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EFFECT OF ETHANOLIC STEM EXTRACT OF NELSONIA CANESCENS ON SELECTED BIOCHEMICAL PARAMETERS IN MALE WISTAR RATS INDUCED WITH SODIUM ARSENITE

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Abstract

Medicinal plants are those that have curative qualities or have positive pharmacological effects on the human body. The effect of ethanolic stem extracts from Nelsonia canescens was studied in relation to Sodium arsenite-induced toxicity in wistar rats. Fresh stem extract of Nelsonia canescens were obtained behind rice mill area, Wukari, Taraba state and was shade dried at room temperature and was homogenized into powder and measured at 300g into 100ml of absolute ethanol for 72 hours. 15 healthy male rats of 70g to 90g weight were obtained from animal house Makurdi, Benue state. Animals from Group 1 were used as control. 5mg/kg body weight of Sodium arsenite was administered to Group 2 animals while animals in Groups 3, 4 and 5 were administered with Nelsonia canescens ethanolic stem extracts 50 mg/kg,

100mg/kg and 200 mg/kg as well. At the end of 3 weeks the animals were sacrificed and serum sample were collected and analysed using standard methods. The results indicate that, when compared to those who received Sodium arsenite, those who received ethanol stem extracts of Nelsonia canescens showed a comparatively considerable liver protection against Sodium arsenite -induced damage. The levels of biochemical parameters: Albumin, Total protein, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total bilirubin, Urea, Creatinine of rats administered with Sodium arsenite only was also observed. The Nelsonia canescens extract's activity at 200mg/kg bw (higher dose) give a reasonable decrease in the amount of these liver enzymes. Deducing from study results, it indicates that Nelsonia canescens leaf extracts could be an effective agent in Sodium arsenite mediated liver toxicity in adult wistar rats and drug development.

Keywords: Medicinal plants, Nelsonia canescens, Sodium arsenite-induced toxicity, Wistar rats, Liver protection, Biochemical parameters

INTRODUCTION

Medicinal plants and herbs contain substances known to modern and ancient civilizations for their healing properties. A number of plants have been used in traditional medicine for many years; some do seem to work, although there may not be sufficient scientific data to confirm their efficacy (Yakubu *et al.*, 2022)[a]. The medicinal value of these plants lies in some chemical substances they contain, that produce definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Yan *et al.*, 2004). Plant derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs (Yakubu *et al.*, 2022)[b].

Traditional medicines include practices, such as herbal medicine, ayurvedic medicine, unani medicine, acupuncture, as well as other medical knowledge and practices (not orthodox) all over the globe. Inappropriate use of traditional medicines or practices can have negative or

dangerous effects and so, further research is needed to ascertain the efficacy and safety of the medicinal plants used in traditional medicine system (David *et al*., 2014).

Therefore, this study aimed at determining the effect of ethanolic stem extract of *Nelsonia canescens* on selected biochemical parameters in male wistar rats induced with Sodium arsenite.

METHODS

Sample collection

Fresh stem of *Nelsonia canescens* were obtained behind rice mill area, Wukari, Taraba state.

Animal collection

Fifteen (15) healthy male wistar rats of 6 to 8 weeks old with the weight range of 70g to 90g were obtained from an animal house in Wukari, Taraba state and used for the study.

Sample preparation

The stem of *Nelsonia canescens* was shed dried for three (3) weeks and pounded into powder. Plant isolation and standardization extraction of *Nelsonia canescens* stem 300g of stem powder was weighed using weighing balance put in a glass bottle which was extracted using 1200ml of 70% ethanol using measuring cylinder then the sample was stirred every two hours for 3 days, filtering of sample using filter paper then evaporating of ethanol using glass plate to get the actual yield of the plant sample.

Study Area

This study was conducted in Wukari situated on longitude 9° 47'E and latitude 7° 51'N in Taraba State, Northeastern Nigeria. The vegetation of the area is predominantly characteristics of savannah zone and with major climatic seasons of wet or rainy seasons, which starts in March or April, and ends in October and the dry season, which starts in November and ends in March or April. Wukari covers an area of 4,308 km^2 and with a population of about 241,546 at the 2006 census, traditional state rich with various cultures, norms and value. Fishing, farming and trading are the major occupation of the people.

