

## Alterations of Evans Healthy Bitters on Some Biochemical Parameters of Wistar Albino Rats

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**Abstract:** This present research deals with the effect of Evans Healthy Bitters on some biochemical parameters of wistar albino rats. Twenty (20) wistar albino rats weighing between 90-110g were divided into four (4) groups (A, B, C, and D) and were allowed to acclimatize to laboratory condition for seven (7) days. Rats in group A served as the control and were administered with normal saline throughout the experimental period of twenty eight (28) days, while rats in group B, C and D were administered oral dosage of 1, 2 and 3 mg/kg body of the drug respectively throughout the experimental period. Twenty four (24) hours after the last administration, the wistar albino rats were sacrificed. Blood was obtained by cardiac puncture for analysis of serum ALP, AST, ALT, total protein, bilirubin, glucose, urea, creatinine,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$  concentrations using standard methods. The drug produced a significant increase ( $P < 0.05$ ) in serum total protein, total bilirubin, conjugated bilirubin, unconjugated bilirubin and glucose levels following the administration of the drug throughout the experimental period. A significant ( $P < 0.05$ ) dosage dependent elevation was also displayed in serum ALP, AST and ALT following the administration of 1, 2 and 3 mg/kg body weight of the drug. The administration of the drug produced a significant ( $P < 0.05$ ) decrease in serum  $\text{HCO}_3^-$  but a significant increase ( $P < 0.05$ ) was shown by serum  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ , Urea and Creatinine concentrations. Despite the therapeutic efficacy of the drug, results suggest clear manifestation that the combined effect of the drug might produce both hepatic and renal injury.

**Key words:** *Evans healthy bitters, hepatic damage, Oral administration, renal injury, therapeutics.*

### INTRODUCTION

The use of plants, plant extracts, essential oil and pure compounds isolated from natural sources provided the foundation of modern pharmaceutical compounds. Nature has been a source of medicinal agent for thousands of years and impressive numbers of modern drugs were isolated from natural sources, many of these isolations were based on the use of the agents in traditional medicine (Doughari *et al.*, 2008). Medicinal plants contain numerous biologically active compounds such as nutrients and phytochemicals which have physiological actions on human bodies (Edeoga *et al.*, 2005; Olokudejo *et al.*, 2008). The active ingredients are used to manage diverse diseases such as diabetes, cancer, diarrhoea, cholera, obesity among others.

Research on alternative and comparative medicine using single or combination of herbal formulations has led to the formulation of Evans Healthy Bitters. Evans Healthy Bitters is a multipurpose herbal formulation or preparation made from *Alhaji cametorum* (22.20mg), *Cassia angustifolia* (16.66mg), *Commiphora myrrha* (5.33mg), *Andrographis paniculata* (22.20mg), *Picrorhizakurroa* (22.20mg), *Aloe barbadens* (40.0mg), *Crocus sativus* (02.00mg).

*Alhajimaurosum* is a species of legumes commonly known variously as *Camelthorn*, *Camthorn-bush*, *Caspian manna* and *peresian manna* plant. It belongs to the family *fabaceae* and medicinally known for its gastro protective, diaphoretic, diuretic, expectorant, laxative, anti-diarrhoea and antiseptic properties and in the management of rheumatism and haemorrhoids (Manm, 2006).

*Commiphora myrrha* (*Buseraceae*) is a sturdy, spiny, glabrous shrub or small tree usually with distinct short trunk up to 4m tall (Swet *et al.*, 2009). Outer bark is slivery, whitish or bluish grey, peeling in large or small papery flakes from the greener under-bark, exudate hardly scented, viscid, producing a hard translucent yellowish gum-resin. (Nada *et al.*, 1997). *Commiphora myrrha* has been known for its antiviral, antibacterial, antidepressant, antidepressive, anti-infection, antiparasitic, expectorant, moderates the thyroid, anti-aphrodisiac and anti-inflammatory; Omer, 2005; Ayedun, 1998; Bando and Manm, 2006; Chudhury, 2006).

