SENNA ALATA WITH SELENIUM MITIGATES LIVER AND KIDNEY INJURY FROM ISONIAZID AND RIFAMPICIN IN MICE

ABSTRACT

Aim: This study aimed to evaluate the ameliorative potentials of *S. alata* in combination with Selenium on liver and kidney injury induced by Isoniazid-Rifampicin (INH-RIF).

Study Design: Original Research work

Place and Duration of Study: Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, between August and October 2023.

Methods**:** Hydroethanolic extract of *S. alata* (EESA)was obtained by maceration, 25 Swiss mice (25-35g) were divided into 5 groups, GP1 received distill water, GP 2-5 INH-RIF 150mg/kg for the first two days; while groups 3-5 received *S.alata* 100mg/kg, 200mg/kg and 400mg/kg in combinAtion with Selenium 1mg/kg respectively from day 3 till day 14 orally. Sera samples were obtained and organs (liver and kidney) for weighing and histopathology on day 15.

Results: This study showed that *S.alata* (EESA) (100mg/kg) and Selenium (1mg/kg) showed no reduction (P>.05) in the biochemical parameters (AST, ALP, ALT, BUN, Uric acid, and creatinine); histo-anatomy of both organs showed injury. The 200mg/kg EESA/Se (1mg/kg) reduced significantly (P<.05) elevated parameters for liver only (ALT, ALP); liver histo-picture showed no lesion; 400mg/kg combination decreased all parameters significantly (P<.05); histo-pathology showed no lesion in both organs. Weights of the liver showed significant reduction (P=.0025, .0052, and .0027) at 100, 200, and 400mg/kg respectively; but no significant difference in weights of the kidney compared to the toxic group.

Conclusion: It was concluded that EESA200mg/kg with Selenium 1mg/kg can mitigate the toxic effects of Isoniazid-Rifampicin on the liver, while EESA/Se 400mg/kg combination has ameliorative potentials on liver and kidney toxicity from INH-RIF. These effects may be dose-dependent. It was recommended that 400mg/kg of EESA and 1mg/kg of Selenium combination be considered in drug development.

Keywords: Ameliorative, EESA, Selenium, Histopathology, Biochemical, Toxic

1. INTRODUCTION

Over the years, the relevance of ethnopharmacology has gained global recognition with its predominance in developing countries of the world as practiced by about 80% of the population [1,2]. Medicinal plants have been utilized for ages as cures for human ailments because they contain phytochemicals such as alkaloids, flavonoids, tannins, saponins whIch are components of therapeutic potentials [3]. These phytochemical compounds are reported to have diverse pharmacological activities like anticancer, aphrodisiac, antimalarial, antidiabetic, and abortifacient. They also serve as “lead compounds” or templates for the rational development of drugs [4].

One of the plants that has shown great medicinal potential is *Senna alata or Cassia alata* [5]. It is native to Mexico but widely dispersed in the humid and tropical regions [6]. It is of the family *Fabaceae* and is known by other names such as craw-craw plant, ringworm bush, and candle bush based on its features or usage. It has active ingredients such as anthraquinones, steroids, phenolics, terpenoids, and fatty acids that have been used in the treatment of varying diseases and conditions [7,8,5]. In African traditional medicines, they are used for hepatitis, skin disorders, jaundice, gastroenteritis, ringworm, dermatitis, and diarrhea [5].

Selenium is an important element with an antioxidant role, binding the active site of glutathione peroxidase (GSHPx). The most essential metabolic roles of Se in mammalian cells occur due to its action in the active site of selenoenzyme-GSH-Px [9]. GSH-Px not only shields cells against damage by free radicals but also promotes the regeneration of a membrane lipid molecule through reacylation [10]. Antioxidants may reduce oxidative damage induced by free radicals in biological structures. Interactive connections between antioxidants may affect the toxicity of hepatotoxic substances [11]

Drug-induced organ injury has been implicated in the treatment of tuberculosis using isoniazid and rifampicin which is usually the first line of action. The use of these drugs has been shown to have deleterious effects on the liver [12]. Chang [13] reported that acute kidney injury (AKI) could occur in some tuberculosis patients treated with rifampicin. *Senna alata* has been widely studied in the treatment of various health problems but there is a dearth of information on its use in combination with any antioxidant to mitigate liver and/or kidney injuries that could occur as a result of treating tuberculosis with isoniazid and rifampicin (INH-RIF). This is a combination study to investigate the effect of *Senna alata* and Selenium on the liver and kidneys of mice treated with isoniazid and rifampicin (INH-RIF).

2. METHODS AND MATERIALS

The study was carried out at the Department of Pharmacology and Toxicology of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. 9°02′51″N 7°20′29″E/ 9.0475°N 7.3415°E / 9.0475; 7.3415.

2.1 Plant material

About 1000g of fresh leaves of *Senna alata* were collected from Karu LGA, Nasarawa State, Nigeria. The leaves were washed by rinsing in distilled water and air-dried completely at room temperature (30 ± 2ºC), protected from heat and direct sunlight for about 2 weeks. The dried leaves were homogenized to a fine powder using a Laboratory Hammer mill (Zhen Chang Equipment SFS P66) and extraction of the powder of the leaves was done using 70% ethanol for 72 hours, and dried using a rotatory evaporator. The dried extract was transferred into air-tight glass vials and stored at 4^{o}C in a refrigerator.

2.2 Pilot Toxicity Study

The animals were administered 2000mg/kg body weight of EESA and were observed for 15 minutes, 30 minutes, 1 hour, 24 hours, and a week for signs of toxicity and mortality.

2.3 Experimental Animals

Twenty-five male Swiss mice, weighing 20g-35g were sourced from the Animal Facility center of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD). The animals were maintained under standard environmental conditions

(temperature 24 ± 2 °C) with free access to a standard rodent diet and water. The animals were acclimatized to laboratory conditions for two weeks before the studies. Ethical approval for animal experiments was granted by the Animal Care and Ethics Committee with approval number (NIPRD:05:03:05-44). .

2.3.1 Induction of toxicity in animals

The animals were grouped into 5 of 5 Swiss mice for the study.

Group 1 received distilled water daily as control, Group 2 served as negative control received (INH-RIF) (150mg/kg) orally for the first two days, Group 3 received INH-RIF (150mg/kg) for the first two days before EESA (100mg/kg) and Se (1mg/kg) on day 3 till day 14, Group 4 received INH-RIF (150mg/kg) for the first two days before EESA (200mg/kg) and Se (1mg/kg) on day 3 till day 14, Group 5 received INH-RIF (150mg/kg) for the first two days before EESA (400mg/kg) and Se (1mg/kg) on day 3 till day 14.

