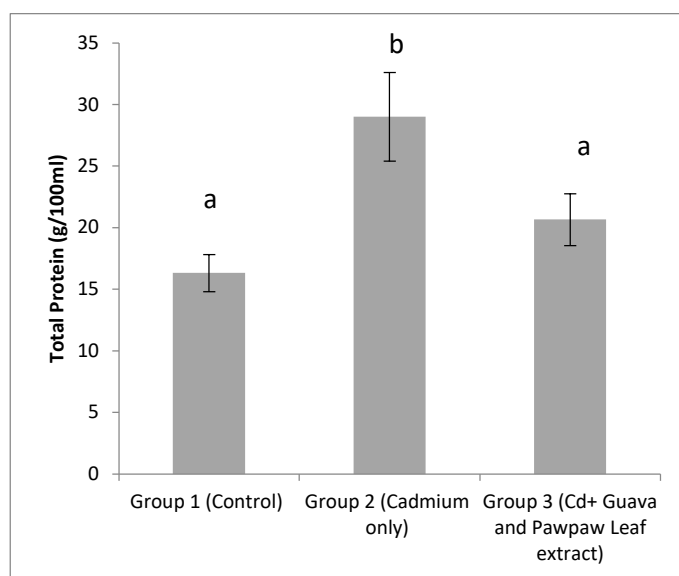
Rats in group two (2) recorded significant rise ( $p < 0.05$ ) in the level of serum total protein and ALP compared to rats in the control group (Group 1). Serum total protein level observed in Cd-exposed rats treated with the extract (Group 3) was considerably lower than that recorded for rats exposed to Cd alone (Group 2). This was also observed in ALP activity. However, no noteworthy difference was observed in serum total protein levels in control rats and rats exposed to Cd and the extract, an indication that the extract successfully normalized protein levels disturbed by intoxication with Cd. This trend was also seen in the activity of serum lactate dehydrogenase (Figure 5).



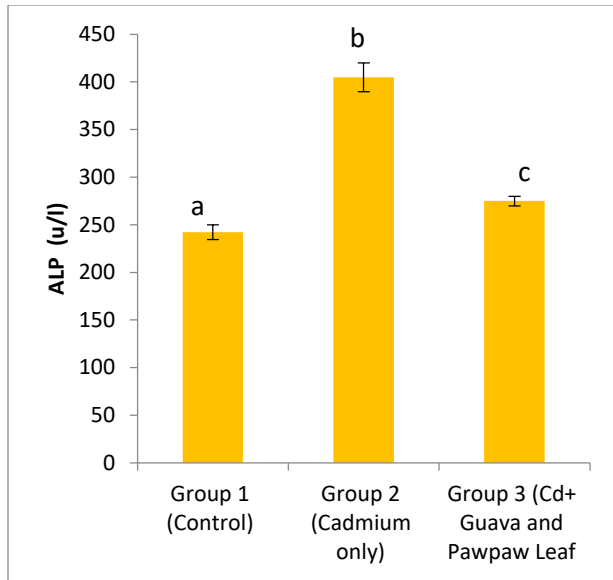
**Figure 1:** Influence of combined ethanolic extract of *Psidium guajava* and *Carica papaya* leaves on serum ALT activity in rats challenged with Cd. Values are mean  $\pm$  SD. Noteworthy difference ( $p < 0.05$ ) exist between bars with dissimilar alphabets.



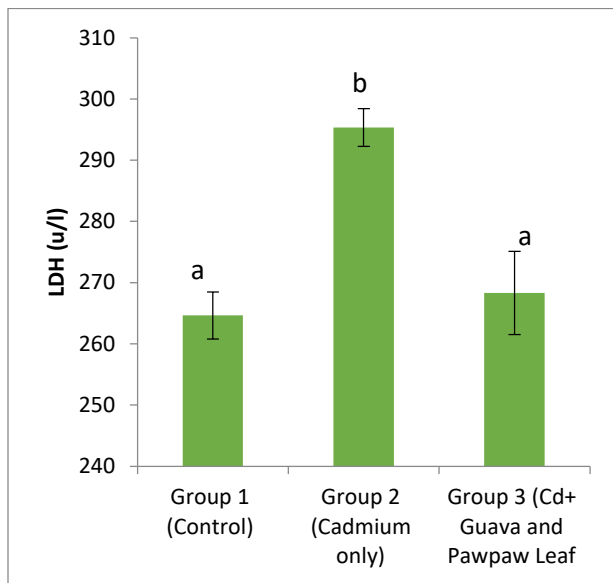
**Figure 2:** Influence of combined ethanolic extract of *Psidium guajava* and *Carica papaya* leaves on serum AST activity in rats challenged with Cd. Values are mean  $\pm$  SD. Noteworthy difference ( $p < 0.05$ ) exist between bars with dissimilar alphabets.