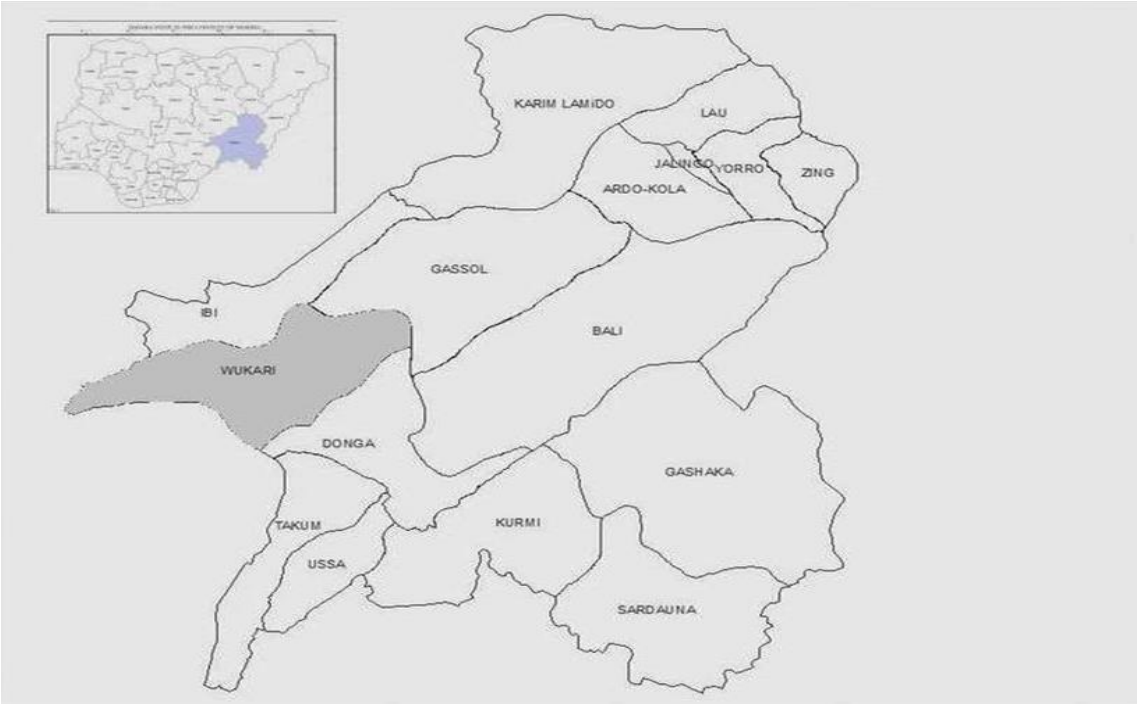

Figure 3: Map of Nigeria and Taraba showing the location of Wukari, study area.

Animals grouping

Table 1: Treatment for the experimental rats

Group	No. of Rats	Treatment
1	3	Only water
2	3	SA 5mg/kg + water
3	3	SA 5mg/kg + *N.C* 50g/kg body weight
4	3	SA 5mg/kg + *N.C* 100g/kg body weight
5	3	SA 5mg/kg + *N.C* 200g/kg body weight

Key: SA= Sodium arsenite; *N.C*= *Nelsonia canescen*

The treatment continued for 21 days

Biochemical parameters

Liver function test; Serum aspartate transaminase (AST), alanine transaminase (ALT) activities, alkaline phosphatase (ALP), Total protein, Albumin and total bilirubin and renal

function tests; urea and creatinine were analysed in the serum The measurements of these parameters were carried out by spectrophotometric determination of their absorbance's using analytical grade laboratory reagent kits from HORIBA ABX SAS (Parc Euromedecen-Rue du caducee, France) and by Pentra C400 automated clinical chemistry analyzer. All biochemical analysis for this study was according to manufactures protocol (Tsegay *et al.*, 2020).

Methods of Phytochemical Screening

The freshly prepared crude extract was quantitatively tested for the presence of biochemical constituents as described by Ihenetu *et al.* (2020).

Screening for Alkaloids: 20ml of aqueous extract was added to 4ml of HCl. To this acidic medium; 2ml of Wagner's reagent was added. A reddish brown precipitate showed the existence of alkaloids.

Screening for Glycosides: To a small quantity of the extract, 2ml of Fehling's solution was added and heated, orange precipitate showed the presence of glycoside.

Screening for Flavonoids: To 2ml of the extracts, a few drops of sodium hydroxide solution were added. Disposition of deep yellow colour, which turns into colourless on addition of dilute hydrochloric acid, signifies the occurrence of flavonoids.

Screening for Terpenoids: To 2ml of the extracts was treated with chloroform, acetic anhydride and drops of sulphuric acid was supplemented, the formation of dark green colour specified the existence of terpenoids.

Screening for Steroids: 2ml of the extract was diluted with chloroform, acetic anhydride and drops of sulphuric acid was added and dark pink colouration indicates the existence of steroids.

Screening for Saponins: 2ml of the extract was diluted with 40ml of decontaminated water and it was agitated in a graduated cylinder for 20mins. The disposition of 1cm layer of foam showed the presence of saponins**.**

Screening for Phenolic Compounds: 2ml of the extract was taken discretely in water and tested for the incidence of phenolic compound with dilute ferric chloride solution. Violet colour showed the presence of phenolic components.

Screening for Tannins: 2ml of the extract was treated with acetic acid solution and it was discerned for the formation of red colour solution.

Statistical analysis

Data collected was subjected to one-way analysis of variance using graphpad prism version 8.0 and mean separation was done using Fisher LSD.

RESULTS

Effect of *Nelsonia canescens* stem extracts on albumin levels in male wistar rats.

Result of the effect of *Nelsonia canescens* stem extracts on albumin levels in male wistar rats (Fig. 1) showed that there was a statistical significantly increased of albumin in arsenite, 50mg/kg, 100mg/kg and 200mg/kg *Nelsonia canescens* stem extracts groups when compared to the control group at P≤0.05. 50mg/kg of the extract showed the least amount of albumin, while the highest amount was found in 200mg/kg of the extracts.