2.3.2 Biochemical Analysis

After each experiment, animals were anaesthesized with chloroform, and blood samples were collected from the heart into plain bottles for the determination of biochemical parameters such as Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT), Blood urea nitrogen (BUN), Creatinine (CRT), and uric acid (UA). Well-labeled plain bottles were used to collect 5 mL of the blood sample, which was allowed to clot for 4 hours before centrifuging using Uniscope Laboratory Centrifuge (Model SM 112, Surgifriend Medicals, England) at 2000 revolutions per minute for 20 minutes to separate the sera from clotted blood cells. Each serum was carefully separated into plain bottles that were well labeled accordingly at room temperature of 23-26^{o}C. The activities of AST, ALP, and ALT were estimated using a Randox test kit (UK). The blood urea was determined using urease-Berthelot (enzymatic) colorimetric method. Uric acid, and creatinine were also investigated using standard procedures.

2.3.3 Gross and Histopathology

The organs were identified (kidney and liver) and weighed, then sectioned and rinsed in normal saline. The tissues were fixed in 10% formal saline, dehydrated with 100% ethanol solution embedded in paraffin, and sectioned at 5µm, stained using the H&E method, cleared in xylene, and mounted in a mountant (14). Using the magnification of 400x.

2.4 Data analysis

The results were expressed as the mean± standard error of the mean (SEM) of 5 animals. One-way analysis of variance (ANOVA) was used to compare the means between groups. It was followed by a Dunnet test using GraphPad Prism software. P<.05 was considered statistically significant.

3. RESULTS

3.1 Pilot study

The pilot study showed that there were no signs of toxicity and mortality during the period of the study

3.2.1 Biochemical parameters of mice treated with EESA (100mg/kg) and Selenium (1mg/kg)

The 100mg/kg of EESA in combination with Se (1mg/kg) showed a significant decrease (P<.05) in ALP and ALT only and no significant difference in AST, as described in Fig 1.

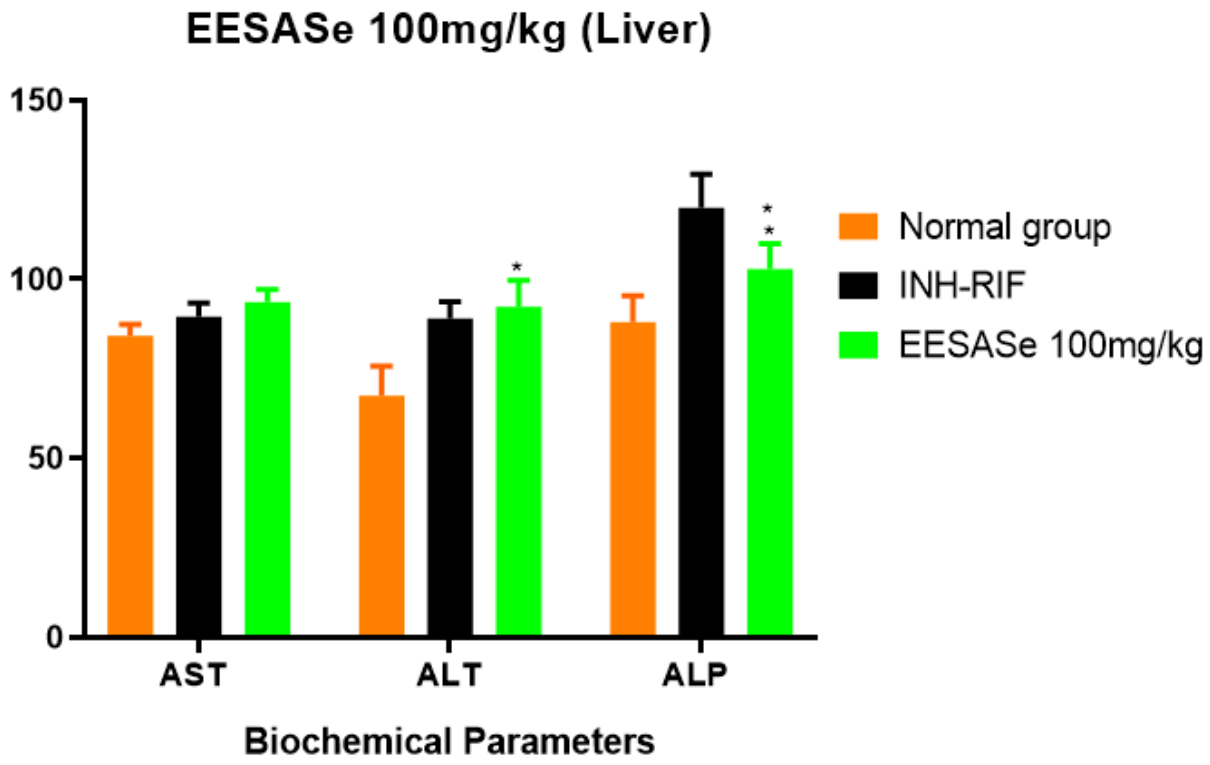


Fig 1. Effects of *S. alata* (100mg/kg) and Se (1mg/kg) on liver biomarkers of mice with liver injury induced by Isonazid-Rifampicin, INH-RIF. AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline Phosphatase, Normal control: normal untreated group.

3.2.2. Urinalysis of mice treated with of EESA (100mg/kg) and Selenium (1mg/kg).

The results of the biochemical analysis on the kidney parameters showed no significant difference in BUN, CRT, and Uric acid as shown in Fig 2.