**Figure 3:** Influence of combined ethanolic extract of *Psidium guajava* and *Carica papaya* leaves on serum total protein levels in rats challenged with Cd. Values are mean  $\pm$  SD. Noteworthy difference ( $p < 0.05$ ) exist between bars with dissimilar alphabets.



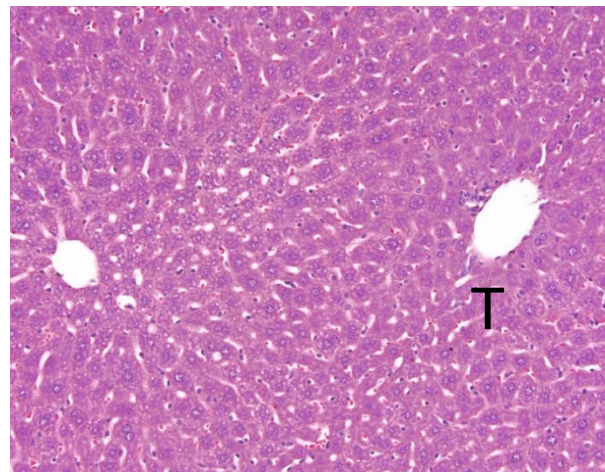
**Figure 4:** Influence of combined ethanolic extract of *Psidium guajava* and *Carica papaya* leaves on serum ALP activity in rats challenged with Cd. Values are mean ± SD. Noteworthy difference ( $p < 0.05$ ) exist between bars with different alphabets.



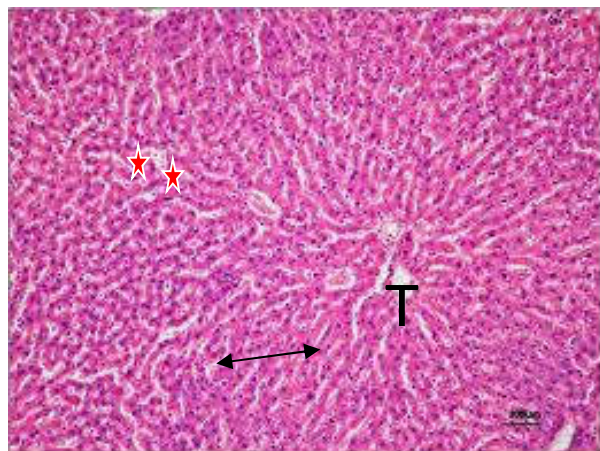
**Figure 5:** Influence of combined ethanolic extract of *Psidium guajava* and *Carica papaya* leaves on serum LDH activity in rats challenged with Cd. Values are mean ± SD. Noteworthy difference ( $p < 0.05$ ) exist between bars with different alphabets.

**The outcome of combined ethanolic extract of *Psidium guajava* and *Carica papaya* leaves on the histology of the liver of rats challenged with Cd**

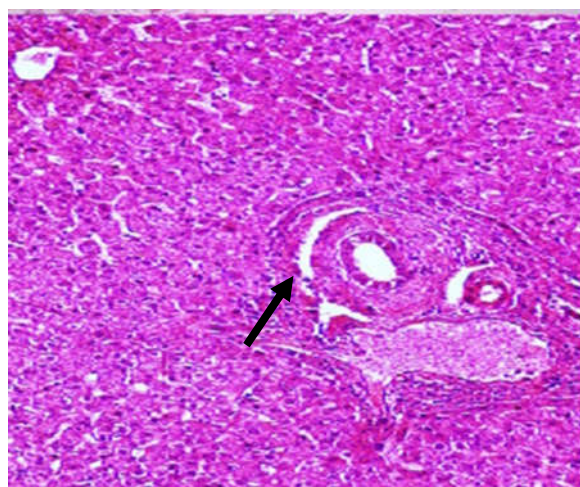
The outcome of combined ethanolic extract of *Psidium guajava* and *Carica papaya* leaves on the histology of the liver of rats challenged with Cd is depicted in the micrographs in Figures 6-8. Section of liver of rats in Group A: (control), depicted normal histological features: central portal triads (T) with intact liver epithelia lining indicating normalcy. Conversely, portions of the liver of rats exposed to Cd alone (Group B) showed mild congestion of vesicular nuclei (indicated by the red stars), with central portal triads (T) of hepatic lobules within sheets of hepatocytes and intact epithelia lining with little granulation (double arrow) an indication of toxic effects. Treatment of Cd-challenged rats with the biherbal extract of *Psidium guajava* and *Carica papaya* leaves brought about mild hepatic necrosis shown by infiltration of leucocytes (large arrow).



