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LONG TERM ADMINISTRATION OF KOLAVIRON ON BILE SECRETION AND BILIARY COMPOSITION IN HERBAL ALCOHOL-TREATED WISTAR RATS

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ABSTRACT

This study was aimed at investigating the effect of long term administration of kolaviron and herbal alcohol on rate of bile secretion and biliary composition in Wistar rats. Fifteen (15) Wistar rats of female sex were weighed (83-150g). They were housed in modern cages under standard temperature condition of (± 25^0C) and the room was properly ventilated. The animals were given rat chow (type of feed) and provided with water throughout the duration of the experiment. The animals were divided into three (3) groups: **Group I:** control-0.2ml normal saline; **Group II:** Herbal alcohol (0.3mg/kg); **Group III:** Herbal alcohol (0.3mg/kg) +kolaviron (100mg/kg). After 21 days of administration of kolaviron and herbal alcohol, the rats were starved for 18 hours. Thereafter, the rats were weighed and anaesthetized with urethane (6mg/100g body weight). A laparotomy was performed and the bile was collected for 3 hours for each rat into a sample bottles. Alcohol+kolaviron treatment group was significantly higher ($P<0.05$) in bile secretion and bicarbonate compared to the control and herbal alcohol group. The herbal alcohol dose had significantly lower ($P<0.05$) cholesterol compared to the control**.** Herbal alcohol +kolaviron dose significantly increased ($P<0.05$) conjugated bilirubin, total bilirubin but decreased ($P<0.05$) sodium and potassium compared to the control, and herbal alcohol group. In conclusion, extract of kolaviron and herbal alcohol may boost biliary secretion and composition and can help reduced the conjugated bilirubin and total bilirubin levels.

Keywords: Kolaviron, Herbal Alcohol, Bile, Bilirubin, Garcina Kola

1.0: INTRODUCTION

1.1: BACKGROUND OF STUDY

Kolaviron is an extract of *garcinia* kola seeds which has anti-inflammatory activity and has been used in the treatment and prevention of diseases like bronchitis, diarrhea (Adedara *et al*, 2012). Kolaviron an extract (*Garcinia kola*) is an indigenous to Cameroon, Nigeria and other parts of central and West Africa, where it grows in the rain forests of west Africa mostly in sub-tropical or tropical moist low land forest (Ayepola *et al*, 2014). The rapid growing tree of bitter kola was utilized by the ancient Romans Greeks and Egyptian it is now widely cultivated and has become naturalized in many locations in tropics (Fahey, 2005). *Garcinia* kola is a tropical flowering plant which belong to the family of, Guttiferal. It is an herb grown in Nigeria with a characteristic bitter and resinous taste, the seed is eaten raw by people with the belief that it promotes longevity. Extracts of the plant (Kolaviron) are used in traditional African medicine for the treatment of laryngitis, cough and liver diseases (Adedara *et al,* 2012). Bitter kola is used by traditional men and women for entertainment, ritual practices, festival, and traditional marriages. The plant has been reported to have medicinal properties, although many have not been scientifically substantiated. It has been used in the treatment and prevention of disease such as bronchitis, diarrhea and throat infections by the local population. The most active ingredient of Bitter Kola is Kolaviron. Several investigations using Kolaviron have shown its potential for use in chemoprevention and protection against many health threatening diseases of the liver (Faromi *et al*, 2013) and brain (Adaramoye, 2012). The demonstration of dietary Kolaviron supplementation reduces cardial infarct size and improves myocardial function.

Human nutritional studies has shown that kolaviron has several benefits. According to Fahey 2005, nutritional properties of koloviron have been discovered that there seems to be little doubt of the substantial health benefits to be realized by consumptions of bitter kola in situation where starvation is eminent. Bitter kola contains a high source of vitamins such as Vitamin A, C, E, B1,

B2, B3, and fibres, calcium, potassium and ions. They are used to treat impotence, improve sexual performances, promotes male fertility, prolong life, Anti poison effect, improve immunity, remedy for cold, relives Arthritis, throat cleaning, improves lung function, prevent Glacoma, Malaria, remedy for osteo-arthitis.

JUSTIFICATION OF STUDY

The use of herbal alcohol and kolaviron (in the form of bitter kola) is very rampant particularly in rural areas of Cross River State, Nigeria. Sometimes it is taken alone or in combination for recreational and medical purposes. Little or no research has been done on the effects of long term administration of these drugs could result to this on rate of bile secretion and composition of bile, thus the need for this study.

