Ameliorative Effect of Activated Charcoal on Tannic Acid Induced Hepatotoxicity in Rats

Abstract

Background: There is an increase in substance toxicity and abuse with tannic acid in developing countries in the past few years. Different substances both chemical and traditional have been used by different individuals to neutralize or reduce the toxic effects from tannic acid poisoning. **Aim:** This study aimed at assessing the ameliorative effect of activated charcoal in tannic acid induced liver poisoning in rats. **Methodology:** The experimental study included 20 male wistar rats that were divided into 5 groups (negative control NC), positive control (PC), activated charcoal group 1 (ACG 1), activated charcoal group 2 (ACG 2), activated charcoal group 3 (ACG 3) with each group having 5 rats. NC was neither induced with tannic acid nor treated with activated chacoal. PC was only induced with 2260mg/Kg tannic acid while ACG1, ACG2 and ACG3 were not only induced in same dose of tannic acid but were treated with 0.01mg/ml, 0.02mg/ml and 0.03mg/ml doses respectively on the 6^{th} day after the tannic acid poisoning. Blood samples were collected via cardiac puncture for assessment of liver function. **Results:** The result showed a significant increase ($p<0.05$) in liver parameters (AST, ALT, ALP and albumin) after tannic acid poisoning while a significant reduction ($p<0.05$) in the same parameters were observed after the administration of activated charcoal. **Conclusion:** The study has demonstrated that tannic acid causes hepatotoxicity and activated charcoal has the potential to ameliorate the toxic insult.

Keywords: *biochemical parameters, enzymes, liver, toxicity*

Introduction

Tannic acid is a naturally occurring plant polyphenol and can be found in practically all aerial plant tissues. The tannins are applied widely, with uses ranging from tanning, known over millennia (Mediterranean since *ca*. 1500BC), through medicinal uses in the food industry. It is mainly used as a fixative of dyes and as a chemical intermediate and reagent in the manufacture of inks, rubber, wine, medicine, imitation horns and tortoise shells [1]. Ingestion of tannic acid can cause hardening of the gastrointestinal mucosa [2], which can result in reduced gastrointestinal absorption of nutrients as well as of xenobiotics.

Traditionally, tannins are considered to have anti-nutritional properties [3]. Tannins have greater tendency to form complexes with proteins than carbohydrates and other food polymers because of the strong hydrogen bond affinity of the carboxyl oxygen of the peptide group. Complexes formed by tannins and proteins have been reported to be responsible for growth depression, low protein digestibility, decreased availability of amino acid, increased fecal nitrogen and other toxic effects [4-7].

Activated charcoal is an odorless, fine powder which is black in color it has been known to be effectively used to cure overdose in emergency rooms[8]. Activated charcoal possess lots of medicinal and other characteristics which shows their effectiveness in several fields in which includes cosmetics and many more [8]. One of the effective characteristics of activated charcoal showed toxin absorbing activities [9]. The formation of activated charcoal done after heating the common charcoal in the existence of gas which causes charcoal to produce pores and inside spaces so, with the help of these pores, the trapping of chemicals occurs through activated charcoal [10]. Reports have shown that it can effectively treat flatulence, poison, cholestasis, and reduce the level of cholesterol [11]. From research, it was concluded that people who used activated charcoal for long duration are prevented from many health-related problems. It not only prevented stomach and intestine problems, but also showed interaction and absorbent effects for drugs, toxins, bacteria, viruses, chemicals & fungus, which are present in water [12].

The benefits of tannin have increased the consumption of tannins especially as supplements. This may expose individuals to tannin toxicity. Activated charcoal had shown usefulness in health related problems.

There is an increase in substance toxicity and abuse with tannic acid in developing countries in the past few years. Different substances both chemical and traditional have been used by different individuals to neutralize or reduce the toxic effects from tannic acid poisoning. This study intends to evaluate the biochemical (hepatic) effects of activated charcoal on acute toxicity poisoning with tannic acid, thus establishing the level of its efficacy. This may be useful in the management of tannic acid poisoning.