**Figure 6:** Segment of the liver of control (Group 1)



**Figure.7:** Segment of the liver of rats exposed to Cd alone (Group 2)



**Figure.8:** Segment of the liver of rats exposed to Cd and biherbal extract of *Psidium guajava* and *Carica papaya* leaves (Group 3)

### Discussion

It has been published that herbal extracts can protect intoxicated hepatocytes (Mansur *et al.*, 2015). In this investigation, the ability of combined ethanolic extract of *Psidium guajava* and *Carica papaya* leaves to defend liver cells against Cd intoxication was examined. Cd is a known hepato-toxic heavy metal whose metabolism precipitates oxidative stress and tissue damage (Daisy *et al.*, 2013; Ansah *et al.*, 2016;

Orororo *et al.*, 2018b). Assessment of certain liver marker enzymes such as LDH, ALP, ALT and AST, is routinely deployed in estimating the extent of liver injury and obstruction of bile production and transport (Arshad *et al.*, 2021).

A rise in the activities and levels of LDH, ALP, ALT, AST and total protein in the serum, as observed in this study, points to the fact that the integrity of hepatocyte membranes may have been breached leading to their leakage into the blood strain (Ebong *et al.*, 2012; Nili-Ahmadabadiet *al.*, 2018; Kandemir *et al.*, 2020). This result agrees with established studies on Cd-induced hepatotoxicity in which Cd exposure brought about major elevation in the serum activities of the aforementioned enzymes (Abu-El-Zahab *et al.*, 2019; Zhijie *et al.*, 2021; Noor *et al.*, 2022). In line with Zafar *et al.* (2009) and Kolawole *et al.* (2014) an elevation in the level of serum total protein can come from the disruption and removal of polyribosome present in the endoplasmic reticulum which eventually brings about flaws in the protein biosynthesis apparatus of cells. The observed hepato-toxic effects of Cd in this study were also confirmed by the damages noticed in the histology of tissues of rats exposed to Cd (mild congestion of vesicular nuclei, hepatic necrosis and infiltration of leucocytes). Again, this is in consonance with previous reports (Shen *et al.*, 2017; Refaie *et al.*, 2018; Ojo *et al.*, 2021).

The treatment of Cd-challenged rats with the combined extract caused an appreciable reversal of the Cd-induced alteration of the liver marker enzymes and histopathological changes. This is not surprising as the ability of *C. papaya* and *P. guajava* leaf extract (singly administered) to ameliorate liver function markers altered by exposure to Cd and other heavy metals have been published in literature (Vijayakumar *et al.*, 2017; Molla and Azene, 2017; Mohammad *et al.*, 2018). Interestingly, the combined extract restored the activity of LDH and level of total protein in Cd exposed rats to levels not statistically different

from that of the control. This observed restoration may have arisen from the increase in the grouping of ribosome on endoplasmic reticulum to facilitate normalized protein biosynthesis (Ayeni *et al.*, 2017; Okoro, 2020). The impact of the pooled ethanolic extract of *Carica papaya* and *Psidium guajava* leaves is certainly as a result of its huge content of bioactive compounds and their possible synergistic properties (Karole, 2019). Biherbal formular of *Carica papaya* and *Psidium guajava* leaves can be employed effectively in the management of liver damage caused by Cd intoxication.

### Conclusion

Hepato-toxicity as a result of Cd intoxication this investigation was defined by a major elevation in serum levels of basic liver biochemical markers such as LDH, ALT, ALP, AST and TP as well as histopathological changes in hepatocytes. However, treatment with biherbal extract of *P. guajava* and *C. papaya* leaves significantly reduced the elevated levels of the liver biomarkers in the serum alongside histopathological restoration. The study has established that liver injury occasioned by Cd exposure can be successfully managed through administration of pooled extract of *P. guajava* and *C. papaya* leaves.

