



## Evaluation of the Effect of Action Bitters (Herbal Mixture) on Some Biochemical Indices of Albino Rats

Nwachuku, Edna Ogechi<sup>1</sup> and Elekima, Ibioku<sup>1\*</sup>

<sup>1</sup>Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

### Authors' contributions

This work was carried out in collaboration between both authors. Author NEO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author EI managed the analyses of the study, performed the statistical analysis and managed the literature searches. Both authors read and approved the final manuscript.

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### ABSTRACT

**Aim:** The aim of the study was to evaluate the effect of action bitters on some liver and renal function indices of Albino rats.

**Study Design:** Experimental rats were divided into two groups A and B. Group A (control rats) consist of 10 rats and were not given herbal drugs while group B (treated rats) consist of 25 rats and were treated orally with 0.68 ml/kg of Action Bitters on a daily basis for 30 days.

**Place and Duration of Study:** The experiment was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria within a period of four months (July 2018 – October, 2018).

**Methodology:** A total of 35 male albino rats weighing an average of 0.18 kg were separated into two groups namely A and B. Group A had 10 rats and were used as control while Group B had 25 rats and were treated as the test group. Group A rats were fed normally while Group B received water, rat feds and Action Bitters. The action bitters were administered orally using galvage tube at a dose of 0.68/kg bodyweight. After 30<sup>th</sup> day of treatment, blood samples were collected for

\*Corresponding author: E-mail: [asaboasa@rocketmail.com](mailto:asaboasa@rocketmail.com);

analysis of serum Electrolytes, Urea, Creatinine, AST, ALP and ALT. The biochemical parameters were analysed statistically using GraphPad Prism 5.03 version.

**Results:** Results of renal indices obtained showed that control rats had values of  $9.567 \pm 6.396$ ,  $74.59 \pm 40.93$ ,  $153 \pm 25.67$ ,  $111.7 \pm 20.23$  and  $5.767 \pm 1.819$  for Urea, Creatinine, Potassium, Sodium and Chloride respectively while the treated rats had  $11.72 \pm 6.142$ ,  $122.9 \pm 82.16$ ,  $152.8 \pm 27.57$ ,  $116.6 \pm 24.98$  and  $6.156 \pm 1.843$  for Urea, Creatinine, Potassium, Sodium and Chloride respectively. More so, when liver enzymes were considered, control rats had values of  $134.2 \pm 52.07$ ,  $35.44 \pm 13.82$  and  $169.2 \pm 71.92$  for AST, ALT and ALP respectively while treated rats had values of  $139.4 \pm 44.6$ ,  $35.39 \pm 14.39$  and  $196.9 \pm 65.92$  for AST, ALT and ALP respectively. The comparison of control rats and treated rats showed no significant differences in the biochemical indices considered at  $P = .05$ .

**Conclusion:** The study indicated that Action Bitters did not induce any obvious biochemical derangement in liver and renal indices at a dose of 0.68ml/kg bodyweight.

*Keywords: Action bitters; liver enzymes; renal parameters; albino rats.*

## 1. INTRODUCTION

The therapeutic use of herbal medicine started about 5000 years ago by the Indian, Egyptians, Greek, Chinese, Roman and Syrian [1]. These people presumably became aware that a strange change (metamorphosis) occurred when many fruits, cereals, berries and other plants materials were mixed with water and left in the warmth of the sun. The product made was the first meads (perhaps the earliest beverages known to human being [1,2]. These products were made from fermentation of honey (meads), fruits and berries (wines) and those from cereals were the first beers [3,4]. These beverages were known, accepted and prized as foods, as magic fluids and as medicines [5] until the 19<sup>th</sup> and early 20<sup>th</sup> century when there was a steady decline in the therapeutic use of herbal medicine. Plants serves as a basic for medical treatment and their different parts have been a source of herbal medicine which has been shown to be effective to about 80% of population as primary healthcare [6,7]. Therefore, the medicinal plants are the most important source of life saving drugs for the majority of the world's population [6,7].