*Andrographis paniculata* is a herbaceous plant in the family of *Acanthaceae* with anti-hepatotoxic, anti-malarial, anti hepatitis, anti thrombogenic, anti-inflammatory, antsnake venom, antipyretic properties (Burgos *et al.*, 2009; Thiagarajan *et al.*, 2011; Schultz 2010; Coon and Ernst, 2004).

*Tinospora cordifolia* (menispermaceae) is a large extensively spreading glabrous, perennial deciduous twiner with succulent stems and papery bark leaves simple, alternate, cordate, entire, 7-9 nerves flowers in clusters, female flowers usually solitary fruit. This plant act as antidote to fever, diabetes, dyspepsia, jaundice, urinary problem, skin diseases and chronic diarrhoea and dysentery, heart diseases, leprosy helmenthiasis and rheumatoid arthritis(Sham *et al.*,2001).They are also known for their antioxidant,anti ulcer, hypolipidemic and immunological activities (Grover and Verts,2002,Pahadiya and Sharne,2003).

Evans Healthy Bitters has a characteristics bitter taste and proven potentials against poor digestion, jaundice, chronic constipation, painful digestion, poor appetite, anaemia, immunological disorder among others. Therefore, paucity demands that the effect of this preparation be determined on some biochemical parameters associated with both the kidney and liver since no documented information are available on folkloric usage of this herbal formulation.

## MATERIALS AND METHODS

### **Samples:**

The drug sample (Evans Healthy Bitters) was obtained from Evans Medical Plc,Ogun State ,Nigeria while other samples, kits and reagents used were of analytical grade.

### **Animals And Treatment Protocols:**

Twenty (20) wistar albino rats weighing **90-110g** were used for this work. The animals were obtained from the animal holding unit of the Department of Human Physiology, University of Calabar, Calabar, Cross River State. The animals were housed in plastic cages and were allowed acclimatization period of Seven (7) days in a well-ventilated room with a temperature and pressure of  $29\pm 2$  °C and 70% respectively. The wistar albino rats were maintained with rat chow (Vital Feeds Ltd,Aba) and water *ad libitum*. The animals were exposed to 12hours light -dark cycle and handled according to standard protocols. At the end of acclimatization period, they were divided into four groups A, B , C and D of five (5) rats each. Group A served as control while B ,C and D were the test group .The control group was treated with 0.5mls of normal saline while B - D were treated with 0.5 mls corresponding to 1,2, and 3 mg/kg body weight of the drug(Evan Healthy Bitters) respectively and were administered orally for Twenty eight days (28) days. The wistar albino rats were sacrificed 24 hours after the last administration in accordance with the guide lines of the European Convention for the protection of vertebrate animals and other scientific purposes-ETS-123(European Treaty Series, 2005).

### **Preparation of Serum:**

The animals were anaesthetized in a jar containing cotton wool soaked in ether and chloroform in ratio1:1. When the animal became unconscious, they were brought out quickly of the jar, the abdominal region was opened along the linear Alba and diaphragm cut with scalpel blade to expose the organs and blood was collected into a sterile sample container by cardiac puncture. Blood was collected into a clean, dry centrifuge tube and allowed to clot for 30 min before centrifuging at 300rpm x 10min using Uniscope Laboratory Centrifuge. The serum was thereafter aspirated into clean, dry, sample bottles using Pasteur pipette and was kept or stored in sample bottles and used within 12hours of preparation as described by (Malomo, 2000). Later it was transferred into specimen bottles before being used for biochemical analysis.

### **Statistical Analysis:**

Statistically analysed data used was presented as mean  $\pm$  SD of five (5) determinations. Statement analysis was carved out using one way analysis of variance (ANOVA). Differences were statistically significant at  $P<0.05$  (Mahajan, 1997).