### References

- Abraham, R. and Paridhavi, M. (2013). A review of comprehensive study on medicinal plants of polyherbal formulation- Churna. *Asian Journal of Pharmaceutical and Clinical Research*; 6(4):11-18.
- Abu-El-Zahab, H., Hamza, R.Z., Montaser, M.M., El-Mahdi, M.M. and Al-Harhi, W.A., (2019). Antioxidant, antiapoptotic, antigenotoxic, and hepatic ameliorative effects of L-carnitine and selenium on cadmium-induced hepatotoxicity and alterations in liver cell structure in male mice. *Ecotoxicol. Environmental Safety*; 173, 419–428.
- Annino, J. S. and Giese, R. W. (1976) *Clinical Chemistry. Principles and procedure*, 4th edn. Boston: Little Brown.
- Ansah, C., Aggrey, A. J., Boadu, B. M. and Mante, P. K. (2016). Aqueous leaf Extract of *Carica papaya* (caricaceae) causes liver injury and reduced fertility in rats. *International Journal of Pharmaceutical and Pharmacological Sciences*; 8:261-265
- Arige, S. S., Arige, S.D. and Lakshmana Rao, A.(2017). A review on Hepatoprotective activity, *International Journal of Current Research*. 9(06): 51876-51881.
- Arshad, S., Azeem, M. A., Muzahir, J., Mehreen, A. and Khan, M. A. (2021). Effects of *Carica Papaya* and *Psidium Guajava* Extract on Hepato-Renal Performance of Streptozotocin-Induced Diabetic Male Albino Rats. *Pakistan BioMedical Journal*; 4(1): 62-72
- Ayeni, M. I., Akanmu, M. A., Bolajim, O., Osasan, S. A. Olayiwola, G., Afolabi, M. O. and Morohunfolo, A. M. (2017). Bilirubin Lowering Potential of Aqueous *Carica papaya* Extract in Induced Jaundice in Rats. *Journal of Pharmaceutical and Pharmacological Research*; 7:457-466.
- Branca, J. J., Morucci, G. and Pacini, A. (2018) Cadmium-induced neurotoxicity: still much ado. *Neural Regeneration Research*; 13(11): 1879.
- Che, C. T., Wang, Z. J., Chow, M. S. S. and Lam, C. W. K. (2013). Herb-herb combination for therapeutic enhancement and advancement: Theory, practice and future perspectives. *Molecules*; 18(5):5125–41.
- Daisy, P., Feril, G. and Kani, J. (2013). Hypolipidemic and hepato-

protective effects of *Cassia auriculata* (L.) barkextract on streptozotocin induced diabetic rats. *Asian Journal of Pharmaceutical and Clinical Research*; 6: 43-48.

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Ebong, P. E., Igile, G. O., Mgbeje, B.I.A., Iwara, I. A., Odongo, A. E., Onofiok, U. L., Oso, E. A., Ezekiel, A. and Florence, M., (2012). Papain, a plant enzyme of biological importance: A review. *American Journal of Biochemistry and Biotechnology*; 8(2):99-104 Ekakitie, L. I., Okpoghono, J., Orororo O. C. and Ekakitie, O. A. (2021) Ameliorative prowess of bee honey in the tissues of rats administered aluminium nitrate. *Scientific African*; 12 (2021) e00782:1-9

Ezekwe, A., Constance, N., Ijeoma, E., Prince, O. and Samuel, N. (2017). Studies on biochemical effects of aqueous extract of *Carica papaya* leaf on alloxan-induced diabetic albino rats. *Food Biology*. 6: 28-35.

Genchi, G., Maria, S. S., Lauria, G., Carocci, A. and Catalano, A. (2020). The effects of Cadmium toxicity. *International Journal of Environmental Research and Public Health*, 17:3782–3806.

Henry, B.(1979). *Clinical Diagnosis and Management by Laboratory Methods*. Philadelphia,PA: W.B. Saunders and Company.

Kandemir, F.M., Yıldırım, S., Kucukler, S., Caglayan, C., Darendelioğlu, E. and Dortbudak, M. B. (2020). Protective effects of morin against acrylamide-induced hepatotoxicity and nephrotoxicity: A multi-biomarker approach. *Food Chemistry and Toxicology*; 138 (2020), 111190.

Karole, S., Shrivastava, S., Thomas, S., Soni, B., Khan, S., Dubey, J., Dubey, S. P., Khan, N. and Jain, D. K. (2019). Polyherbal

Formulation Concept for Synergic Action: A Review. *Journal of Drug Delivery and Therapeutics*; 9(1-s):453-466.

