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Total Protein, Bilirubin and AST Levels in Rat Models Treated with Ethanolic Extract of *Eleusine coracana* during Arsenic Trioxide Induced Hepatotoxicity

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Authors' contributions

This work was carried out in collaboration among all authors. Author OMO designed the study and wrote the protocol. Authors BJO and NJ managed the analyses of the study. Author NOA performed statistical analysis, managed the Literature search and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Eleusine coracana is considered one of the most nutritious cereals. It has different names in local languages. It is known as Ragi in Telugu and Kannada/aariyam in Tamil, and Madua in Hindi and in Nigeria, it is known as Okababa in Yoruba, Dawa in Hausa, etc. This study sought to investigate the protective ability of ethanolic extracts of *Eleusine coracana* in Arsenic trioxide induced hepatotoxicity using rat models. Animals were grouped into four (4). Group A received only distilled water, in group B,C and D hepatotoxicity was induced using 5 mg/dl Arsenic Trioxide solution for 14 days, followed by treatment in group C and D daily with 200 mg and 500 mg per kg body weight respectively for 14 days, and changes in body weight and Liver Function parameters were

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determined. Eleusine coracana contains Tannins, phlobatannins,Falavonoids and Terpenoids but not Steriods and Saponins, Eleusine treated groups had a significant decrease in the organ-body weight index. The mean weight and Total Protein was significantly reduced in the intoxicated-untreated group (group B). The AST, direct and total Bilirubin level was significantly higher in group B compared to control and other treated groups. *Eleusine cocarna* as a plant and source of food contains certain phytochemicals which are capable of managing hepatic cell injury, this serve as a point for pharmacological intervention.

Keywords: Eleusine coracana; hepatoprotective; liver function test; finger millet; Dawa; Okababa; acute; hepatotoxicity.

1. BACKGROUND

Eleusine coracana is an annual plant widely grown as a cereal in the arid area of Africa and Asia [1]. Despite its importance as a food crop, many policy makers in countries that grow finger millet generally regard it as a poor person's crop, and the scientific community has largely ignored it. Many farmers are giving up growing the labor-intensive *eleusine coracana* in favor of maize, sorghum, and cassava [2]. The plant is high in iron, calcium, fibre, starch and is considered “superior” to wheat in that its proteins are more easily digested, contains mainly unsaturated fatty acids [1]. *Eleusine coracana* is especially valuable as it contains the amino acid methionine, which is lacking in the diets of hundreds of millions of the poor who live on starchy staple such as cassava, polished rice, or maize meal [3]. It is easy to digest and does not contain gluten; people who are sensitive to gluten can easily consume the plant [4]. The incidence of arsenic contamination of ground water used for both irrigation as well as for human consumption or industrial activities has taken the dimension of an epidemiological problem. It has been established that inorganic arsenic is extremely toxic both acute and chronic. Humans are now unavoidably exposed to this element. Medicine used for remedy of arsenicosis has been found to be unsatisfactory by repeated application and experience [5]. Arsenic is a protoplastic poison due to its effect on sulphydryl group of cells interfering with cells enzymes, cell respiration and mitosis and it is one of the major cause of hepatic cell injury [6]. The hepatic tissue is considered the hub of metabolism, because it functions in the metabolism and excretion of almost all substances that go into the body (drug and nutrients). Liver cell injury can be caused by various toxicants, such as Arsenic trioxide, carbon tetrachloride (CCl_4), thioacetamide, chronic alcohol,e.t.c, affecting the overall state of health of the subject [7]. Patients with chronic liver disease often suffer from unspecific symptoms and report severe impairment in the quality of life [8], and this contributes majorly to mortality and morbidity rate. Therefore there is a need to establish a natural element which can be consumed alongside this "unavoidable toxicant” when aware, so as to manage side-by-side possible symptoms that could result from the toxicity. This, therefore, aims at investigating the ability of *Eleusine coracana* grain extract to manage arsenic trioxidenduced hepatotoxicity.

2. MATERIALS AND METHODS

2.1 Animal Treatment

32 wistar albino rats with average weight of 130 g were obtained from the University animal house for the study. According to the ethics of the experimentation on animals [9], rats were housed in groups in clean capacious plastic cages (seven per cage) under standard laboratory conditions including well aerated room, good lighting, with suitable temperature (28ºC ± 2ºC) in a neat environment and at a 12-hour light/dark cycle. The animals were divided into four (4) groups, eight per cage and acclimatized for two (2) weeks, where they had access to standard rat chow and water ad libitum; Group A (Control group) received distilled water, Group B received 5mg/dl arsenic trioxide solution only (Negative control), Group C received 5mg/dl Arsenic trioxide solution + 200 mg of the extract, per kg body weight and Group D received 5mg/dl Arsenic trioxide + 500 mg of the extract per kg body weight. All groups were administered orally using a metal cannula.