Methodology

Experimental Animals

Twenty-five (25) male albino rats that weighed 120-200g were selected for the study. The animals were obtained from University of Port Harcourt College of Health Sciences. They were transported in well ventilated wired cage to the animal house at Department of Medical Laboratory Science, Rivers State University, Port Harcourt. They were allowed for 2 weeks to acclamatize before the study. The rats were maintained with solid pellets as feed and water from the tap.

Experimental design and grouping

The 25 male albino rats weighing between 120-200g were assigned into 5 groups, containing 5 rats per group. The rats were acclimatized for 14days.

List 1-Grouping

Groups	Details
NC	Negative control: No tannic acid given and no artivated charcoal given
NP	Positive control: 2260mg/Kg of tannic acid was given
ACG 1	Treatment group 1: Administered 2260mg/Kg and treated with 0.01mg/ml of activated charcoal
ACG 2	Treatment group 2: Administered 2260mg/Kg and treated with 0.02mg/ml of activated charcoal

ACG 3	Treatment group 3: Administered 2260mg/Kg and treated with 0.03mg/ml of activated charcoal

AC: Activated charcoal

After the acclamatization, the rats were administered with a single oral dose of 2260mg/Kg of tannic acid as presented in Table 1 above. After the oral administration of tannic acid to the appropriate rats groups the rats were observed for 6 days. After which, activated charcoal was administered orally in a one-time dose-dependent fashion as described in the Table 1 above.
After the treatment period, the rats were sedated using chlorofoam and their blood samples were collected via cardiac puncture for biochemical analysis of liver function parameters [13,14].

Blood collection and preparation
By means of syringe and needle 5ml of blood samples were collected aseptically into lithium heparin bottles. The whole blood samples were spun using a centrifuge at 3000RPM for 5minutes. The plasma was collected into a plain bottle for analysis of protein, albumin, bilirubin, AST, ALT and ALP using biuret method for protein estimation, Bromocresol green (BCG) method for albumin estimation, Jendransik and grof method for bilirubin estimation, Reitman-Frankel method for AST and ALT, and King Amstrong method for ALP [14].

Statistical Analysis
Data generated from this study was statistically analyzed using Graph pad prism (Version 6.0). Analysis of variance (ANOVA) and Tukey's multiple comparison was done to obtain mean, standard deviations, *p*-value and statistical significance between groups. A *p*-value of <0.05 was considered statistically significant in all statistical comparison.

RESULTS

The results of the mean blood biochemical parameters for the control groups are shown in Table 1. The result showed that there were significant differences in AST, ALP, ALT and albumin in the negative control group when compared with the positive control group ($p<0.05$). However significant differences were not observed in protein and bilirubin parameters.

Table 1: Comparison of Biochemical Markers in Negative and Positive Control Groups

Parameter	Negative control	Positive control	P. Value	Inference
ALP (U/L)	20.33±1.53	40.67±3.786	0.0010	S
AST (U/L)	31.33±4.51	64.00±6.00	0.0017	S
ALT (U/L)	22.00±2.65	31.33±3.22	0.0178	S
Protein (g/L)	34.67±2.52	35.33±2.08	0.7415	NS
Albumin (g/L)	30.33±0.58	33.67±1.53	0.0241	S

T.BIL (umol/L)	2.280±0.99	3.990±0.99	0.1012	NS
C.BIL (umol/L)	1.710±0.00	2.850±0.99	0.1161	NS

The results of the mean blood biochemical parameters for the groups are shown in table 3. The result showed that there were significant differences in AST, ALP, ALT and albumin in the groups (p<0.05). However significant differences were not observed in protein and bilirubin parameters.

Table 2 Comparison of Biochemical Parameters in The Study Groups

	NC	PC	ACG 1	ACG 2	ACG 3	F-value	p-value	Remark
DB	1.71±0.00	2.85±0.99	2.28±0.99	2.85±0.99	1.71±0.00	1.667	0.23	NS
TB	2.28±0.99	3.99±0.99	2.85±0.99	2.28±0.99	2.28±0.99	1.70	0.22	NS
ALB	30.33±0.57	33.67±1.53	30.33±0.58	32.00±1.00	31.67±1.53	4.53	0.024	S
PR	34.67±2.52	35.33±2.08	37.33±2.O8	35.33±1.53	34.0±1.00	1.273	0.343	NS
ALT	22.0±2.65	31.3±3.22	25.33±2.51	25.67±3.51	25.67±0.57	4.66	0.02	S
ALP	20.33±1.53	40.67±3.8	32.33±3.77	33.67±0.58	28.67±1.53	24.67	<0.0001	S
AST	34.67±3.21	64.0±6.0	46.32±4.0	32.0±2.6	32.0±1.7	39.03	<0.0001	S