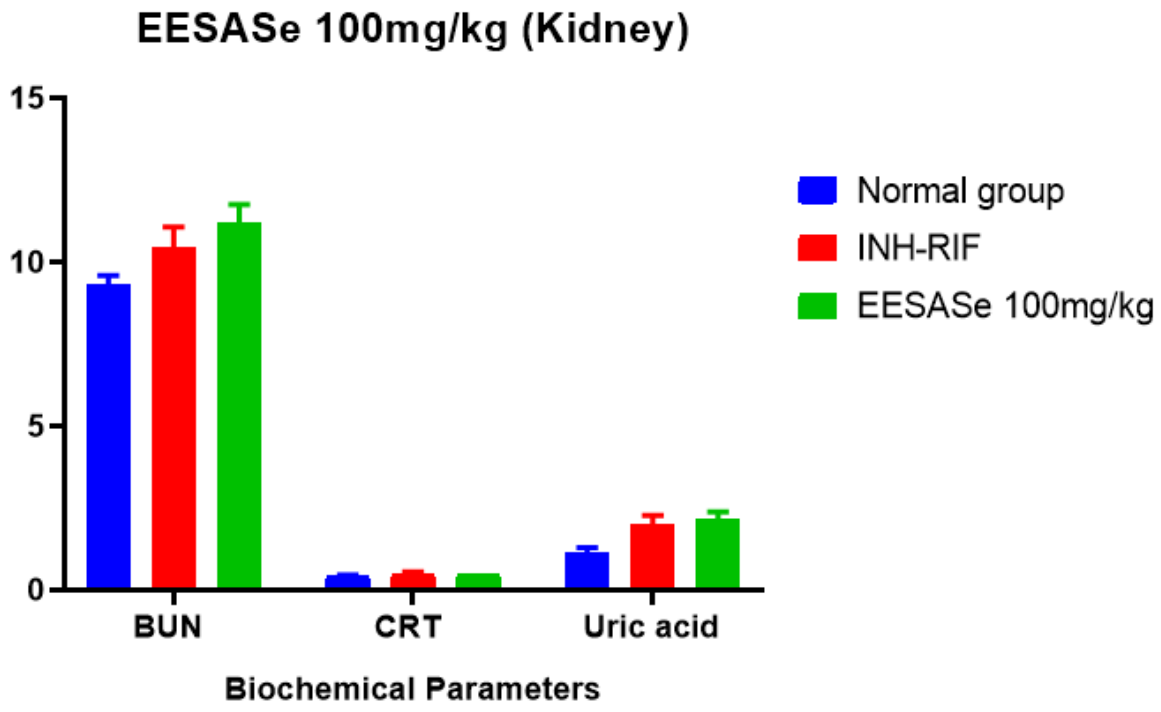


Fig 2. Effects of EESA (100ng/kg) and Selenium (1mg/kg) on the kidney injury induced by Isoniazid-Rifampicin (INH-RIF). Blood Urea Nitrogen (BUN), Creatinine (CRT), and Uric acid.

3.3.1 Biochemical parameters of mice treated with EESA 200mg/kg and Selenium (1mg/kg) on the liver

The result of EESA (200mg/kg) and Se (1mg/kg) showed a significant reduction (P<.05) in ALT and ALP, with no significant decrease in AST as described in Fig 3 below.

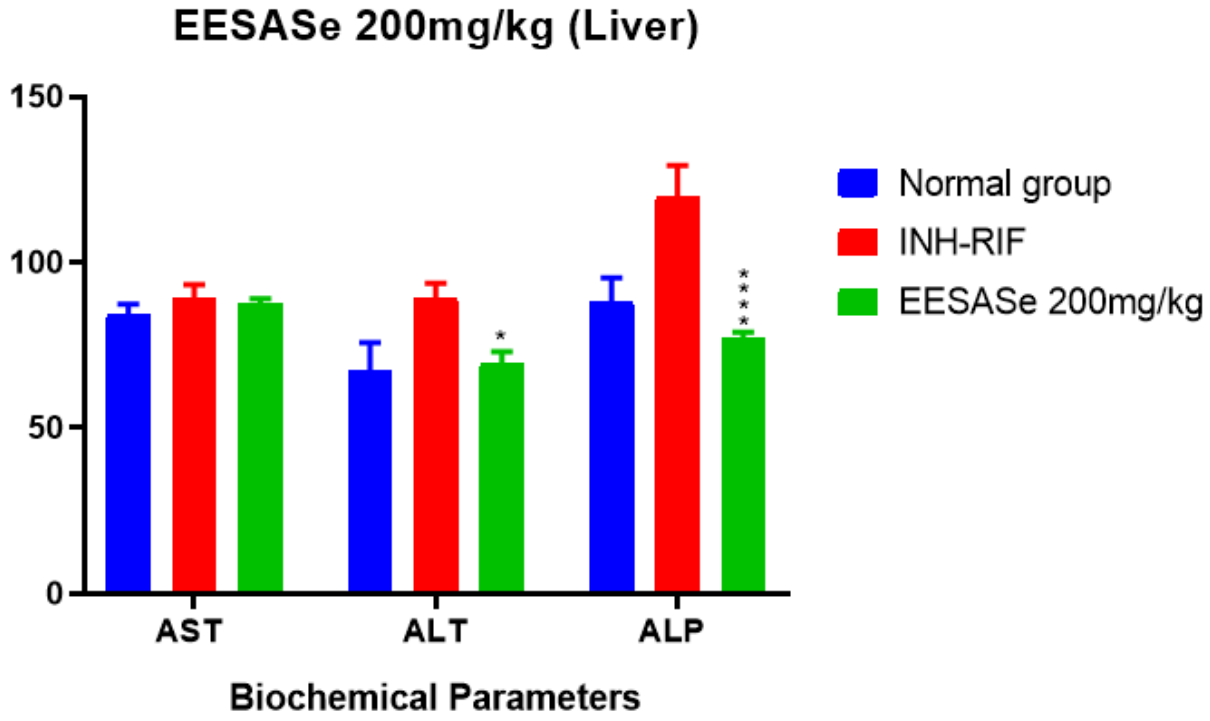


Fig 3. Effects of EESA (200mg/kg) and Selenium (1mg/kg) on the liver biomarkers of mice with liver injury from Isonazid-Rifampicin, INH-RIF. AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline Phosphatase, Normal control: normal untreated group.

3.3.2. Urinalysis of mice treated with EESA (200mg/kg) and Selenium (1mg/kg)

There was a slight decrease in the BUN and Uric acid but not significantly different when compared with the toxic group respectively. There was no significant difference in CRT when compared to the toxic group as shown in fig 4.