2.2 Plant Treatment

Dried *Eleusine coracana* grains were purchased at Olu-ode Market, Oshogbo in Osun State. The grains were decorticated into powdered form, using mortar and pestle. After which, it was defatted using petroleum ether as the solvent and soxhlet apparatus was used for extraction.

2.3 Preparation of Ethanolic Extract

600 g of the deffated grain was soaked in 3000 ml of ethanol for 3 days in ratio. The filtrate was freeze dried and the freeze-dried crude extract from this ethanolic extraction was used for the reconstitution into solution for administration.

2.4 Phytochemical Screening

The condensed extract was used for the screening of phytochemicals such as Tannis, Phlobatannis, Flavonoids, Steriods, Terpenoids, and Saponins, using standard procedures. [10,11].

2.5 Biochemical Analysis

Blood was collected on day 15, by cardiac puncture and centrifuged at 3000rpm for 20 min to obtain the serum. Organs such as the Liver, Kidney and Heart were carefully harvested and homogenised for further analysis.

2.6 Determination of Total Bilirubin Concentration

Total bilirubin concentration was estimated following the colourimetric method modified by [12]. Total bilirubin laboratory kit was obtained from Randox laboratory Ltd. Total Bilirubin (mg/dl) =10.8 X $A_{TB(578nm)}$

2.7 Determination of Direct Bilirubin Concentration

Direct bilirubin concentration was estimated according to the colorimetric method modified by [12]. Direct bilirubin laboratory kit was obtained for Randox laboratory Ltd. Direct bilirubin (µmol/l) = 246 X $A_{DB\ (546nm)}$ Direct bilirubin (mg/dl) = 14.4 X $A_{DB\ (546nm)}$.

2.8 Determination of Indirect Bilirubin Concentration

Indirect bilirubin (unconjugated) concentration was determined by the subtraction of the values for direct bilirubin concentration from total bilirubin concentration estimated above, therefore; Indirect conc. (g/dl) = Total bilirubin conc – Direct Bilirubin conc.

2.9 Determination of Total Protein Concentration

Total protein concentration was estimated using the Biuret method as modified by Hanne et al. [13].

2.10 Quantification of Aspartate Aminotransferase (AST)

The assay was performed using Randox Kit. AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine [14].

2.11 Statistical Analysis

Data were analyzed for significance by Analysis of Variance (ANOVA), followed by Post HOC to compare significance between groups. Results were expressed as mean ± Standard error (SE). Values were considered significant at $P<0.05$.

3. RESULTS AND DISCUSSION

The medicinal phytochemicals present in ethanolic extract of *Eleusine coracana* grains are tannins, phlobatannis, flavonoids and terpenoids but steroids and saponins were found to be absent, as presented in the table below;

Table 1. Phytochemical constituents of *Eleusine coracana*

S/N	Phytochemical constituents	Observation
1	Tannis	+
2	Phlobatannis	+
3	Flavonoids	+
4	Steriods	_
5	Terpenoids	+
6	Saponins	_

Key: + Signifies the presence of the phytochemical
- Signifies absence Phytochemical

There was a significant increase ($p\geq0.05$) in liver-body ratio and brain-body ratio of arsenic trioxide treated rats (group B) when compared to control group A, and the *Eleusine c*oracana extract treated groups had a significant decrease in the organ-body weight index, close to the index observed in control group after 14days of treatment. Eleusine coracana contains tannins, phlobatannins, Flavonoids and Terpenoids but not Steriods and Saponins, this is in line with earlier report by [15], although steroid was reported to be present. This variance may be due to the diversity in the solvent of extraction. Bailey et al. 2004 [16] has revealed that an increased organ-body weight in rats denotes an abnormality. Therefore arsenic trioxide induced the alteration of the cytoarchitecture in the liver

while *Eleusine coracana* was reversed the effects.