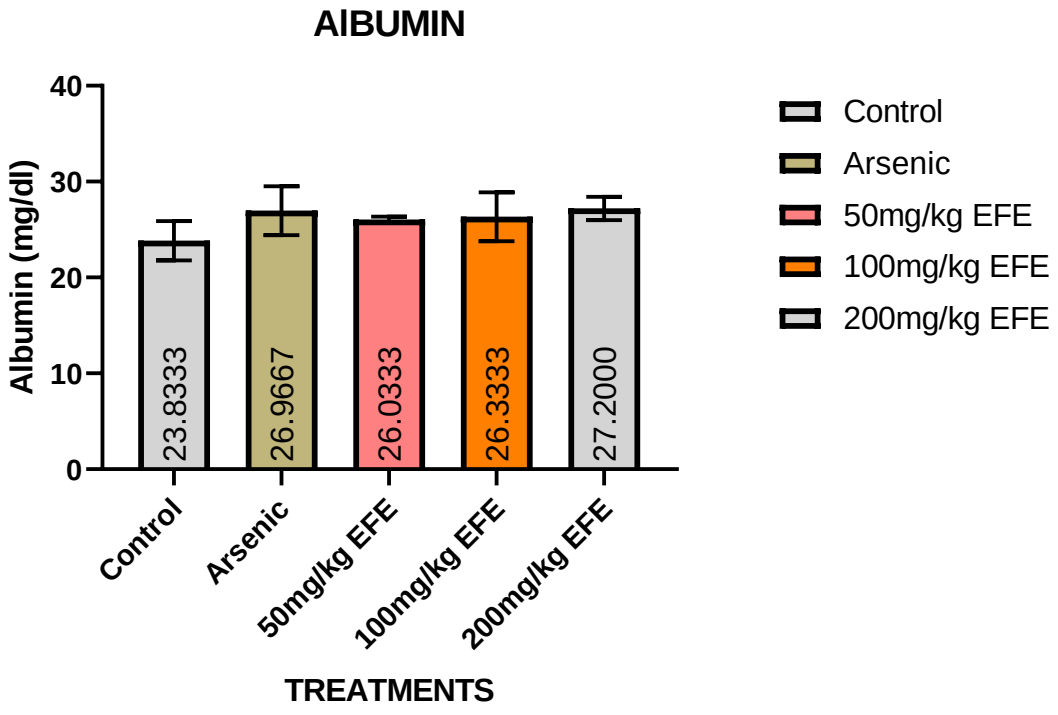

FIG. 1: A chart showing Albumin levels against treatments.

Key: EFE= Stem extract of *Nelsonia canescens*

Effect of *Nelsonia canescens* stem extracts on total protein levels in male wistar rats.

Result of the effect of *Nelsonia canescens* stem extracts on total protein levels in male wistar rats (Fig. 2) revealed that there was an appreciable increased in the level of total protein in arsenite group and all the *Nelsonia canescens* stem extracts groups when compared to the control. The arsenite group showed no statistical significant difference (P≥0.05) with the 50mg/kg and 200mg/kg *Nelsonia canescens* stem extracts groups, while 100mg/kg *Nelsonia canescens* stem extracts group showed statistical significant difference (P≤0.05) .

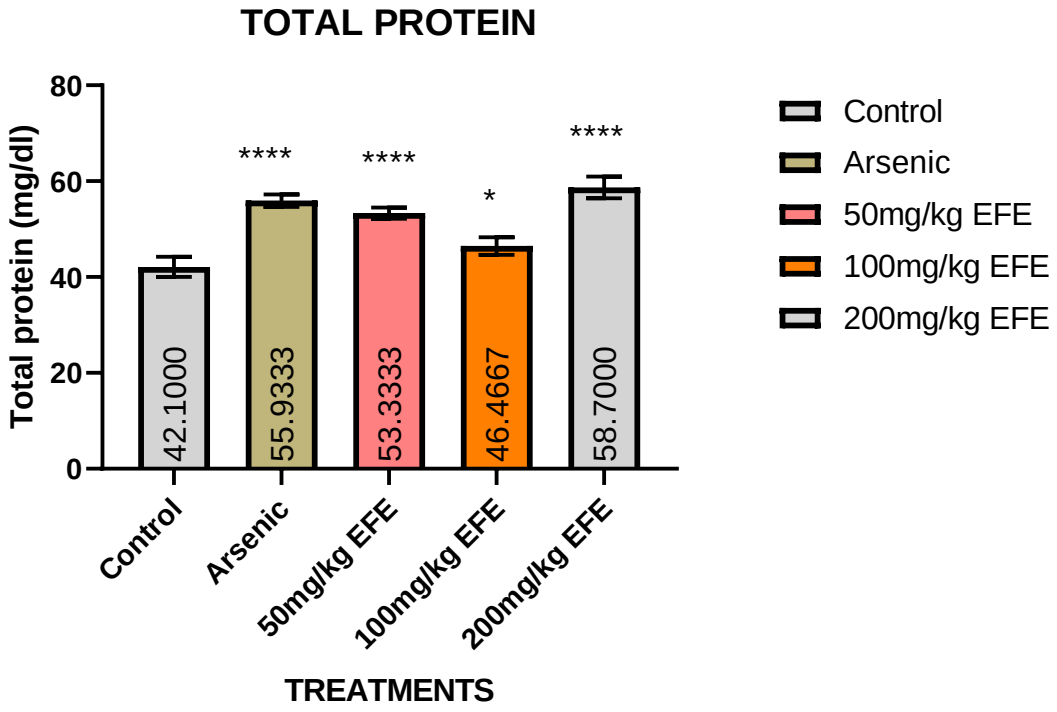

FIG. 2: A chart showing total protein levels against treatments.