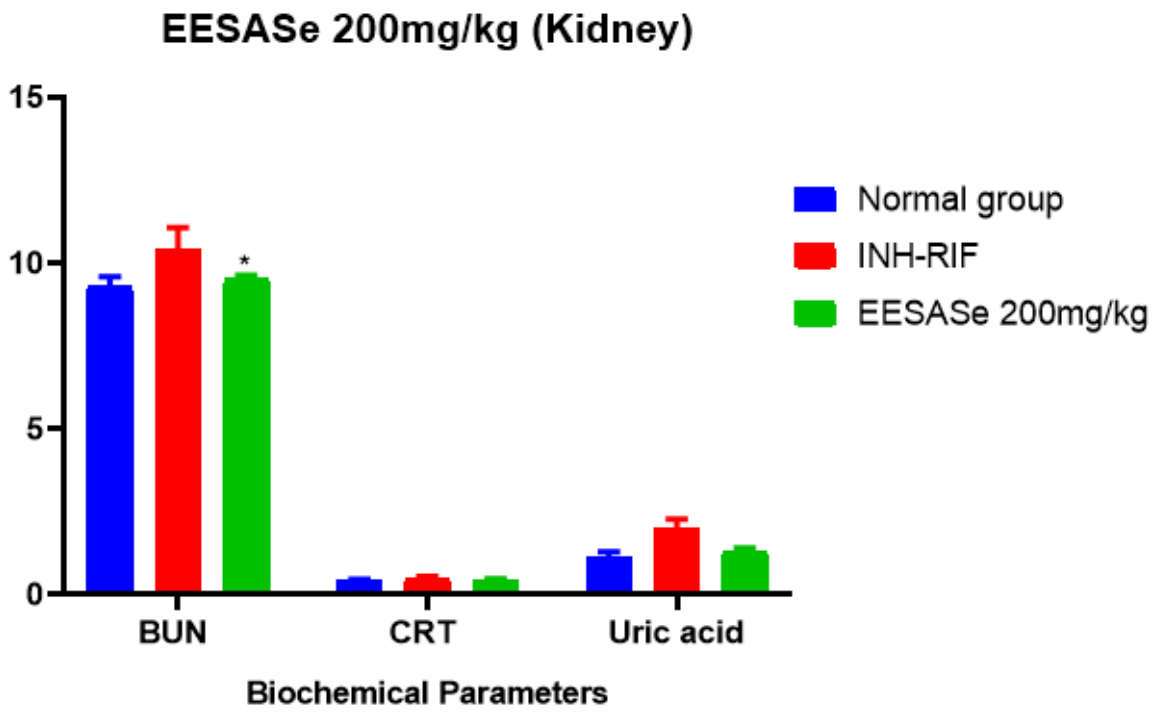


Fig 4. Effects ofEESA (200mg/kg) and Se (1mg/kg) on the kidney injury induced by Isoniazid-Rifampicin (INH-RIF). Blood Urea Nitrogen (BUN), Creatinine (CRT), and Uric acid.

3.4.1 Biochemical parameters of mice treated with EESA (400mg/kg) and Selenium (1mg/kg)

The 400mg/kg EESA and Se (1mg/kg) showed a significant decrease (P<.05) in ALT and ALP, with no significant difference (P>0.05) in AST, T.bil and D.bil when compared with toxic group as described in fig 5.

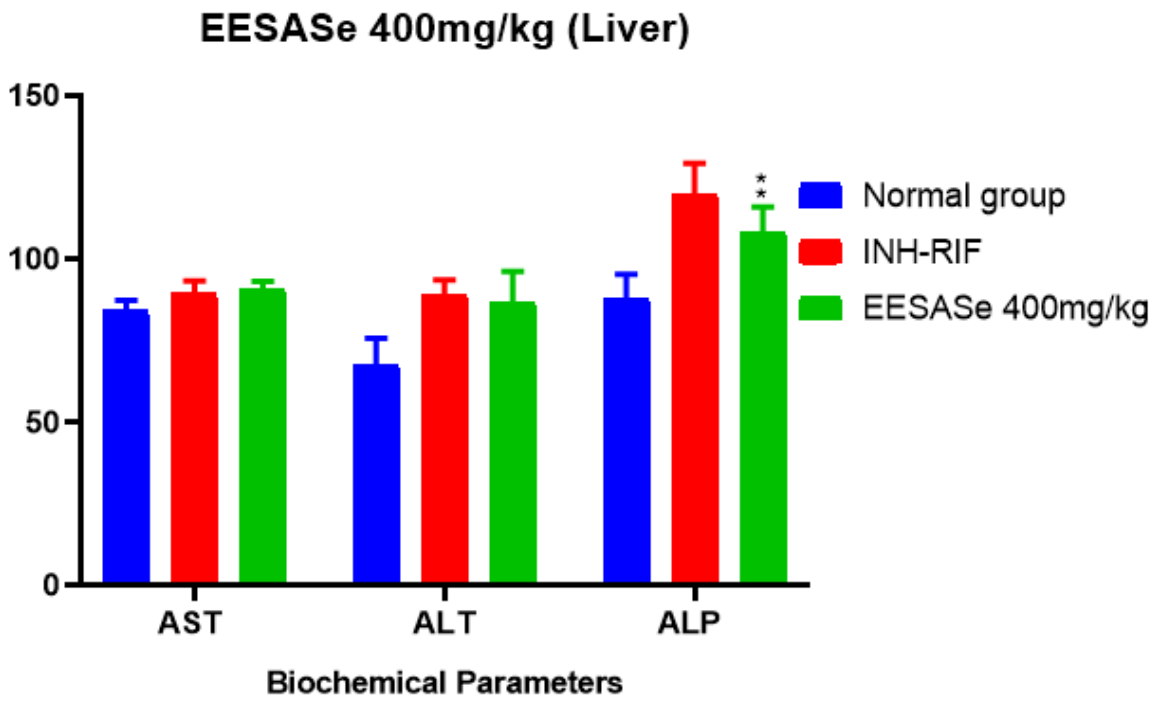


Fig 5. Effects of EESA (400mg/kg) and Selenium (1mg/kg) on the liver biomarkers of mice with liver injury induced by Isonazid-Rifampicin, INH-RIF. AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline Phosphatase, Normal control: normal untreated group.

3.4.2 Urinalysis of mice treated with EESA (400mg/kg) and Selenium (1mg/kg)

The 400mg/kg of EESA with Se (1mg/kg) showed a significant reduction ($P<.05$) in BUN and Uric acid with no significant reduction ($P>.05$) in CRT when compared to the toxic group as shown in fig 6.

