Original Research Article

TOTAL POTEIN, BILIRUBIN AND AST LEVELS IN RAT MODELS TREATED WITH ETHANOLIC EXTRACT OF ELEUSINE CORACANA DURING ARSENIC TRIOXIDE INDUCED HEPATOTOXICITY

ABSTRACT

Background: *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.

Objective: This study sought to investigate the protective ability of ethanolic extracts of *Eleusine coracana* in Arsenic trioxide induced hepatotoxicity using rat models.

Methods: Animals were grouped into four (4). Group A received only distilled water, in group B,C and D hepatotoxicity was induced using 5mg/dl Arsenic Trioxide solution for 14days, followed by treatment in group C and D daily with 200mg and 500mg per kg body weight respectively for 14days, and changes in body weight and Liver Function parameters were determined.

Results: 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.

Conclusion: *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.

Key words: *Eleusine coracana,* Hepatoprotective, liver function test, Finger millet, Dawa, Okababa, Acute, Hepatotoxicity.

INTRODUCTION

Eleusine coracana is an annual plant widely grown as a cereal in the arid area of Africa and Asia (Sood *et* al., 2017).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. One hurdle in finger millet production is that it is a labor intensive crop (Sakamma *et al*., 2018). The plant is high in iron, calcium, fiber, starch and is considered "superior" to wheat in that its proteins are more easily digested, contains mainly unsaturated fatty acids (Sood *et al.,* 2017). *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 (Shibairo *et al*., 2014). It is easy to digest and does not contain gluten; people who are sensitive to gluten can easily consume the plant (D.Chandra *et al*., 2016). The hepatic tissue is considered the hub of metabolism, because it function in the metabolism and excretion of almost all substances that goes into the body (drug and nutrients). Liver cell injury 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 (Liangyou, 2014). This study is aimed at investigating the ability of *Eleusine coracana* grain extract to manage a hepatic injury caused by arsenic trioxide intoxication.

MATERIALS AND METHODS

Animal Treatment

32 wistar albino rats with average weight of 130g were obtained from the university animal house for the study. According to the ethics of the experimentation on animals (Hammond,

1994), rats were housed in groups in clean capacious plastic cages (seven per cage) under standard laboratory conditions including good aerated room, good lighting, with suitable temperature ($28^0C \pm 2^0C$) in a neat environment and at a 12-hour light/dark cycle. The animals were divided into four (4) groups, eight per caged and acclimatized for two 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, Group C received 5mg/dl Arsenic trioxide solution and 200mg of the extract, per kg body weight and Group D received 5mg/dl Arsenic trioxide and 500mg of the extract per kg body weight of the orally using a metal canula and syringe respectively.

PLANT TREATMENT

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

Preparation of Ethanolic Extract

600g of the deffated grain was soaked in 3000ml of ethanol for 3days in ratio. The filtrate was

freeze dried and the The freeze-dried extract crude extract from this ethanolic extraction was used for the reconstitution into extract solution for administration.

Phytochemical Screening

The condensed extract was used for the screening of phytochemicals such as Tannis , Phlobatannis, Flavonoids, Steriods, Terpenoids, and Saponins, using standard procedures. (Harborne, 2005; Trease & Evans, 2002; Sofowora, 1993).

BIOCHEMICAL ANALYSIS

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

Determination of Total Bilirubin Concentration

Total bilirubin concentration was estimated according to the colorimetric method as modified by Jendrassik and Grof, (1938). Total bilirubin laboratory kit was obtained from Randox laboratory Ltd. Total Bilirubin (mg/dl) =10.8x A_{TB} (578nm)

Determination of Direct Bilirubin Concentration

Direct bilirubin concentration was estimated according to the colorimetric method as in modified Jendrassik and Grof, (1938). Direct bilirubin laboratory kit was obtained for Randox laboratory Ltd. Direct bilirubin (µmol/l) = 246x A_{DB} (546nm) Direct bilirubin (mg/dl) = 14.4 X A_{DB} (546nm).