Key: EFE= Stem extract of *Nelsonia canescens*

Effect of *Nelsonia canescens* stem extracts on ALT levels in male wistar rats.

Figure 3 showed an increased in ALT concentration in 50mg/kg and 100mg/kg *Nelsonia canescens* stem extracts groups above the control group and decreased in ALT concentration in arsenite and 200mg/kg *Nelsonia canescens* stem extract group below the control group. Statistically, there was no significant difference (P≥0.05) in the ALT

concentrations in arsenite, 50mg/kg *Nelsonia canescens* stem extract and 100mg/kg *Nelsonia canescens* stem extract groups and the 200mg/kg showed statistical difference (P≤0.05) with other treatment group and arsenite.

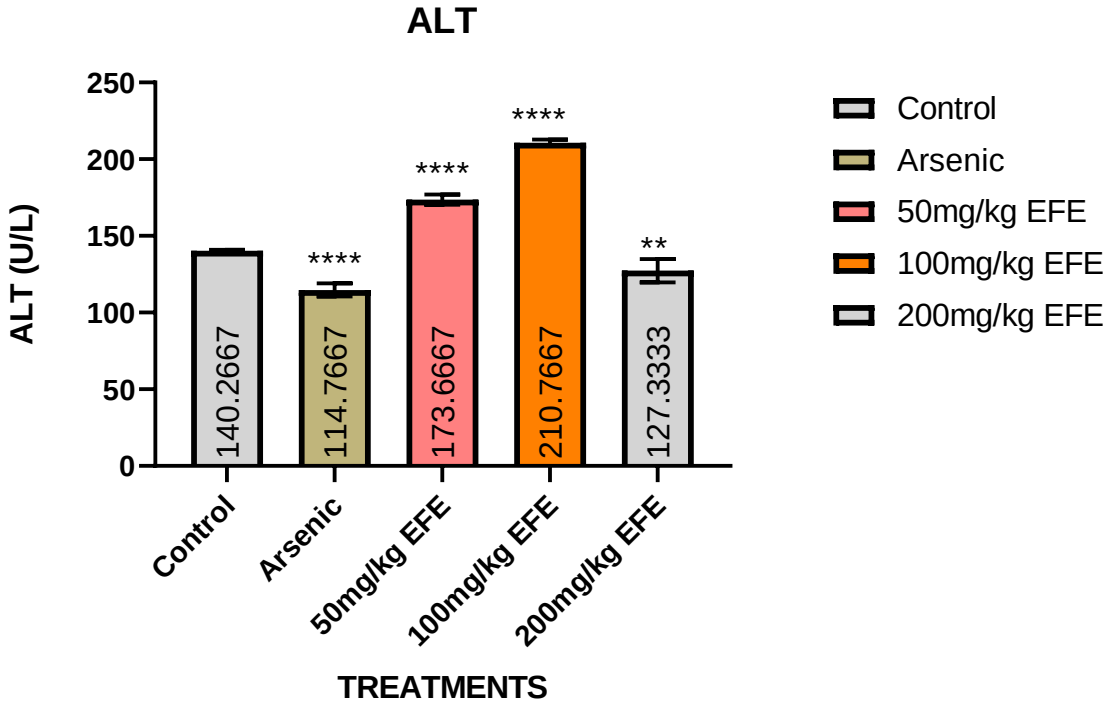

FIG. 3: A chart showing ALT levels against treatments.

Key: EFE= Stem extract of *Nelsonia canescens*

Effect of *Nelsonia canescens* stem extracts on AST levels in male wistar rats.

Result of the effect of *Nelsonia canescens* stem extracts on AST levels in male wistar rats (Fig. 4) revealed an increased in AST concentration in 100mg/kg and 200mg/kg *Nelsonia canescens* stem extracts group above control group, while a decreased in AST concentration in arsenite and 50mg/kg *Nelsonia canescens* stem extract group was observed. Statistically, there was no significant difference (P≥0.05) in AST concentration in 100mg/kg and 200mg/kg *Nelsonia canescens* stem extracts groups, while statistical significant difference (P≤0.05) of AST concentration was observed in the arsenite group.