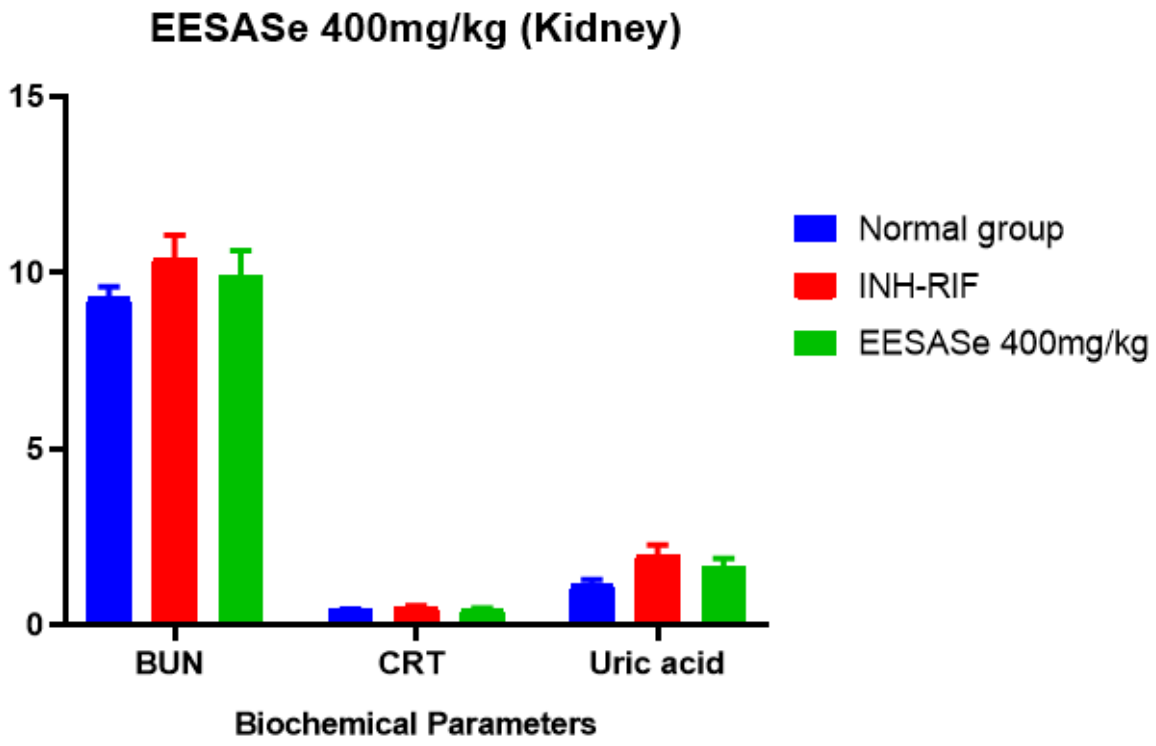


Fig 6. Effects of EESA (400mg/kg) and Selenium (1mg/kg) on the kidney injury induced by Isoniazid-Rifampicin (INH-RIF). Blood Urea Nitrogen (BUN), Creatinine (CRT), and Uric acid.

3.5 Effects of EESA and Selenium (1mg/kg) on the weight of Liver and Kidney

The weight of the liver showed a significant decrease (P=.0025, .0052, and .0027) at 100,200 and 400mg/kg respectively; but no significant difference in the weight of the kidney when compared with the toxic group