3.1: MATERIALS

The materials used for this research work include the following:

- Electronic weighing balance
- Electronic blender
- Cotton wool
- Distilled water
- Albino Wistar rats
- 2ml& 1ml syringes
- Sample bottles
- Dissection set
- Hand gloves
- Cannula for rats

- Urethane
- Herbal alcohol (1960)
- Kolaviron
- Cages
- Feed (vital feed)
- Disinfectant (dettol)
- Methylated spirit
- Tissue paper
- PH meter
- Tween 80 solution

3.2 COLLECTION OF PLANT MATERIAL

Fresh seeds of Garcinia kola were purchase from from Bodiga market in Ibadan, Oyo state, Nigeria. At the department of Botany, University of Ibadan. A voucher specimen (FHI-109777) is available at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan. There was ethical approval of the work by the ethical committee, University of Cross River State.

3.3 EXPERIMENTAL ANIMALS

Fifteen (15) albino Wistar rats of female sex were obtained from the disease free stock of the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, (UNICROSS) Okuku Campus, Cross River State, Nigeria. The animals weighed between 83-150 body weights, and were allowed to acclimatize for one week. They were housed in modern cages under standard temperature condition of (± 25^{0}C) and the room was properly ventilated. The animals were given rat chow (type of feed) and provided with water throughout the duration of the experiment.

3.3.1 ANIMAL GROUPING

The animals were divided into three (3) groups respectively as shown below:

Group I: control.

Group II: Herbal alcohol (0.3mg/kg).

Group III: Herbal alcohol (0.3mg/kg) +kolaviron (100mg/kg).

Each group consisted of Fifteen (15) rats, all the animals were allowed free access to food and water daily, were housed in different cages for proper identification.

3.4 PREPARATION OF EXTRACTION FRACTION

Garcinia kola seeds were peeled, sliced and air-dried (25-28^{0}C). Kolaviron was isolated according to the method of Iwu *et al* 1990. The powdered seeds (600g) were defatted with 800ml of light petroleum ether (bp 40-60^{0}C) in a soxhlet for 24 hour (Adaramoye, 2013). The defatted powder was spread in thin layers on trays and air dried at room temperature for 24 hour, repacked in the soxhlet and extracted with acetone (500ml) at a temperature of 40$^{\mathbf{0}}$C. The extract was concentrated and diluted twice its volume with distilled water and extracted with ethylacetate. The concentrated ethylacetate yielded kolaviron (12g).

3.5 KOLAVIRON ADMINISTRATION

Kolaviron were administered to the rats at 100mg/kg rat body weight using 1ml syringe, by using an oropharyngeal cannula. Kolaviron was administered once a day for 21 days, the animals were allowed free access to food and water.

3.6 HERBAL ALCOHOL ADMINISTRATION

Herbal alcohol (1960) were administered to the rats at 0.3ml/100g body weight using 2ml syringe, by using an oropharyngeal canula. The herbal alcohol was given once a day for the 21 days and the animals were allowed free access to food and water.

3.7 COLLECTION OF BILE

After 21 days of administration of kolaviron and herbal alcohol, the rats were starved for 18 hours. Thereafter, the rats were weighed and anaesthetized with urethane (6mg/100g body weight). They were fastened into the dissecting board for tracheotomy to clear the dead space. An incision was made along the linear alba to expose the stomach. A laparotomy was performed and the liver lobes deflected anterolateral to expose the common bile duct. The common bile duct was then cannulated (0.5mm in diameter) after a small incision was made. The cannula was fastened with a white cotton thread. The bile was collected for 3 hours for each rat into a sample bottles.

3.8: BILIARY COMPOSITION DETERMINATION

3.8.1: DETERMINATION OF BILIARY SODIUM AND POTASSIUM

Sodium and potassium in the bile were determined using a flame photometer (model 410C, Petra court Ltd England). The bile was sprayed into a non-luminous gas flame which became colored by the characteristic emission of metallic ions in the sample. The wavelength of the metals 598nm and 767nm for sodium and potassium respectively, were selected by the light prism system and allowed to fall on a photosensitive detection system. The amount of light emitted is dependent on the concentration of the metallic ion present by comparing the amount of light emitted from the sample with that from the standard solution. The amounts of sodium and potassium were determined.