Key: S- Significant; NS-Non-significant; ACG: Activated Charcoal Group

Comparison of biochemical parameters as presented in Table 4, shows significant change (p<0.05) in AST, ALP, ALT and albumin when compared between negative control, positive control and treatment groups.

Table 3: Tukey's Multiple Comparisons for Biochemical Parameters

	AST (U/L)	ALT (U/L)	ALP (U/L)	Protein (G/L)	Albumin (G/L)	T. BIL (UMOL/L)	C. BIL (UMOL/L)
NC vs. PC	S	S	S	NS	S	NS	NS
NC vs. ACG1	S	NS	S	NS	NS	NS	NS
NC vs. ACG2	NS	NS	S	NS	NS	NS	NS
NC vs. ACG3	NS	NS	S	NS	NS	NS	NS
PC vs. ACG1	S	NS	S	NS	NS	NS	NS
PC vs. ACG2	S	NS	S	NS	NS	NS	NS
PC vs. ACG3	S	NS	S	NS	NS	NS	NS

S + 1 vs. ACG2	S	NS	NS	NS	S	NS	NS
S + 1 vs. ACG3	S	NS	NS	NS	NS	NS	NS
S + 2 vs. ACG3	NS	NS	NS	NS	NS	NS	NS

KEY: S- significant; NS-non-significant; NC-negative control; PC- positive control;

Discussion

The present study evaluated the effects of activated charcoal on hepatic parameters of tannic acid toxicity in albino rats. As presented in Table 1, there was a significant difference ($p<0.05$) in the concentrations of AST (aspartate aminotransferase), ALP (alkaline phosphates), ALT (alanine aminotransferase) and albumin between negative and positive control groups. Turkey's multiple comparison between these groups also presented significant increase in AST parameters. The significant increase in enzyme activities is an indication that tannic acid may be capable of inducing liver toxicity. Reports have shown that Tannic acid is capable of causing toxicity in rats as it is capable of causing hepatic necrosis, thus leading to the death of liver cells causing toxicity [15]. This result is in agreement with the work by Samanta and his colleagues in 2004 [15], who reported an increase in death of liver cells after exposure to tannic acid when administered to rats. However, there was no significant difference in protein and bilirubin concentrations. This may be due to the concentration of tannic acid and the duration of study.

AST (aspartate aminotransferase) is an enzyme that is found mostly in the liver, but also in the heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. When the liver is damaged, it releases AST into the bloodstream [14]. An AST blood test can help in the diagnosis of liver damage or disease.The significant increase in AST may indicate hepatitis, cirrhosis or other liver diseases which in this case may be caused by tannic toxicity. Although, high AST levels can also indicate heart problems or pancreatitis (Pratt & Kaplan 2000). However, the rise of all studied liver enzymes; AST, ALT and ALP is a clear implication of liver pathology.

Furthermore, the findings from this study in Table 3 showed a significant difference ($p<0.05$) in the levels of almost all the liver function parameters among the different treatment groups signifying that activated charcoal may be capable of ameliorating the toxic effect which was induced by tannic acid as evident in the parameters (AST, ALT, ALP and albumin) on Table 3. This could suggest that activated charcoal has some protective effects over tannic acid toxicity.

However, Protein and bilirubin did not show significant different in the study groups in both toxicity induction and activated charcoal treatment. This may be due to administration of inadequate dose to elicit an effect in the parameters studied.

Conclusion

The study has shown that tannic acid caused changes in AST, ALP, ALP and albumins levels. The results obtained suggested that activated charcoal showed an effect in

ameliorating the hepatotoxicity induced by tannic acid. This may be used in the management of tannic acid toxicity.

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