This curve shows that there is an increase in the growth of rats in groups (C and D) that were administered extract of *Eleusine coracana* compared to the control. Group B that received only Arsenic trioxide without treatment showed a significant decrease ($p \leq 0.05$) in the mean body weight as compared to control and the extract treated groups. Therefore, the consumption of *Eleusine coracana* could help manage side effects that goes along with hepatic cell injury and the injury itself.

The organ-body weight ratio of group B (Untreated) is relatively high compared to the control and other treated groups. The revealed that the extract was able to ameliorate the growth decline observed in the toxicity group, the growth in the treated and control group are not significantly different. This further support earlier finding that *Eleusine coracana* has the components capable of managing arsenic trioxide toxicity which in turn suggests hepatoprotective effects.

Result reveals that there was no significant difference ($p \geq 0.05$) in mean values of total protein concentration of treated and control groups but group B (Ar only). Also, the mean serum AST level in group B (5mg/dl/body weight of Arsenic trioxide) was significantly higher (indicating hepatic cell injury) when compared to the Eleusine treated groups and control. Total bilirubin, direct bilirubin and AST serum levels were significantly increased in group B but drastically and significantly reduced in the treated groups in a dose-dependent manner. The high bilirubin and Aspartate Aminotransferase levels in group B, shows that the liver's capacity to process the bilirubin has declined due to the Arsenic toxicity, The aminotransferases, AST and ALT are the most frequently utilized indicators of hepatocellular necrosis, as earlier reported by [17]. The total protein in arsenic treated group is significantly low, compared to the treated groups, Total protein in the serum of treated is improved and significantly higher than that of the untreated-intoxicated group, this decrease in serum total protein signifies an impaired function of the liver, which has been managed and reversed in the *Eleusine coracana* treated groups.

The plant extract exhibits the afore mentioned clinical response partly due to its free radical mop-up capacity. The ability of *Eleusine coracana* to protect the Liver can be further supported by its rich bioactive constituents among which are the phenolic compounds revealed by phytochemical screening to be present and other important constituents such as antioxidants reported earlier in previous findings [18].

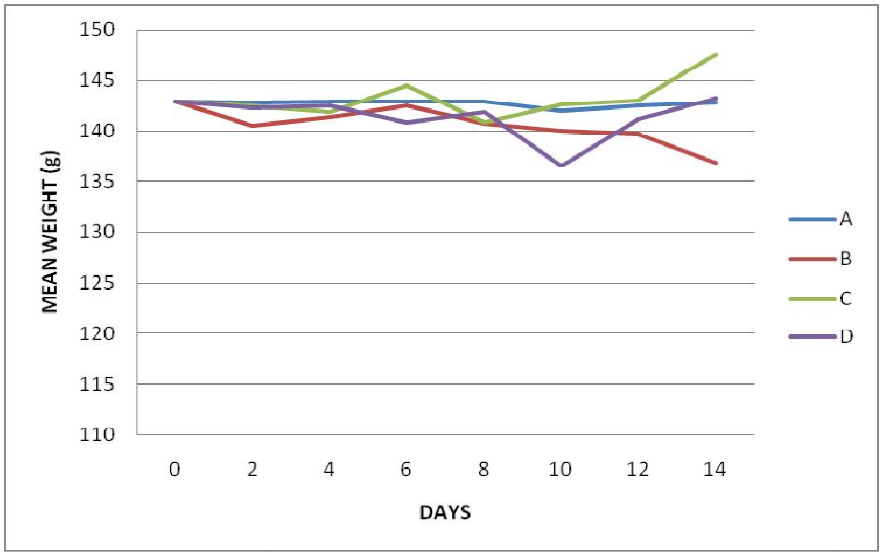

Fig. 1. Mean Weight of the different groups during the 14 days treatment

Table 2. Mean of organs-body weight index of the experimental rats

S/N	Groups	Organs-body weight index (%)	
		Brain	Liver
1	A(Control)	4.25±0.03	1.12±0.03
2	B (Arsenic trioxide)	5.45±0.04[a]	1.81±0.04[a]
3	C (Arsenic trioxide +200 mg/kg Extract of *Eleusine Coracana*)	4.35±0.04	1.04±0.06[b]
4	D(Arsenic trioxide+500 mg/kg Extract of *Eleusine coracana*)	4.38±0.02	1.44±0.04[b]

Key: "a" means significantly different from control.
"b" means statistically different from group B