Kolawole, O. T., Akiibinu, M. O. and Akanji, M. A. (2014) Assessment of the effect of aqueous extract of calyx of *Hibiscus sabdariffa* on some biochemical indices of renal function in rats. *International Journal of Pharmaceutical Sciences*, 4 (3):587-590.

Manikandaselvi, S., Ezhilarasi, S. and Vaithehi, R. (2012). Modulation of lysosomal enzymes activity by *Carica papaya* Linn. and *Solanum torvum* Linn. in carbon tetrachloride vapour induced liver damage in rats. *Journal of Chemical and Pharmaceutical Research*, 4(2) 1235-1238.

Mansur, M., Seyed, H. H., Mohammad, K., Majid, E. G., Noubarani, M. and Mohammad, R. E. (2015). Ameliorative effects of quince fruit on diabetes. *Iranian Journal of Pharmaceutical Research*, 14(4): 1207-1214.

Maximas, H., Rose, H. R., Sudha, P. N., Vinayagam, A. and Sudhakar, H.(2013). A Review on Hepatoprotective Activity of Commonly Consumed Vegetables. *Der Pharmacia Lettre*, 5 (5):290-304.

Mohammad, N. S., Tangpong, J. and Rahman, M. M. (2018). Toxicodynamics of lead. Cadmium. Mercury and arsenic-induced kidney toxicity and treatment strategy, a mini review. *Toxicology Reports*, 5:704–713 .

Molla, T. and Azene, H.(2017). A Systemic Review on Antioxidant and Hepatoprotective Effect of *Psidium Guajava* Leaf and Fruit Extract. *Food Science and Quality Management*; 60:28-46

Nili-Ahmadabadi, A., Alibolandi, P., Ranjbar, A., Mousavi, L., Nili-Ahmadabadi, H., Larki-Harchegani, A., Ahmadimoghaddam, D.



- and Omidifar, N. (2018). Thymoquinone attenuates hepatotoxicity and oxidative damage caused by diazinon: an in vivo study. *Research in Pharmaceutical Sciences*; 13 (6) (2018) 500–508.
- Noor, K. K., Ijaz, M. U., Nazia, E., Arfa, T., Derya, K. Y., Neamul, S. M., Kabir, Z., Shaikh, J., Uddin, D., Asma, A. and Simal-Gandara, J. (2022). Hepatoprotective role of vitexin against cadmium-induced liver damage in male rats: A biochemical, inflammatory, apoptotic and histopathological investigation. *Biomedicine and Pharmacotherapy*; 150 (2022) 112934
- Odewumi, C., Latinwo, L. M., Badisa, V. L. D., Suenita, S., Cobb-Abdullah, A., Marijo, K. F. and Osborne, D. (2018). Modulation of Cadmium Induced Apoptotic, Cancer and Inflammation Related Cytokines by Diallyl Disulfide in Rat Liver Cells. *Annals of Toxicology*; 1(1):1-8.
- Ojo, O. A., Damilare, R., Ayomide, E. B., Omowumi, T. K., Adebola, B. O., Omokolade, O. A., Basiru, O. A. and Olarewaju, M. O. (2021). Gallic acid protects against cadmium chloride-induced alterations in Wistar rats via the antioxidant defense mechanism. *Journal of Pharmacy & Pharmacognosy Research*, 9 (5), 668-676.
- Okoro, I. O. (2020). Hepatoprotective effect of hydroethanol extracts of *Allium sativum* and *Carica papaya* on CCl<sub>4</sub>- induced liver damage in rats. *Nigerian Journal of Pure and Applied Sciences*; 33(1): 3639-3640
- Orororo O. C., Asagba, S. O., Oghri, E. and Egbune, E. O. (2017) Comparative Effect Of Garden Egg, Carrot And Oat On Biochemical Parameters In Cadmium Exposed Rats. *African Journal of Biochemistry Research*, 12(3): 28-34
- Orororo, O. C., Asagba, S. O., Tonukari, N. J., Okandeji, O. J., and Mbanugo J. J. (2018). Effects of *Hibiscus Sabdarrifa* L. Anthocyanins on Cadmium-Induced Oxidative Stress in Wistar Rats. *Journal of Applied Science and Environmental Management*; 22(4): 465 – 470.
- Orororo, O. C., Asagba, S. O., Tonukari, N. J., Okandeji, O. J., and Mbanugo J. J. (2018b). Cadmium-Induced Testicular Damage in Wistar Rats: Protective Effects of *Hibiscus sabdariffa* L. Anthocyanins. *International Journal of Biochemistry Research and Review*; 21(4): 1-8
- Orororo, O. C., Efekemo, O. and Udi, O. A. (2022). Changes in liver histomorphology, hematological parameters and lipid profile of cadmium-exposed rats treated with combined leaf extract of *Verninia amygdalina* and *Ocimum gratissimum*. *Asian Journal of Medicine and Health*; 20(11):195- 203
- Parasuraman, S., Thing, G. S. and Dhanaraj, S. A. (2014). Polyherbal formulation: concept of ayurveda. *Pharmacognosy Review*; 8(16): 73-80.
- Porwal, V., Sing, P. and Gurjar, D. (2012). A Comparative Study on Different Methods of Extraction from *Guajava Leaves* for Curing Various Health Problems. *IJERA* 2(6): 490-96.
- Rafati, M. R., Rafati, M. R., Kazemi, S. and Moghadamnia, A. A. (2017). Cadmium toxicity and treatment: An update. *Caspian Journal of Internal Medicine*; 8:135–145.
- Ramaiah, M., Chakravathi, G. and YasaSwini, K. (2013). *In vitro* biological standardization, formulation and evaluation of directly compressed polyherbal anthelmintic tablets. *Pharmacognosy Journal*; 5(3):130-4.