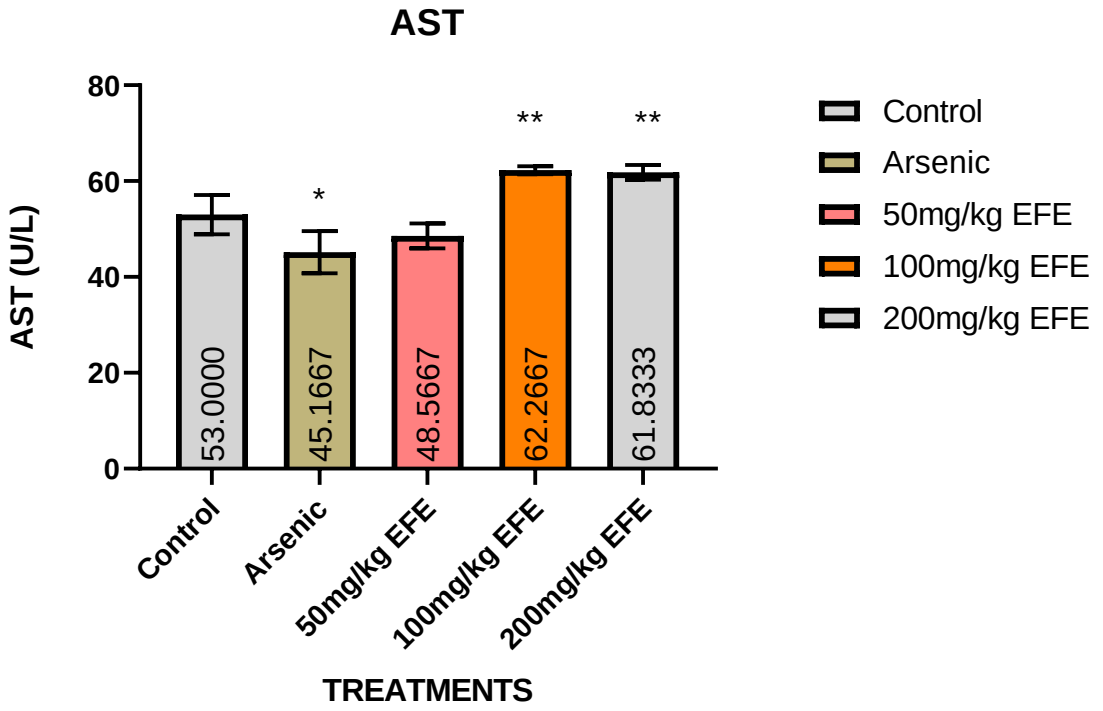

FIG. 4: A chart showing AST levels against treatments.

Key: EFE= Stem extract of *Nelsonia canescens*

Effect of *Nelsonia canescens* stem extracts on ALP levels in male wistar rats.

Figure 5 showed that the ALP concentrations in arsenite group and all the administered *Nelsonia canescens* stem extract groups were significantly increased ($P \leq 0.05$) above control group. Although, there was no statistical difference ($P \geq 0.05$) in ALP concentrations in arsenite and all *Nelsonia canescens* stem extract groups but a lower concentration of ALP was observed in 100mg/kg *Nelsonia canescens* stem extract group when compared to others.

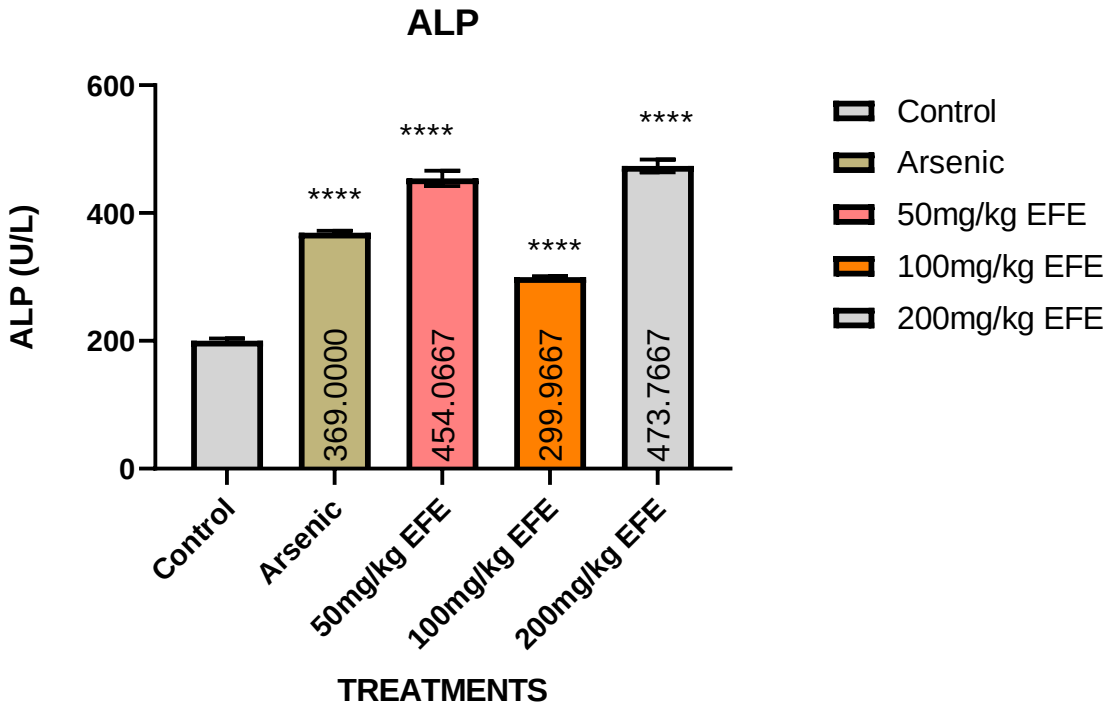

FIG. 5: A chart showing ALP levels against treatments.