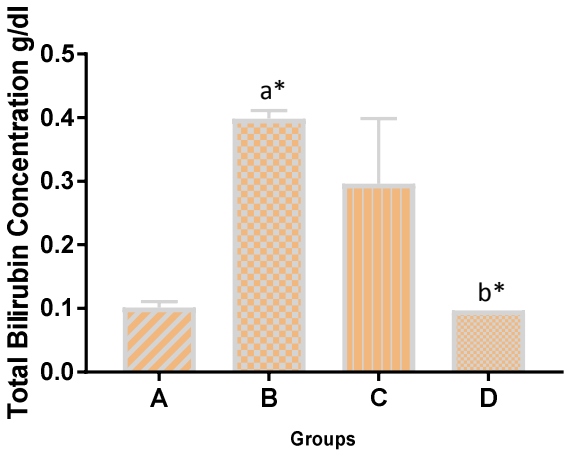

Fig. 2. Mean serum total bilirubin concentration of the different treatment groups after 14 days
Key: "a" means significantly different from control.
"b" means statistically different from group B
P <0.05

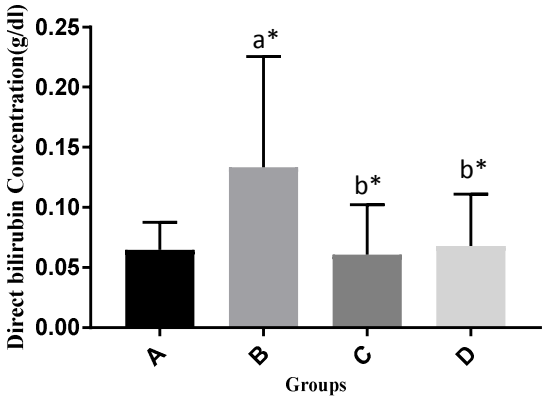

Fig. 3. Mean serum direct bilirubin concentration of the different treatment groups after 14 days
Key: "a" means significantly different from control.
"b" means statistically different from group B
P <0.05

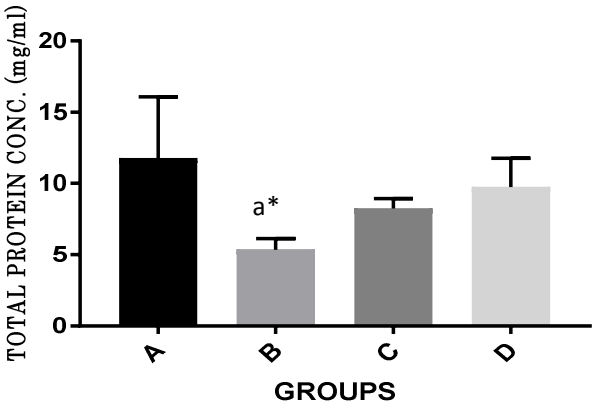

Fig. 4. Mean serum total protein concentration of the different treatment groups after 14 days

Key: "a" means significantly different from control.
"b" means statistically different from group B
P <0.05

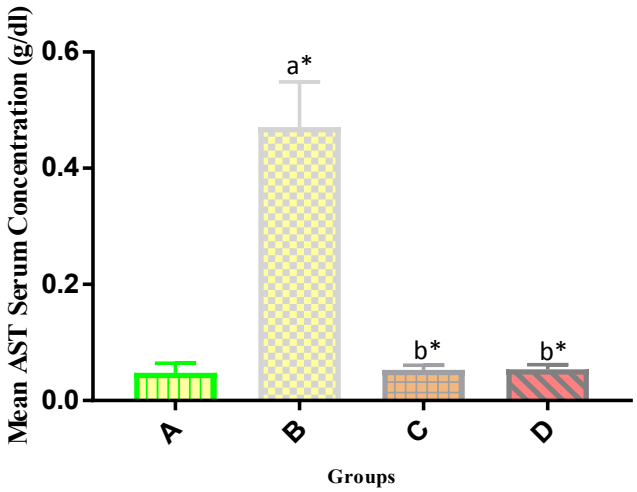

Fig. 5. Mean aspartate aminotransferase (AST) concentration in the serum of the different treatment groups after 14 days

Key: "a" means significantly different from control.
"b" means statistically different from group B
P <0.05

4. CONCLUSION

Eleusine coracana as a food crop and source of bioactive compounds contains certain phytochemicals which are capable of optimizing the invivo defense system in managing hepatic cell injury by boosting the endogenous cellular antioxidant capacity, this study substantiate other findings on the hepatoprotective potentials of *Eleusine coracana* with focus on the arsenic trioxide induced hepatic cell injury.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per University standard guideline, ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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