- Rane, J., Kadhai, R. and Bakal, R.L. (2016). Liver diseases and herbal drugs: A review. *Journal of Innovations in Pharmaceutical and Biological Sciences*. 3: 24-36.
- Refaie, M., El-Hussieny, M. and Zenhom, N.M. (2018). Protective role of neбиволol in cadmium-induced hepatotoxicity via down regulation of oxidative stress, apoptosis and inflammatory pathways. *Environmental Toxicology and Pharmacology*; 58: 212–219.
- Reitman, S. and Frankel, S. (1957). Glutamic – pyruvate transaminase assay by colorimetric method. *American Journal of Clinical Pathology*; 28: 56-65
- Shen, R., Liu, D., Hou, C., Liu, D., Zhao, L., Cheng, J., Wang, D. and Bai, D. (2017). Protective effect of *Potentilla anserina* polysaccharide on cadmium-induced nephrotoxicity in vitro and in vivo. *Food Functions*; 8, 3636–3646.
- Uboh, F. E., Iniobong E. O. and Ekong, M. B. (2010). Effect of Aqueous Extract of *Psidium Guajava* Leaves on Liver Enzymes, Histological Integrity and Hematological Indices in Rats. *Gastroenterology Research*; 3(1):32-38
- Udi, O.A., Igbigbi, P.S., Chris-ozoko, L.E. and Oyeleke, A.A. (2018). Lead II Acetate Induced Physio-Morphological Changes in Prefrontal Cortex of *Ocimum gratissimum* Fed Wistar Rats. *AJORIN*, 1 (1) (2018):1-10.
- Udi, O. A, Oyem, J. C., Ebeye, O. A., ChrisOzoko, L. E., Igbigbi, P. S., and Olannye DU. (2022). The effects of aqueous extract of *Ocimum gratissimum* on the cerebellum of male wistar rats challenge by lead acetate. *Clinical Nutrition Open Science*. 2022; 44: 28-41.
- Vijayakumar, K., Rengarajan, R. L., Radhakrishnan, R., Anand, A. V. (2018) Hypolipidemic effect of *Psidium guajava* Leaf extract against hepatotoxicity in rats. *Pharmacognosy Magazine* 2018;14:4-8.
- Zafar. S. M., Ah, Naeem-ul-Hassan, N. M. and Kaim, K. Z. A. (2009). Altered liver morphology and enzymes instreptozotocin-induced diabetic rats. *International Journal Morphology*; 27:719-
- Zhang, C. Tian, X. and Zhang, K. (2017). Protective effects of *Foeniculum vulgare* root bark extract against carbon tetrachloride-induced hepatic fibrosis in mice, *World Journal of Gastroenterology*. 23 (2017) 5722–5731 .
- Zhijie, Y., Yuqin, H., Haifang, W. and Qiong, Z. (2021). Protective effect of melatonin against chronic cadmium-induced hepatotoxicity by suppressing oxidative stress, inflammation, and apoptosis in mice. *Ecotoxicology and Environmental Safety*; 228 (2021) 112947