Key: EFE= Stem extract of *Nelsonia canescens*

Effect of *Nelsonia canescens* stem extracts on total bilirubin levels in male wistar rats.

Result of the effect of *Nelsonia canescens* stem extracts on total bilirubin levels in male wistar rats (Fig. 6) revealed increased in total bilirubin concentrations in arsenite, 50mg/kg *Nelsonia canescens* stem extract and 200mg/kg *Nelsonia canescens* stem extract groups above control group. There was equal amount of total bilirubin concentration in 100mg/kg *Nelsonia canescens* stem extract group with the control group.

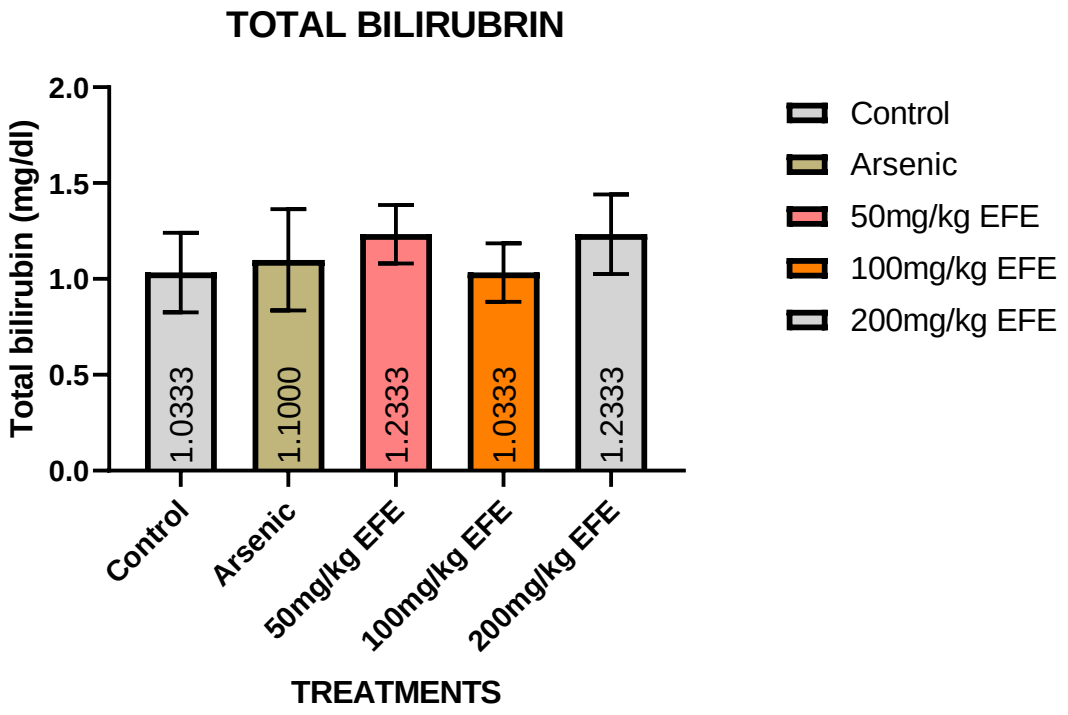

FIG. 6: A chart showing bilirubin levels against treatments.

Key: EFE= Stem extract of *Nelsonia canescens*

Effect of *Nelsonia canescens* stem extracts on urea levels in male wistar rats.

Figure 7 showed that the amount of urea was analysed to be higher in arsenite and all the administered *Nelsonia canescens* stem extract groups above the normal control group. There was no significant difference ($P \geq 0.05$) observed in the amount of urea in 100mg/kg and 200mg/kg *Nelsonia canescens* stem extract groups.