Determination of Indirect Bilirubin Concentration

Indirect bilirubin (unconjugated) concentration can be determined by the subtraction of the values for direct bilirubin concentration from total bilirubin concentration gotten above, therefore; Indirect conc. (g/dl) = Total bilirubin conc,

Determination of Total Protein Concentration

Total protein concentration was estimated according to the Biuret method as modified by Donninger et al., (1972).

Quantification of Aspartate Aminotransferase

The assay was performed using Randox Kit. AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine. (Reitman and Frankel, 1957).

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 $P<0.05$.

RESULTS

The medicinal phytochemicals present in ethanolic extract of *eleusine coracana* grains are tannis, phlobatannis, flavonoids and terpenoids but doesn't contain steroids and saponins, 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 Phytochemical's absence

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 +200mg/kg Extract of *Eleusine Coracana*)	4.35±0.04	1.04±0.06[b]
4	D(Arsenic trioxide+500mg/kg Extractof*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

There was a significant increase ($p \geq 0.05$) in liver-body ratio and brain-body ratio of arsenic trioxide treated rats (group B) when compared to control group A, and the Eleusine treated groups had a significant decrease in the organ-body weight index, close to the index observed in control group after 14days of treatment.

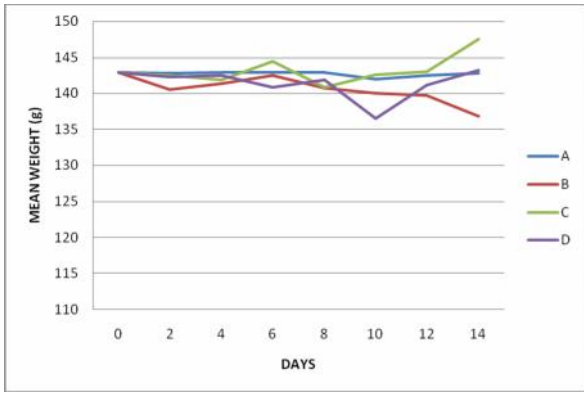

Fig 1: Mean Weight of the different groups During 14days of treatment.

This curve shows that there is increase in growth of rats in groups (C and D) that were administered extract of *Eleusine coracana* grains compared to the control and groups (A)

significant decrease ($p \leq 0.05$) in rat in group (B) that were administered arsenic trioxide only, when compared control group (A).

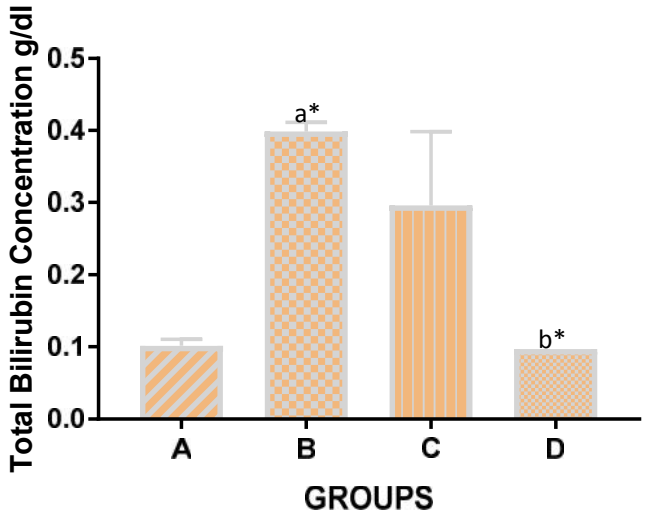

Fig 2: Mean Serum Total Bilirubin concentration of the different treatment groups after 14days.

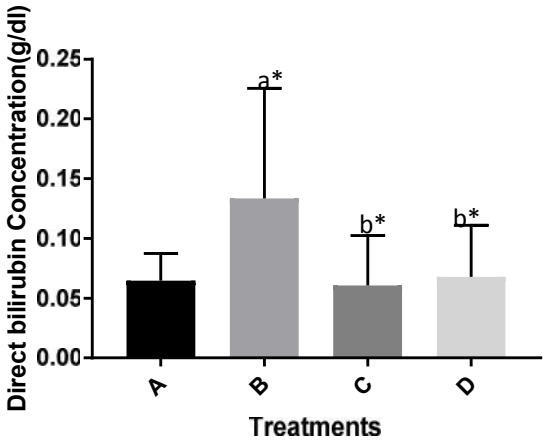

Fig 3: Mean Serum Direct Bilirubin concentration of the different treatment groups after 14days.