According to World Health Organization (WHO) herbal medicine is a therapeutic practice that have been in existence for hundreds of thousands of years ago before the development and spreading of modern medicine and are still in use today [7]. Herbal drugs constitute only those traditional medicine which primary use medicinal plant preparation for therapeutic purposes [1]. Traditional medicine including herbal drugs played a vital role in the past and will continue to do so in the future [8]. Recently, consumers of alcohol preferred local herbal concoctions in the

name of bitters which mimic alcoholic beer of international standard; the age-long consumption of beer was on its way out of fashion. This is because consumers of bitters believe that it contains body purifiers, it is anti-malaria, contain ingredients that strengthen the virility of men, anti-diabetic, hypo-lipidemic and non-toxic to the kidney [8,9], as a result, big brands were struggling for sales and manufacturers of smaller brands were smiling to the banks on a daily basis. There was a serious crisis in the marketing of alcoholic beer and other alcoholic drinks because herbalist has increased in population.

Alcoholic beverages such as Alomo bitters, Action bitters, Origin bitters, Pasa bitters, Osomo bitters etc. makes an impact in the alcoholic beverages market despite the initial fears over the hygiene level of their product and their composition [8,9]. The claim that it is restorative and sex energy boosting, continue to lure customers to patronize them in mass. The term 'bitters' as it is used today, is a beverage, often alcoholic, flavored drink with herbal essences that gives it a bitter or bittersweet flavor [10]. The generic term applies for all bitter liquors and herbal bitters. Bitters are produces from root extracts and herb, from the narcotic content of (primarily) tropical and subtropical plant and spices [10]. Bitters are usually dark in color and valued for their ability to promote appetite and digestion hence they are use as patent medicine, aid in digestion and as flavoring in cocktails [11]. Bitters have a common characteristic of a bitter taste and act to increase the vital energy centres in the body. Bitters are also made up of numerous groups of chemical compounds extracted from the herbs and roots (medicinal plant) [12].

Historically, the botanical ingredients used in preparing bitters consist of bark, aromatic herbs roots, and/or fruit for their flavor and medicinal properties [12]. Some of the common ingredients are; orange peel, gentian, cassia, cascarilla, cinchona bark etc. Most bitters also contain water and alcohol, the latter functions as both solvent for botanical extracts and as preservative while the alcoholic strength varies across different brands. Indeed, plant extracts, now popularized and publicized as an herbal medicine in form of bitters have been shown to treat, manage and cure several diseases [12].

Unorthodox traditional medicine practice which employ the use of herbs (medical plant) have in recent time been gaining much recognition and publicity for their solution to ailment seemingly elusive to the system of orthodox medical practice modern medicine may have widened for sometimes but there are little differences in terms of medication between orthodox and unorthodox/traditional medicine, this gap seems to be closing fast as the current trends is that most modern pharmacology had its origin in these medicinal plants/unorthodox and both are adopting practices from each other.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Materials used in this experiment include Polypropylene gavage tubes (Intech Laboratory Incorporated, Plymouth Meeting, USA), Haier thermocool refrigerator (China), Vis spectrophotometer (Axiom Medical Limited, United Kingdom), MPW bucket centrifuge Model 351 (MPW Medical Instruments, Warsaw, Poland) and Ohaus Scout-Pro Electronic weigh balance (Ohaus Corporation, New Jersey, USA). Action bitters (produced by Intercontinental Distillers Limited, Ogun State, Nigeria) were purchased from pharmaceuticals stores at Mile 3 market, Rivers State, Nigeria. The actions bitters were brought as liquid formulations and stored at room temperature of 18°C throughout the period of the experiment. AST, ALT, Urea and Creatinine reagents were purchased from Randox Laboratories Limited, Crumlin, United Kingdom while ALP, Potassium, Sodium and Chloride reagents were purchased from Atlas Diagnostics, Cambridge, United Kingdom.

### 2.2 Experimental Rats

A total of 35 experimental albino rats weighing approximately 0.18 kg were used in this

experiment. The rats were kept in the Department of Medical Laboratory Science animal house, caged in a polycarbon plastic with a solid bottom, and had a top covering of stainless-steel grid with provisions to hold rat chow and water drinking bottles on these lids. The cages were well-ventilated rat cage with a uniform temperature with fed (grower chicken marsh) and water *ad libitum*. The cages were lined with bedding of clean, hard wood shavings mixed with shredded pieces of paper for environmental enrichment. The bedding provided for the rats was changed twice a week.