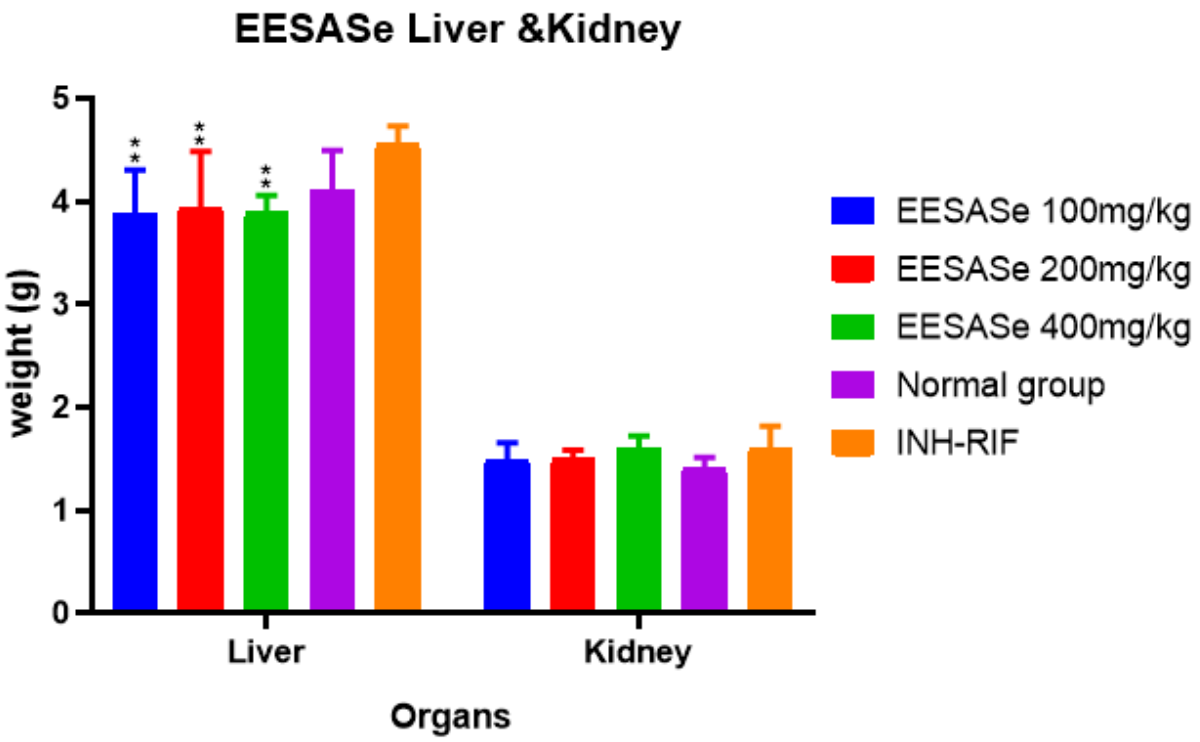
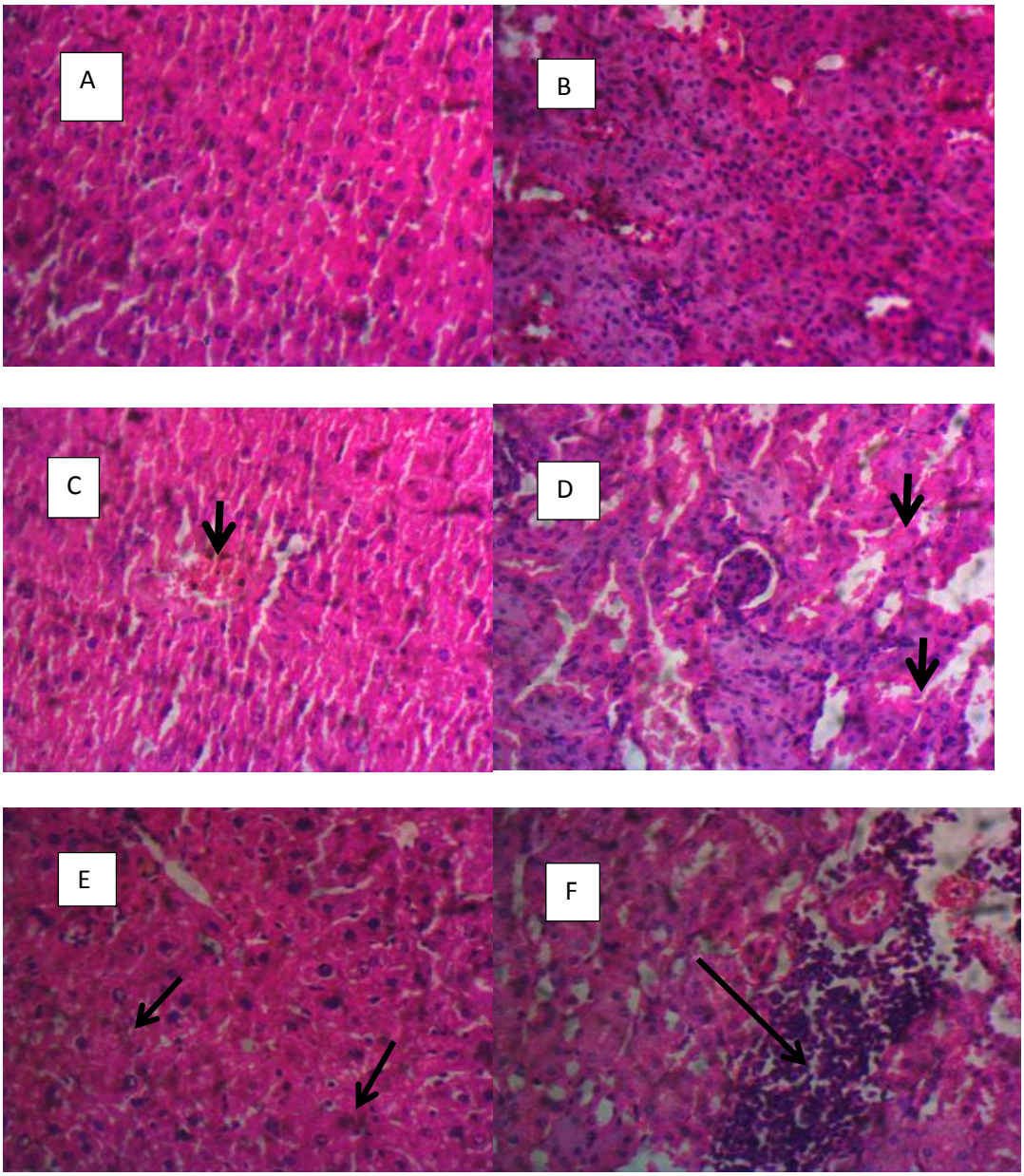
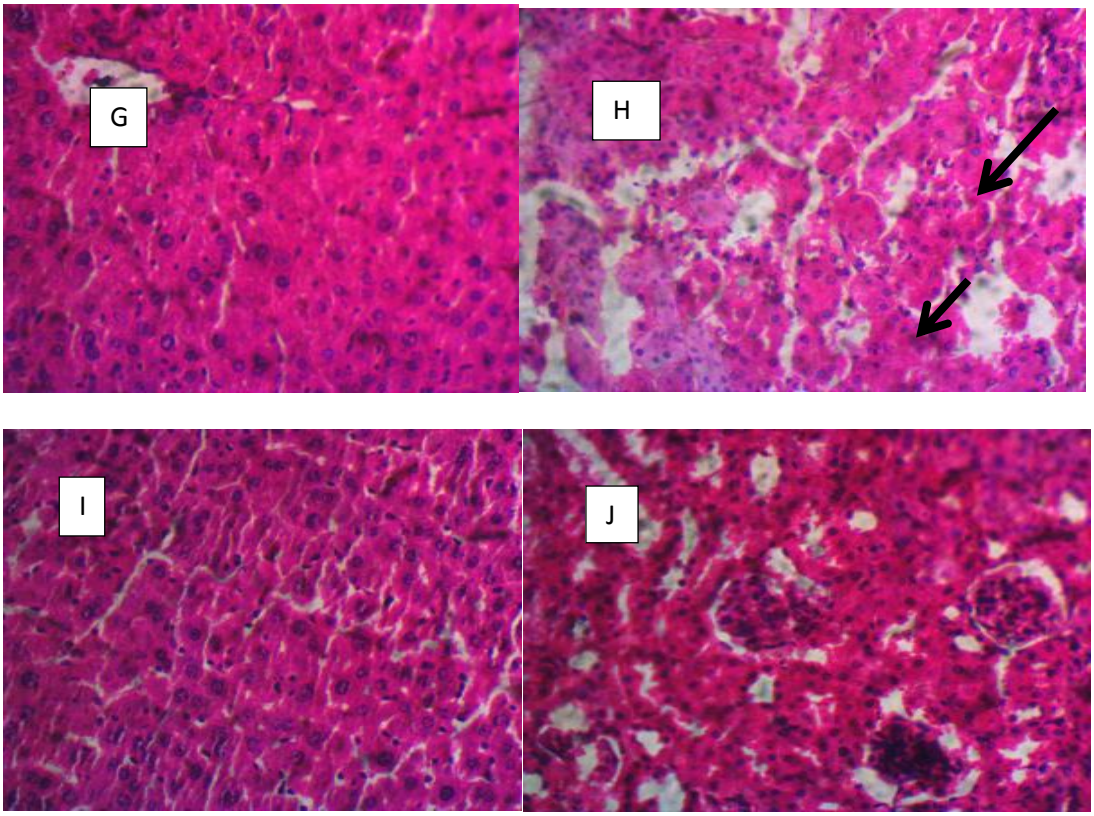


Fig 7. Effect of EESA and Selenium (1mg/kg) on the weight of the liver and kidney at 100mg/kg, 200mg/kg, and 400mg/kg

3.6 Histopathology of the liver and kidney





(A) NC: Liver- No visible lesion. (B) NC: Kidney- No visible lesion. (C) INH-RIF: Liver- There is centrilobular hepatocellular atrophy (arrow). (D) INH-RIF: Kidney- There is patchy tubular epithelial necrosis (arrows). (E) EESASe 100mg/kg: Liver- There is moderate hepatocellular atrophy (arrow). (F) EESASe 100mg/kg: Kidney-There is interstitial inflammation (arrow). (G) EESASe 200mg/kg: Liver- There is no observable lesion. (H) EESASe 200mg/kg: Kidney- There is patchy tubular epithelial necrosis (arrow). (I) EESASe 400mg/kg: Liver- There is no observable lesion. (J) EESASe 400mg/kg: Kidney- There is no observable lesion. HE stained, x400 magnification

4. DISCUSSION

There are many drugs when used for their therapeutic action tend to produce toxicity of some organs of the body [15]. The liver and the kidney by being involved in the biotransformation of drugs are often susceptible to drug toxicities [16,17]. Isoniazid and rifampicin are chemotherapeutic agents used for anti-tuberculosis effects but they are reported to be toxic [18]. It is reported thatliver toxicity due to other anti-tuberculosis drugs is increased by Rifampicin. Rifampicin activates the xeno-sensing pregnane X receptor (PXR), which is a member of the nuclear receptor, a superfamily of ligand-dependent transcription factors. In the activated promotors, PXR binds to response elements and also upregulates transcription of phase I and II enzymes that are involved in drug metabolism like glutathione S-transferases (GSTs), cytochrome P450 (CYP)s and transporters that are involved in phase III. Many metabolic enzyme pathways are induced by rifampicin via the hepatocyte PXR in particular cytochrome P450 (CYP3A4) system [19].