### **Results:**

The results below depict the effect of Evans Healthy Bitters on some serum biochemical parameters. The drug produced a significant increase ( $P<0.05$ ) in serum total cholesterol, total protein, total bilirubin, conjugated bilirubin, unconjugated bilirubin and glucose level/glucose concentration following the administration of the drug throughout the experimental period (Table 1).A significant ( $P<0.05$ ) dosage dependent elevation was also displayed in serum ALP, AST and ALT following the administration of 1,2 and 3mg/kg body weight of the drug (Table 2).The administration of the drug (Table 3) produced a significant ( $P<0.05$ ) decrease in serum  $\text{HCO}_3^-$  and a significant increase was shown by serum  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ , Urea and Creatinine concentration.

**Table 1:** Effect of Evans Bitters on hepatic function parameters

Biochemical parameters	Control Grp (A)	GrpB (Treated with 1mg of drug)	Grp C (Treated with 2mg of drug)	Grp D (Treated with 3mg of drug)
Total Protein(g/dl)				
Albumin (g/dl)	82.5±0.63	101.8 ±0.37*	105.7± 0.2*	111.2±0.3*
Globulin (g/dl)	42.0±0.15	53.8± 0.42	59.2 ± 0.48*	66.4 ±1.1*
Total Bilirubin	33.6± 0.40	47.6± 0.67	45.8± 0.20	53.2± 0.29
Conjugated Bilirubin (mmol/L)	4.58± 0.20	5.02±1.66	6.4±1.10*	6.4± 1.10
Unconjugated Bilirubin(mm/l)	2.2±0.06	2.06 ±0.30	2.50± 0.21	3.32± 0.33*
Bilirubin(mm/l)	2.38± 0.05	2.96 ±0.04	2.75± 0.11	3.32± 0.84*
Glucose(mmol/L)	4.12±0.04	19.46±0.04*	8.12±0.04	12.32±0.45*

Results are expressed in mean ± SEM (n=5). \* Significant at P<0.05 compared with the control

**Table 2:** The effect of differing dosage of the drug (Evan Healthy Bitters) on some serum enzymes

Serum Enzymes	Grp A (Control)	Grp B (Treated with 1mg of drug)	Grp C (Treated with 2mg of drug)	Grp D (Treated with 3 mg of drug)
AST(IU/L)	66.8± 0.20	67.2±1.1	71.2±0.9*	77.8± 1.0*
ALT(IU/L)	54.0± 0.1	54.4± 3.4	58.6±2.4*	64.8± 1.3*
ALP(IU/L)	226.8±1.7	236.2±1.2	248.0± 0.3	251.6±0.9

Results are expressed in mean ± SEM (n=5). \* Significant at P<0.05 compared with the control

**Table 3:** Effect of Evans Healthy Bitters on the Electrolyte profile

Serum Electrolyte	Grp A (Control)	Grp B (Treated with 1 mg of drug)	Grp C (Treated with 2mg of drug)	Grp D (Treated with 3mg of drug)
Na <sup>+</sup> (mmol/L)	132.6±0.24	134.8±0.37	137.96±0.04*	134.84±0.46
K <sup>+</sup> (mmol/L)	2.20± 0.27	5.11±0.27*	6.18±0.04*	5.68±0.07*
Cl <sup>-</sup> (mmol/L)	105.96±0.74	107.6±0.74	111.6±0.4*	107.2±0.48
HCO <sub>3</sub> <sup>-</sup>	15.0±0.2	14.36±0.41	13.0±0.06*	14.8±0.48
Urea (mmol/L)	0.68±0.2	0.74±0.24	0.69±0.20	0.88±0.2*
Creatinine (mmol/L)	78.6±0.24	78.8±1.2	79.8±0.2	108.8±0.48. *

Results are expressed in mean ± SEM (n=5). \* Significant at P<0.05 compared with the control