### 2.3 Experimental Design and Treatment of Animals

The rats were divided into two groups A and B. Group A (control rats) consist of 10 rats and were not given any of the drugs except normal rats' feds and water while group B (treated Rats) consist of 25 rats and were fed with rats feed and water in addition with 0.68 ml/kg bodyweight of action bitters administered orally on a daily basis for 30 days using an orogastric gavage tube to ensure satisfactory intake of doses. Treatment was stopped on the 30th day and the animals were fasted overnight. The administered dose was derived from the recommendation of the manufacturer of 50 mls daily of the herbal preparation for an adult human weighing at least 75 kg.

### 2.4 Blood Sample Collection and Preparation

The last dose of the action bitters was administered on the morning of the 30th day. More so, all meals were stopped by 7pm on the 30th day. After an overnight fast, blood samples were collected from the animals (rats) using a 5 ml hypodermic syringe. The rats were anaesthetized with chloroform and before the heart completely stopped beating, 5mls of blood was collected by cardiac puncture into labeled plain bottles without undue pressure to either the arm or the plunger of the syringe. The blood samples were subsequently centrifuged at 4000 rpm for 10 minutes to obtain serum. The sera collected were preserved at -4°C for later analyses of liver and renal indices considered in this study.

### 2.5 Laboratory Analysis of Liver and Renal Indices

Alkaline Phosphatase was determined by colorimetric end-point method as described by

Roy Method [13]. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined based on enzymatic methods as described by Reitman and Frankel, [14]. Urea estimation was also determined based on Berthelot enzymatic method in which urea in the sample is hydrolyzed enzymatically into ammonia (NH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) as described by Fawcett and Scott, [15]. Creatinine was determined by modified Jaffe colorimetric-Kinetic method as described by Bowers and Wong, [16]. Chloride was also determined by method as described by Schrenfeld and Lewellen, [17]. More so, Potassium was determined by colorimetric method as described by Adam and St. John, [18]. Finally, Sodium was determined using modified Muruna and Trinder colorimetric method as described by Arnold and Pray, [19].

## 2.6 Statistical Analysis

Data obtained from the laboratory analysis were statistically evaluated using GraphPad Prism version 5.03 (San Diego, California, USA). Student's t-test, standard deviation and mean were the statistical tools used and results obtained were represented in the form of mean  $\pm$  standard deviation. Inferential statistics made use of student's t-test for the comparative analysis of values from the control and treated

rats as unpaired data. The statistical significance was seen at  $P=.05$ .

## 3. RESULTS

The results of renal indices obtained showed that control rats had values of 9.567 $\pm$ 6.396; 74.59 $\pm$ 40.93, 153 $\pm$ 25.67, 111.7 $\pm$ 20.23 and 5.767 $\pm$ 1.819 for Urea, Creatinine, Potassium, Sodium and Chloride respectively while the treated rats had 11.72 $\pm$ 6.142, 122.9 $\pm$ 82.16, 152.8 $\pm$ 27.57, 116.6 $\pm$ 24.98 and 6.156 $\pm$ 1.843 for Urea, Creatinine, Potassium, Sodium and Chloride respectively. The comparative analysis of the control rats and the treated rats indicated no significant differences in all of the renal function parameters considered (Table 1). More so, when liver enzymes were considered, control rats had values of 134.2  $\pm$  52.07; 35.44 $\pm$  13.82 and 169.2 $\pm$  71.92 for AST, ALT and ALP respectively while actin bitter treated rats had values of 139.4  $\pm$ 44.6; 35.39 $\pm$  14.39 and 196.9 $\pm$  65.92 for AST, ALT and ALP respectively. The comparative analysis of control rats and rats treated with Action Bitters showed no significant differences in AST, ALT and ALP at  $P=.05$ . However, higher values were observed in AST and ALP of treated rats (Table 2). Finally, no obvious changes in the colours of the stool, urine and eye of the action bitters fed rats were seen. In addition, there was absence of sedation, weakness and diarrhea in the treated rats at the 0.68 ml dose daily for 30 days.