Hydrazine is the metabolites of isoniazid and is capable of generating free radicals that induce oxidative stress and thereby cause damage to organs or tissues [15].

This study showed that the combination of the two anti-tubercular induced liver and kidney damage due to an increase in the biochemical parameters such as ALP, AST, ALT, BUN, Creatinine, and Uric acid. The increase in serum biochemicals showed injury to the liver where the enzymes (ALT, AST, and ALP) linked out into the extracellular fluid [20]; and kidney toxicity with an increase in BUN, Creatinine, and Uric acid in the serum [21, 22].

The 100mg/kg of EESA in combination with Selenium 1mg/kg (EESASe 100mg/kg) could not reduce the elevated parameters (Fig 1 and 2), the histopathology showed injury and inflammation (Fig E and F); the lower dose and the active component which are the phytochemicals may are not enough to ameliorate the oxidative effects of the drugs. The combination at 200mg/kg, showed a significant reduction in liver parameters (Fig 3), while that of kidney biomarkers is not significant (Fig 4), the histo-picture confirms this with no lesion on the liver (Fig G) but necrosis in the kidney (Fig H); the gross anatomy also showed no significant different in the weight of the kidney compared to the toxic/anti-tubercular group (Fig 7). This dose may have an ameliorative effect on the liver due to the antioxidant effects of the phytochemicals saponins and anthraquninone in combating the toxic effects of the drugs in the liver this corresponds with the work done by Okwulu [23] which showed that 200mg/kg of *Harungana madagascariensis* with 1mg/kg of Selenium was able to ameliorate liver damage induced by Acetaminophen. EESASe 400mg/kg showed a significant decrease in the level of the elevated biomarkers for both the liver and kidney (Fig 5and 6) because no lesion was seen in either organ in the histo-anatomy (Fig I and J) and this dose showed a significant decrease in the weight of the liver, though the three doses showed no significant different in the weight of the kidney compared to the toxic group (Fig 7). This dose was able to ameliorate the toxic effects of Rifampicin and the oxidative activities of hydrazine which is the metabolite of Isoniazid. Anthraquinones are the major phytoconstituents of *S.alata* [24-27]; they inhibit reactive oxygen species, inflammation and scavenge free radicals [27], thereby ameliorating damage/injury on the liver and kidney by reducing oxidative stress through their antioxidant activities: inhibiting inflammation, cell disruption, atrophy, cell death and organ necrosis [24, 25, 26]. Previous work done by Okwulu (27) showed that 300mg/kg of *S. alata* alonerepaired the injury induced by Acetaminophen on the liver while the 100mg/kg could not and the 900mg/kg was toxic therefore the combination of EESA 200mg/kg and 400mg/kg with Selenium form synergy and increase the efficacy of the plant extract.

CONCLUSION

In conclusion, EESA(100mg/kg) and Selenium (1 mg/kg) combination could not ameliorate the toxic effects of INH-RIF in the liver, while at 200mg/kg combination elicited antioxidant effects on the liver only; and at 400mg/kg combination with Se both organs were managed. Therefore 400mg/kg of EESA and 1mg/kg of Selenium can be recommended for the management of hepato-nephrotoxicity induced by isoniazid and rifampicin in tuberculosis patients, and for drug development.

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COMPETING INTEREST

The authors have declared no competing interests exist.

AUTHORS' CONTRIBUTIONS

'AUTHORS A' and B' designed the study, Author A' performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author A' and C' MANAGED THE ANALYSES OF THE STUDY. 'AUTHORS B' AND D managed the literature searches, AND AUTHORS A' AND D' EDITED THE MANUSCRIPT. All authors read and approved the final manuscript."

ETHICAL APPROVAL

"All authors HEREBY DECLARE THAT "PRINCIPLES OF LABORATORY ANIMAL CARE" (NIH PUBLICATION NO. 85-23, REVISED 1985) WERE FOLLOWED AS WELL AS SPECIFIC NATIONAL LAWS WHERE APPLICABLE. ALL EXPERIMENTS HAVE BEEN EXAMINED AND APPROVED BY THE APPROPRIATE ETHICS COMMITTEE" WHICH WAS THE NATIONAL INSTITUTE FOR PHARMACEUTICAL RESEARCH AND DEVELOPMENT WITH APPROVAL NUMBER NIPRD (05:03:05-44).

REFERENCES

1. Ekor, M. (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontier of Pharmacology,*4:1–10.

2. WHO (World Health Organization) *Global Report on Diabetes.* Geneva, Switzerland: WHO; 2016.

3. Fatmawati, S., Purnomo, A.S., and Bakar, M.F.A. (2020). Chemical constituents, usage, and pharmacological activity of *Cassia alata*. *Heliyon,* 6 (7) 1-10.

4. Okwu U. M., Mitsan, O., Augustine, O. A., and Osazee, E. I. Methicillin-resistant Staphylococcus aureus (MRSA) and anti-MRSA activities of extracts of some medicinal plants: A brief review. *AIMS microbiology.* 2019; 5 (2) 117.

5. Seng C. T., Samuel L., Scholastica R. B., Sui S. L. In vitro, antimicrobial efficacy of *Cassia alata* (Linn) leaves stem and root extracts against cellulitis causative agent Staphylococcus aureus. *BMC complementary medicine and therapies* 2020; 23(1)1-17

6. Oladeji, O.S., Adelowo. F.E., Oluyori, A.P., Bankole, D.T. Ethnobotanical Description and Biological Activities of *Senna alata*. *Evid Based Complement Alternat Med.* 2020; 2580259. doi: 10.1155/2020/2580259.

7. Liu, A. Xu, L., Zou, Z.. Studies on chemical constituents from leaves of *Cassia alata*. *China Journal of Chinese Matters on Medicine,* 2009;34:861–863.