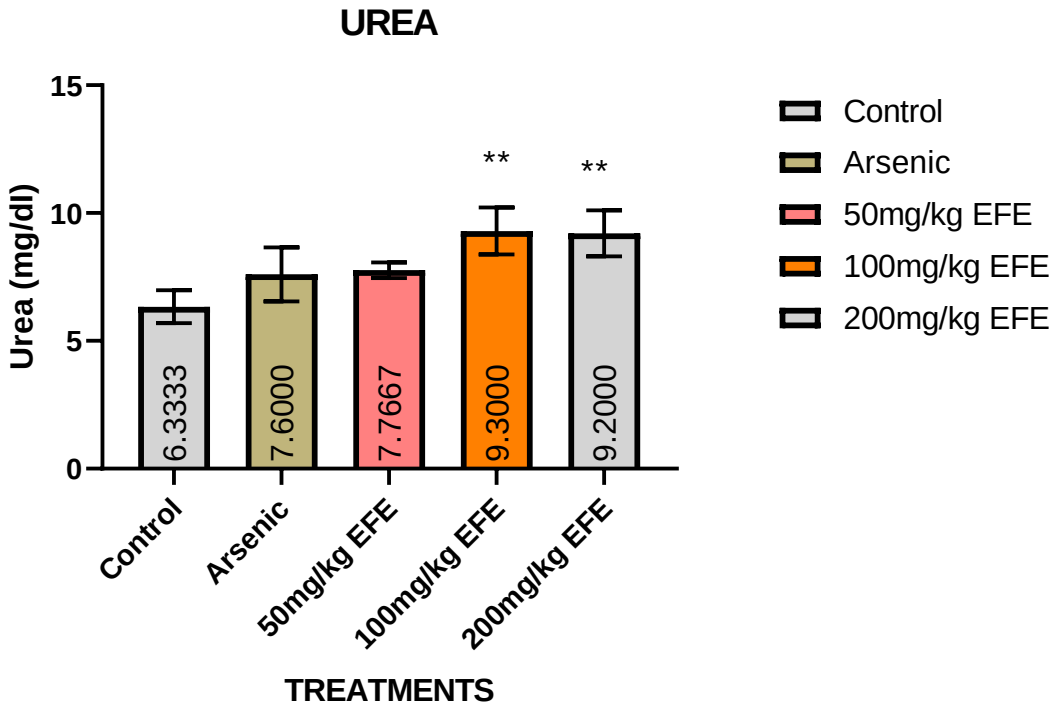

FIG. 7: A chart showing urea levels against treatments.

Key: EFE= Stem extract of *Nelsonia canescens*

Effect of *Nelsonia canescens* stem extracts on creatinine levels in male wistar rats.

Figure 8 showed an appreciable increased in the amount of creatinine arsenite and all *Nelsonia canescens* stem extract administered groups above the control group. Statistically, there was no significant difference (P≥0.05) in the amount of creatinine in arsenite and 50mg/kg *Nelsonia canescens* stem extract groups. The 100mg/kg *Nelsonia canescens* stem extract group showed significant difference (P≤0.05) with 200mg/kg *Nelsonia canescens* stem extract group and also with arsenite and 50mg/kg *Nelsonia canescens* stem extract groups.

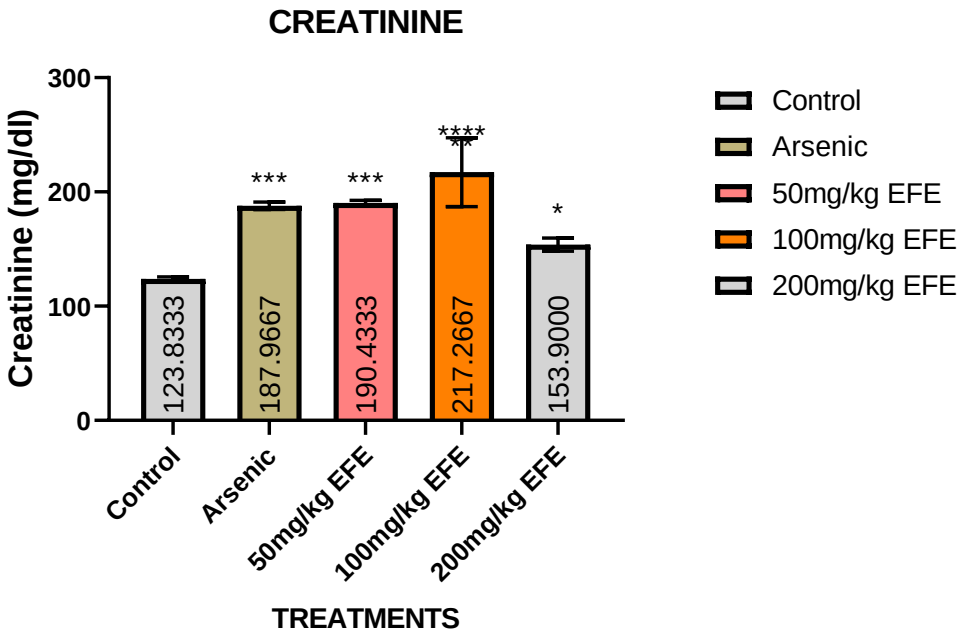

FIG. 8: A chart showing creatinine levels against treatments.

Key: EFE= Stem extract of *Nelsonia canescens*

Qualitative phytochemicals analysis of *Nelsonia canescens* stem extracts.

Table 1 showed the phytochemicals composition of *Nelsonia canescens* stem extracts. From the table it was clear that a lot of active biochemical compounds like alkaloids, flavonoids, phenols, tannins, saponins, and steroids were present in the ethanolic stem extracts of *Nelsonia canescens* whereas terpenoids and glycosides were absent.

Table 1: Qualitative phytochemicals analysis

Phytochemicals	Status
Saponins	+
Tannins	+
Steroids	+
Alkanoids	+
Flavonoids	+
Terpenoids	-
Glycosides	-
Phenols	+

KEYS: + Present - Absent

DISCUSSION

This study aim was to determine the effect of ethanolic stem extract of *Nelsonia canescens* on selected biochemical parameters in male wistar rats induced with Sodium arsenite ($NaAsO_2$). The liver enzymes ALT, AST, and ALP are often low under normal control. The leaking of enzymes into the bloodstream and the resulting damage to the liver cells constitutes an inflammation of the hepatic cells. Small changes in the permeability and transport capacity of the hepatocytes' lipid membrane brought on by oxidation make it easier for enzymes to leave the cells (Yakubu *et al*., 2022)[b]. The release of these enzymes and their increased activity in experimental animals exposed to sodium arsenite may be due to liver cell death and a change in membrane permeability. The *Nelsonia canescens* extracts' ability to stabilize membranes and so limit intracellular enzyme leakage may be responsible for the reversed elevation of serum enzymes seen in sodium arsenite liver cell injury. This study's finding that *Nelsonia canescens* extract has a protective effect.