### Discussion:

The biochemical indices studied in this research are sensitive and useful parameters to indicate the alterations caused by the drugs on the hepatic and renal functional capacity or integrity of the rats. The hepatocyte membrane distortion is associated with membrane leakage of the hepatocyte cytosolic contents which is manifested by significant elevation of serum/plasma enzymes of acute hepatocellular damage namely ALT, AST and ALP as a marker hepatocellular damage (Bhaltacharyya *et al.*, 2003). However of this marker enzymes, ALT is the most reliable. AST is known to be abundant in the cardiac muscles, skeletal muscles, kidneys and testes. Thus, any disease state affecting any of these extra hepatic tissue significantly elevates the serum level of enzymes (Olayinka and Ore, 2013). Therefore, the observed significant increase in serum ALT, AST and ALP when compared with the control at dosages of the treated animals suggest that the drug might induce hepatic damage or hepatotoxicity. These findings are similar to the findings of other researchers (Farombi *et al.*, 2000; Obi *et al.*, 2004; Pari and Anuli, 2005; Dasofunjo *et al.*, 2012). Likewise, the significant increase in the serum level of both total bilirubin, unconjugated and conjugated bilirubin is an indication that the drug might induce injury to the hepatic tissue or caused conjugated hepatobiliary injury on the wistar albino rats. Likewise, the increase in serum glucose level suggest that the drug may hamper the glucose metabolism which might on prolonged administration pose injury to the beta cells of the leading to diabetic conditions.

Renal function tests are usually required to assess the normal functioning units of the kidney the nephron. Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids. Due to their ability to dissociate readily into their constituent ions or radicals, they comprise the single most important factor in the transfer and movement of water and electrolyte: between the divisions of extracellular and Intracellular components (Zilva *et al.*, 1991; Dasofunjo *et al.*, 2013).

The significant increase in serum Sodium ion concentration following oral administration of the drug may be due to excessive loss of heat from the body fluid. It may also be attributed to increased production of aldosterone to other mineral corticoids which will in turns increase the reabsorption of sodium ion concentration (Dasofunjo *et al.*, 2012). Aldosterone can achieve this since its action on the membrane aldosterone receptors has been linked to stimulating Na<sup>+</sup> /H<sup>-</sup> exchanger (Ganong, 2001). Also, the significant increase in serum potassium concentration observed in this study suggests a possible inimical effect on the sodium pump that

maintains the constant of the extracellular potassium. Serum chloride and Bicarbonate ions are group of electrolytes that can be used to assess renal functions therefore, the significant increase in serum chloride and decrease in bicarbonate ions at various doses may be an indication of tubular glomerular function. It might also be that the drug induces a pathological condition resulting in impairment on renal function.

Urea is the major nitrogen containing metabolic product of protein catabolism. The significant elevation in serum urea concentration following the administration of drug at various doses may be attributed to impairment on the urea cycle leading to reduced production of the metabolic product (Yakubu *et al.*, 2003). This is an indication of abnormality in the physiological excretion of urea caused by non-renal factor which is the drug in this study. The consistency of endogenous creatinine production and its release into the body fluids at a constant rate and constancy of plasma levels of creatinine 24 hours of the day, makes creatinine a useful endogenous substance where clearance may be measured as an indication of creatinine content of the serum following the administration the drug may be an indication of glomerular and tubular mass dysfunction. Renal damage reduces the functioning of the tubular mass and may seriously affect the regulatory function (Chawla,1999). The biochemical indices monitored in the kidney are useful 'markers' for the assessment of tissue damage (Dasofunjo, 2012). The measurement of activities of various enzymes in the tissue and body fluids play significant role in disease investigation and diagnosis (Malomo, 2000) assault on the tissue due to toxicity of the drug (Yakubu, 2003). Despite of the therapeutic efficacy of the drug, results suggests clear manifestation that the combine effect of the drug might produce both hepatic and renal injury that is capable of causing renal or bile duct obstruction, intra hepatic cholestasis or infiltrative disease of the liver or the kidney. Therefore, diabetic patients should exercise caution on the usage of this drug but administration at lower dosage might be safer.

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