**Table 1. Comparative analysis of renal function parameters of control rats and rats treated with action bitters (herbal mixture)**

Parameter	Control rats (n=10)	Treated rats (n=25)	P value	T value	Remark
Urea (mmol/L)	9.567 $\pm$ 6.396	11.72 $\pm$ 6.142	0.40	0.85	NS
Creatinine (mmol/L)	74.59 $\pm$ 40.93	122.9 $\pm$ 82.16	0.11	1.65	NS
Potassium (mmol/L)	153.1 $\pm$ 25.67	152.8 $\pm$ 27.57	0.98	0.03	NS
Sodium (mmol/L)	111.7 $\pm$ 20.23	116.6 $\pm$ 24.98	0.62	0.51	NS
Chloride (mmol/L)	5.767 $\pm$ 1.819	6.156 $\pm$ 1.843	0.61	0.52	NS

NS= Not significant

**Table 2. Comparative analysis of liver enzymes of control rats and rats treated with action bitters (herbal mixture)**

Parameters	Control Rats (n=10)	Treated Rats (n=25)	P value	T value	Remark
AST (U/L)	134.2 $\pm$ 52.07	139.1 $\pm$ 44.62	0.80	0.25	NS
ALT (U/L)	35.44 $\pm$ 13.82	35.39 $\pm$ 14.39	0.99	0.01	NS
ALP (U/L)	169.2 $\pm$ 71.92	196.9 $\pm$ 65.92	0.33	0.99	NS

NS= Not significant

#### 4. DISCUSSION

The results obtained when control group was compared with treated group showed that there was no significantly increase in the urea and creatinine level of the test (i.e. bitters fed rats) when compared to the control group. The non-significant difference seen in urea and creatinine is in line with the work done [20] but disagreed with the findings of [21,22,23]. Anionye et al. [20], reported that creatinine levels of bitters treated rats were not different from the control rats, and therefore super bitters did not interfere with renal capacity of the rats to excrete metabolites. However, Anyasor et al. [21], reported increased oxidative stress on the kidneys when antioxidant and anti-inflammatory properties of polyherbal preparations such as Swedish bitters, yoyo bitters among others were evaluated. More so, Jimmy and Udofia [22], also reported detrimental effect of yoyo bitters on the kidneys with a resultant effect of hypokalemia. The non-significant differences seen in urea and creatinine is an indication that action bitters at this treatment dosages did not interfere with the renal integrity and thus, did not affect its ability to excrete urea and creatinine which are waste products.

When electrolytes were considered, it was observed that sodium, potassium and chloride indicated non-significant differences as well when treated rats were compared with control rats. The non-significant differences seen in the electrolytes (sodium, potassium and chloride) indicates that action bitters did not interfere with the renal capacity to regulate and maintain electrolytes balance in the body. Though, there was a slight increase in sodium ion level in the treated rats compared to the control group. The non-significant increase seen in sodium of the treated rats might suggest a slight effect on sodium pump that maintains the constancy of the extracellular concentration of potassium probably due to water deprivation. Our finding also supports the work of [20,22]. Anionye et al. [20], reported that sodium levels of bitters treated rats were not different from the control rats. More so, Jimmy and Udofia [22], also reported that yoyo bitters did not induce any significant change in the electrolyte levels of diabetics however, hypokalemia (low potassium) was seen. Therefore, the non-significant increase in sodium observed may imply that consumption of action bitters orally may increase plasma sodium levels due to the actions of the flavonoid content of the bitters. The lack of nephro-toxic effect of action bitters at the dosage of 0.68 ml/kg bodyweight

could be as a result of the anti-oxidative properties of plant extracts such as *Symphonia globulifera*, *Tetraplura tetraptera*, *Lannea welwitschii* contained in it. Bella et al. [10], Anionye et al. [20] and Olatokunboh et al. [24], in their respective studies reported that the presence of phytochemicals with anti-oxidative capacities in most polyherbal mixtures are prevents or ameliorate the effects of oxidative stress on organs thus, polyherbal drugs such as action bitters are not associated with renal dysfunctions. Hence action bitters at this dosage could be said not to have a nephro-toxic effect and do not interfere with its capacity to excrete metabolites.