According to this research findings, *Nelsonia canescens* stem extracts taken at a dose of 200 mg/kg appear to show elevated levels of albumin than doses given at 100mg/kg and 50mg/kg respectively. Albumin levels were seen to have significant increased ($P \leq 0.05$) in all *Nelsonia canescens* stem extracts groups compare to the control group. This resultant increased in the albumin levels by the plant extract is in conformation with a research conducted by Yakubu *et al*., (2022)[a] which showed that plant extracts poses the ability to elevate the levels of albumin. The levels of albumin in arsenite group were higher when compare to Control, 50mg/kg dose of extract and 100mg/kg dose of extract, whereas the 200mg/kg dose of extract shows higher levels of albumin than the arsenic poisoned group. Elevated levels of total protein were observed in 200mg/kg dose of extract, this value is higher than other treatment groups, control group and the arsenic poison group. *Nelsonia canescens* stem extract tends to have more efficient in elevating albumin and total protein levels at 200mg/kg dose.

The extract at 100mg/kg dose show less efficiency with ALT and AST levels higher when compared to the other treatment groups. Arsenite group reduces the ALT and AST levels below the control sample with extract dose of 200mg/kg following similar pattern. These lower levels of ALT and AST levels in 200mg/kg dose of extract could be attributed that the extract is more efficient in ameliorating the damage caused by the arsenite. 200mg/kg dose of extract tends to increase the levels of ALP more than the other treatment groups.

Although, all the treatment groups and arsenite group show significant elevated levels of ALP compared to control groups; the extract at 100mg/kg dose were observed to be the most efficient dosage as it show a reversal of the elevated level of ALP caused by the arsenite. This research result for ALT, AST and ALP is in agreement with research conducted by Yan *et al.* (2004), Vagvala and O'Connor, (2018) which stated that when sodium arsenite or any poisonous chemical is administered to healthy rats, the serum levels of ALT, AST and ALP rise.

Total bilirubin was found to be more when arsenite poisoned wistar rats were administered with extract dose of 50mg/kg and 200mg/kg. The *Nelsonia canescens* stem extracts administered groups and arsenite group were observed to show significant elevated levels of total bilirubin when compared to the control group. The extract is observed to show efficient ability in liver repair at dose of 100mg/kg. At 200mg/kg dose of the extract it was observed to show elevated levels of bilirubin above the 100mg/kg, this could be attributed that at that dose or concentration the stem extract tends to exhibit some levels of toxicity rather than ameliorating effects.

The kidney helps maintain the body's homeostasis by reabsorbing important material and excreting waste products. Creatinine is a breakdown waste product formed in the muscle by creatinine phosphate metabolism. Creatinine is synthesized in the liver, passes into the circulation, and takes up almost entirely by skeletal muscle for energy production. Creatinine retention in the blood is evidence of kidney impairment (Yakubu *et al.*, 2022)[a].

Creatinine levels were significantly elevated in all treatment groups and arsenite group when compared to the control group. The reversal of the creatinine levels as a result of arsenite poisoning was observed to be more efficient at 200mg/kg dose of the extract. It was observed that at 100mg/kg dose of the extract the creatinine level was more elevated even above the arsenite group.

Urea is the main end product of protein catabolism. Amino acid deamination takes place in the liver, which is also the site of the urea cycle, where ammonia is converted into urea and excreted through urine (David *et al.*, 2014). It represents 90% of the total urinary nitrogen excretion. Urea varies directly with protein intake and inversely with the rate of excretion. Renal diseases that diminish urea's glomerular filtration rate will lead to its retention in the blood (David *et al.*, 2012). *Nelsonia canescens* stem extract showed highest efficient ability in ameliorating liver damage at dose 50mg/kg with a reversal of urea level when compared

with other treatment groups. Generally, *Nelsonia canescens* stem extracts showed potentials of having ameliorating effects in treatment of liver and kidney related diseases poised by arsenite poisoning. At 200mg/kg dose of the plant extract, it was observed to possess the most efficacy ability.

Plants are exhibit medicinal abilities due to its bioactive components or agents. The analysis of the *Nelsonia canescens* stem extracts revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins and steroids. Each of these bioactive compounds play a key role in contributing to the plant medicinal values such as; Flavonoids: possess antioxidant, antimicrobial and anti-inflammatory activities; Alkaloids possess antimicrobial and antioxidant properties; Terpenoids are well known for their anticancer and anti-inflammatory properties and Phenolic compounds exhibit strong antioxidant and anticancer activities. *Nelsonia canescens* stem extracts in overall show a wide range of bioactive compounds that have potentials for various pharmaceutical and medicinal applications (Owoyele *et al*., 2016).

CONCLUSION

The results of the experiments clearly supported the idea that *Nelsonia canescens* may be employed as a source for ameliorating liver or kidney problems or damages caused by arsenic poisoning. The study's findings suggest that *Nelsonia canescens* may be a useful anti-poisoning agent in adult wistar rats with liver damage caused by sodium arsenite. More specifically, the possible health benefits of *Nelsonia canescens* can be used to advance the creation of contemporary medicines, namely contemporary anti-poisoning medicines.

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