8. Varghese, G. K., Bose, L. V., and Habtemariam, S.. Antidiabetic components of *Cassia alata* leaves identification through *α*-glucosidase inhibition studies. *Pharmaceutical Biology.* 2013;51(3):345–349. doi: 10.3109/13880209.2012.729066.

9. Hajinezhad, M.R. Effects of Selenium Nanoparticles on Biochemical Parameters and Histopathological Changes in Lead-intoxicated Rats. *Journal of Human Environment and Health Promotion.* 2021; 7(1):35-40.

10. Bardas, E., Yusuf, K. A., Seher, P., Mine, E., and Gozde A. U. Vitamin E and Selenium reduce prednisolone side effects in rat heart. *International Journal for Vitamin and Nutrition Research* 2019*;* 90(3-4)).

11. Singh K. C., Gagan, C., Mary A. N., Liz, G-P., and Nicholas, J. M. The role of sirtuins in antioxidant and redox signaling. *Antioxidant & redox signaling* 2018;28(8), 643-661.

12. Zhuang X., Li L., Liu T., Zhang, R., Yang, P., et al. Mechanisms of isoniazid and rifampicin-induced liver injury and the effects of natural medicinal ingredients: A review. *Frontiers in Pharmacology* vol.13 DOI=10.3389/fphar.2022;1037814

13. Chang, C.H., Chen, Y.F., Wu, V.C., Shu C.C., Lee C.H., Wang J.Y., Lee L.N., and Yu C.J. (2014) Acute kidney injury due to anti-tuberculosis drugs: a five-year experience in an aging population. *BMC Infect Dis* 14, 23. https://doi.org/10.1186/1471-2334-14-23

14. Akanbi, B. O. and Taiwo, O.V. Mortality and pathology associated with highly pathogenic avian influenza H5N1 outbreak in commercial poultry production system in Nigeria. *International Scholarly Research Notices*, vol 2014,http://dx.dio.org/10.11552014/ 415418, 7 pg

15. Buabeid, M., Arafa, E-S., Rani, T., Ahmad, F., Ahmed, H., and Hassan, W. Effects of Solanum lycopersicum L.(tomato) against isoniazid and rifampicin induced hepatotoxicity in Wistar albino rats. *Brazilian Journal of Biology,* 2022;. 84:e254552.

16. Abd elhameed, M. F, Salama A.A.A., Attia, T.M., Elbatran S.A., Ismaeil, I.E.K., *et al*. Protective effects of Moringa oleifera extract on isoniazid and rifampicin induced hepatotoxicity in rats: Involvement of adiponectin and tumor necrosis factor-α. *Egyptian Journal of Veterinary Sciences,* 2018;49(1):25-34.

17. Kovesdy, C.P. Epidemiology of chronic kidney disease: an update 2022. *Kidney International Supplements.* 2022;12(1):7-11.

18. Apoorva, N., Rao, R.R., Shenoy, P.J., Sindhu, H., and Teerthanath, S. Salvia hispanica (Chia) seeds afford hepatoprotection against Isoniazid and Rifampicin induced toxicity in a murine model. *Research Journal of Pharmacy and Technology.* 2020; 13(10):4805-10

19. Ramachandra, V., Usharani, B., Aaina, M., Anbazhagi, M., and Revathi, M. Assess the diagnostic Accuracy of GeneXpert to Dectect Mycobacterium tuberculosis and Rifampicin-Resistant Tuberculosis among Presumptive Tuberculosis and Presumptive Drug Resistant Tuberculosis Patients. *Microbiology Research* 2023*;* 15(1) 91-108

20. Darvin, S.S., Esakkimuthu, S., Toppo, E., Balakrishna, K., Paulraj, M.G. Hepatoprotective effect of lawsone on rifampicin-isoniazid induced hepatotoxicity in in vitro and in vivo models. *Environmental Toxicology and Pharmacology,* 2018; 61:87-94.

21. Cohen, A., Ioannidis, K., Ehrlich, A., Regenbaum, S., Cohen, M. et al. Mechanism and reversal of drug-induced nephrotoxicity on a chip. *Science translational medicine*, 2021*;* 13(582):eabd6299.

22. Levey, A.S., Eckardt, K-U., Dorman, N.M., Christiansen, S.L., Hoorn, E.J., et al. Nomenclature for kidney function and disease: report of a Kidney Disease: Improving Global Outcomes (KDIGO) Consensus Conference. *Kidney international.* 2020;97(6):1117-29.

23. Okwulu, O.J. and Abatan, M.O. Ameliorative Effects of *Harungana madagascariensis* in combination with Antioxidants in rats. *Tropical Veterinarian* 2021; 39(2),27-43

24. Kamble, S., T. Shinge, and P. Shinde, *A Review Extraction and Standardization Techniques of Senna Leaves.* J Drug Design Discov Res, 2020. **1**(1): p. 33-35.

25. Rahim, N.A., S. Ferdosh, and M.Z.I. Sarker, *Extraction Methodologies, Phytochemical Constituents, and Biological Activities of Senna alata Linn: A Review.* The Natural Products Journal, 2023. **13**(2): p. 2-18.

26. Trung, N.Q. *Radical scavenging activity of natural anthraquinones: A theoretical insight.* ACS omega, 2021. **6**(20): p. 13391-13397.

27. Yeong, Y.L., et al., *Optimisation of microwave-assisted extraction (MAE) of anthraquinone and flavonoids from Senna alata (L.) Roxb.* Natural product research, 2022. **36**(14): p. 3756-3760.

28. Seok, P.R., Kim, J.H., Kwon, H.R., Heo, J.S., Choi, J.R., and Shin, J-H. Protective effects of *Gastrodia elata* Blume on acetaminophen-induced liver and kidney toxicity in rats. *Food science and biotechnology*.2018; 27:1445-54.

29. Barnett, L.M.A., Cummings B.S. Cellular and molecular mechanisms of kidney toxicity. Seminars in nephrology. Elsevier 2019.

30. Birkelo, B.C., Pannu, N., and Siew, E.D. Overview of diagnostic criteria and epidemiology of acute kidney injury and acute kidney disease in the critically ill patient. *Clinical Journal of the American Society of Nephrology*, 2022; 17(5):717-35.

31. Okwulu, O.J and Lawal, I. Evaluation of the Ameliorative Effects of *Phyllantu amarus* and *Senna alata* in Acetaminophen-induced Hepato-nephro toxicity in Rats. *International Journal of Biochemistry Research & Review,* 2023; 32, (7) 53-62

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7. Effect of *S.alata* and Selenium on the liver and kidney at 100mg/kg, 200mg/kg, and 400mg/kg