When the effect of action herbal bitters on liver enzymes of albino rats were considered, significant differences were also not observed when the control and treated rats were compared. However, higher values were seen in AST and ALP of the treated rats. The non-significant increase observed in AST, ALT and ALP support the findings of [20]. Anionye et al. [20], reported a non-significant difference in AST, ALT and ALP when super bitters were administered in rats. They further reported that the non-significant difference in AST and ALT indicates that the super bitters did not cause any hepatocellular damage to the liver of the rats. However, the results of AST and ALT observed in our study contradicts the reports of [9,12,23,25]. Chineke et al. [9], reported increases in liver enzymes (AST and ALT) when 2.7 ml/kg bodyweight of herbal bitters were administered to albino rats. More so, Oluoyomi and Bukola [12], revealed that treatment with fijik herbal bitters resulted in a significant increase in levels of AST and ALP whereas the ALT level was significantly reduced thus indicating hepatocyte damage. In addition, Oyewo et al. [23], reported oxidative induced stress on the liver by herbal bitter therefore affecting the hepatic integrity when yoyo bitters were administered in rats at a dose of 0.462 ml/kg bodyweight. Odesanmi et al. [25], reported significant increase in AST and ALT in their study when *Tetrapleura tetraptera* (a major constituent of action bitters) were administered in rats. Adeyemi et al. [6], also reported that administration of yoyo bitters (a similar herbal mixture like action bitters) induced hepatic derangements at 120 mg/kg bodyweight.

Normally, a basal level of these liver enzymes is found in the plasma [6]. However, when there is cellular damage, the enzymes leak out into the

extracellular fluid, thus raising the concentration in the plasma [6]. The levels of these enzymes indicate that within the period of the action bitters intake, the liver enzymes did not increase significantly in the treated rats compared with the control rats at the dose of 0.68 ml/kg bodyweight. This further indicates that action bitters at this dosage does not have potential for inducing liver disease or toxicity.

The non-significant increase seen in ALP and AST could be as a result of the exerted pressure on the liver due to the alcoholic content (40%) of the Action Bitters but not probably strong enough to induce adverse effect on hepatocytes at the dose of 0.68ml/kg bodyweight for 30 days. These findings are in line with the reports of [20]. Anionye et al. [20], also reported According to Alabi et al. [5], phenolic compounds and flavonoids present in plant extracts enhances hepatoprotective activities and not necessarily derangements by preventing oxidative stress. Alabi et al. [5], further reported that administration herbal Bitters drugs at the dose of 15 ml/kg bodyweight reduced lipid peroxidation and oxidative stress in experimental animals. Also, a study by Udenze et al. [4] revealed that treatment with kolaviron, a major component of *Garcinia kola* (a constituent of action bitters), did not affect hepatic marker indices.

In addition, the absence of diarrhea observed in the treated rats could be an indication of the effect of the bark extract of *Lannea welwitschii* which is an ingredients of action bitters. This finding is in line with the reports of [24]. Olatokunboh et al. [24], reported that aqueous extract of *Lannea welwitschii* (an active component of action bitters) produced an inhibitory action on gastrointestinal motility, thus, preventing diarrhea. The absence of sedation and weakness as well as normal nature of the stool, urine and eye of the bitters fed rats implies that the action bitters at 0.68 ml/kg bodyweight were well tolerated by the rats. This finding is also in agreement with the work done by [20]. Anionye et al. [20], also reported no change in color of stool, urine and eye color.

## 5. CONCLUSION

Biochemical parameters such as ALT, AST, ALP, Electrolytes, Urea and Creatinine were not affected by the Action Bitters when administered in Albino rats at the dosage of 0.68 ml/kg bodyweight for a period of 30 days. Therefore, its (action bitters) toxicity and lethality may likely

depend on the dosage and length of consumption.

## 6. RECOMMENDATION

This research investigated the short-term effect of action bitters at lower dose and therefore recommended that further research should also be carried out on the effect of action bitters on the liver and kidney function indices of albino rats using a higher dose and at prolonged period of time.

## 7. LIMITATION OF THE STUDY

The study was for a short period of time and only one test group treated with 0.68ml/kg bodyweight was compared against the control group. More so, no comparison with other doses were done. Therefore, our findings are subject to further research and validation.

## ETHICAL CLEARANCE

We 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 cleared by the ethics committee of the Rivers State University, Port Harcourt.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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