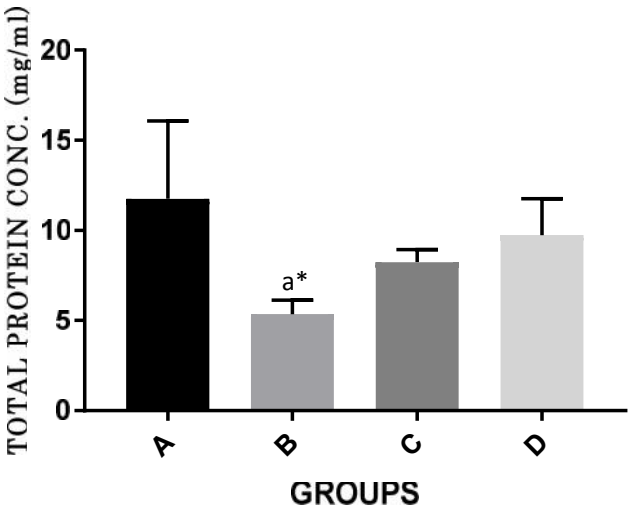

Fig 4: Mean Total protein concentration in the serum of the different treatment groups after 14days

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

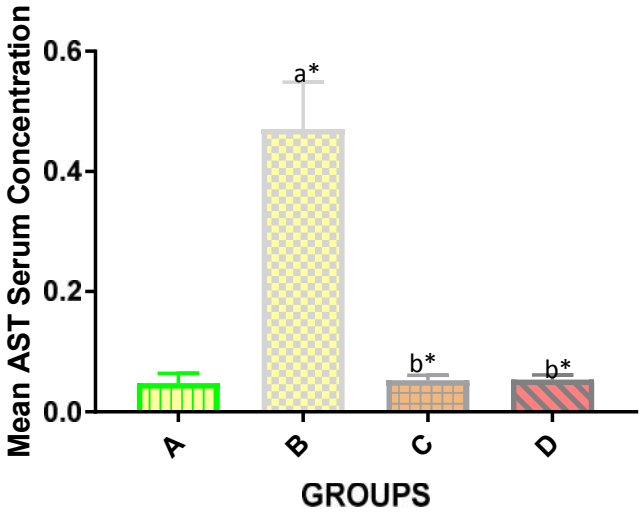

Fig 5: Mean Aspartate Aminotransferase (AST) concentration in the serum of the different treatment groups after 14days

Result reveals that there is a significant difference ($p \geq 0.05$) in mean values of total protein concentration of control as compared to group B and AST concentration in group B (5mg/dl/body weight of Arsenic trioxide) when compared to control group (A).

DISCUSSION

Eleusine coracana contains Tannins, phlobatannins,Falavonoids and Terpenoids but not Steriods and Saponins, this is in line with earlier report by Bwai *et al.,* 2014, although steroid was reported to be present. This variance may be due to the diversity in solvent of extraction. Bailey *et al.,* 2004, has revealed that an increased organ-body weight in rats denote an abnormality. The organ-body weight ratio of group B (Untreated) is relatively high compared to the control and other treated groups. The growth curve revealed that the extract was able to ameliorate the growth decline observed in the intoxicated group, the growth in the treated and control group are not significantly different.

Total bilirubin, direct bilirubin and AST serum levels were significantly increased in the intoxicated group but drastically and significantly reduced in the treated group in a dose-dependent manner. The high bilirubinin and Asparttate Aminotransferase levels in group B(untreated intoxicated) 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 Nannadas *et al*., 2012. 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 in the Eleusine coracana treated group . The ability of Eleusine coracana to protect the Liver can be further supported by its rich 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 (Jignasu *et al*., 2012).

CONCLUSION

Eleusine cocarna as a plant and source of food contains certain phytochemicals which are capable of managing hepatic cell injury, this studies support other findings about the hepatoprotective effects of Eleusine coracana especially in arsenic hepatotoxicity.

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