3.8.2: DETERMINATION OF BICARBONATE IN BILE

Bile bicarbonate ion was measured by a modified method (Rifai, 2023). Phosphoenol pyruvate carboxylase catalysts the reaction between phosphoenol pyruvate and carbon dioxide (bicarbonate) reduced nicotinamide adenine dinucleotide (NADH) to NAD+ the reaction is catalyzed by malate dehydrogenase. This results in decrease absorption at 340nm that is directly proportional to CO2 concentration in the sample. Into 250ml conical flask was place 5ml of CO2- free distilled water. Zero point 2ml (0.2ml) of plasma or serum, two drops of indicator and 2ml H2SO4 or HCl were added and mixed. The mixing and agitation was done for 90s and quickly titrated with NaOH using 1ml automatic burette until pink end-point appears.

3.8.3: DETERMINATION OF BILIARY CHLORIDE

Biliary chloride ion was determined by the principle of end point titration. 2ml of buffer solution was place in a conical flat and 0.2ml of bile was added and mixed. Four drops of diphenyl carbazine indicator was added. This was titrated with mercuric nitrate from a 2ml micropipette. The end point was indicated by a violet color, the standard solution (0.2ml) was added to 2ml of buffer solution with the indicator and similarly titrated. Biliary chloride concentration was obtained from the following calculation and expressed in mEq/L:

Calculation: Biliary chloride (mEq/L) = $\frac{\text{Titrated values of test} \times 100}{\text{Titrated value of standard}}$

3.8.4 DETERMINATION OF BILIARY BILIRUBIN CONCENTRATION

Biliary bilirubin concentrations were determined by colorimetric method. Total bilirubin is the sum of conjugated and unconjugated bilirubin. Conjugated bilirubin reacts with diazotized sulfanilic acid in alkaline medium to form the blue color complex. The total bilirubin is determined in the presence of caffeine which releases albumin bound bilirubin by reacting with diazotize sulfanilic acid. The color produced is read spectrophotometrically at 530-560mm.

3.8.5 EXTRACTION OF CHOLESTEROL FROM BILE SECRETION

Cholesterol was extracted from the bile following the principle of esterification reaction.

Free cholesterol and cholesterol ester are released from lipoproteins by the action of surfactant. Then both existing free cholesterol and cholesterol released from its ester by cholesterylester hydrolase are oxidized by cholesterol oxidase. The resulting hydrogen peroxide reacts in the presence of peroxidase with the chromogenic system 4-ammine antipyrenes phenol to produce a red color compound. The red Quinone formed is proportional to the amount of cholesterol present.

3.8.6 STATISTICAL ANALYSIS

Data obtain were analyzed using one way analysis of variance (ANOVA) followed by post hoc test at $P<0.05$. Statistical package for social science (SPSS) software version 20.0 was use for the analysis and Microsoft Excel 2010 was employed for graphs plotting.

CHAPTER FOUR

RESULT

TABLE 1

Effect of long term administration of Kolaviron and Herbal Alcohol Beverages consumption on bile secretion and bile composition in albino Wistar rats

Parameters	**Normal control**	**Herbal Alc.**	**Herbal**
Alc+Kolaviron			
Total bilirubin	6.25 ± 0.14^{a}	8.47 ± 0.9^{b}	9.30 ± 0.5^{c}
Conjugated bilirubin	3.80 ± 0.23^{a}	4.53 ± 0.15^{b}	5.50 ± 0.12^{c}
Cholesterol	2.20 ± 0.12^{a}	0.96 ± 0.01^{b}	1.40 ± 0.06^{c}
Sodium	140.00 ± 0.00^{a}	142.67 ± 0.67^{b}	139.00 ± 0.58^{bc}
Potassium	5.20 ± 0.23^{a}	4.60 ± 0.12^{b}	4.50 ± 0.58^{ab}
Chloride	106.00 ± 1.16^{a}	102.33 ± 0.33^{b}	98.67 ± 0.67^{c}
Bicarbonate	21.00 ± 0.58^{a}	24.33 ± 0.33^{b}	26.33 ± 0.33^{c}

Values are expressed as Mean ± SEM. Normal control = group administered normal saline; Herbal Alc. = group administered herbal alcohol (0.3ml/g) and Herbal Alc+Kolaviron = group administered herbal alcohol (0.3ml/g) and kolaviron extract (100ml/kg). Different letters in a row = significantly different ($P<0.05$); the same letters = non-significantly ($P>0.05$). n=5

4.1: BILE SECRETION

The mean bile secretion value for control was 0.18±0.02 mmol/L while the herbal alcohol dose and kolaviron+herbal alcohol dose were 0.18±0.01mmol/L and 0.3±0.05mmol/L respectively. The herbal alcohol dose was significantly higher ($P<0.05$) than the control. The alcohol+kolaviron dose was significantly higher ($P<0.05$) compared to the control and herbal alcohol group.

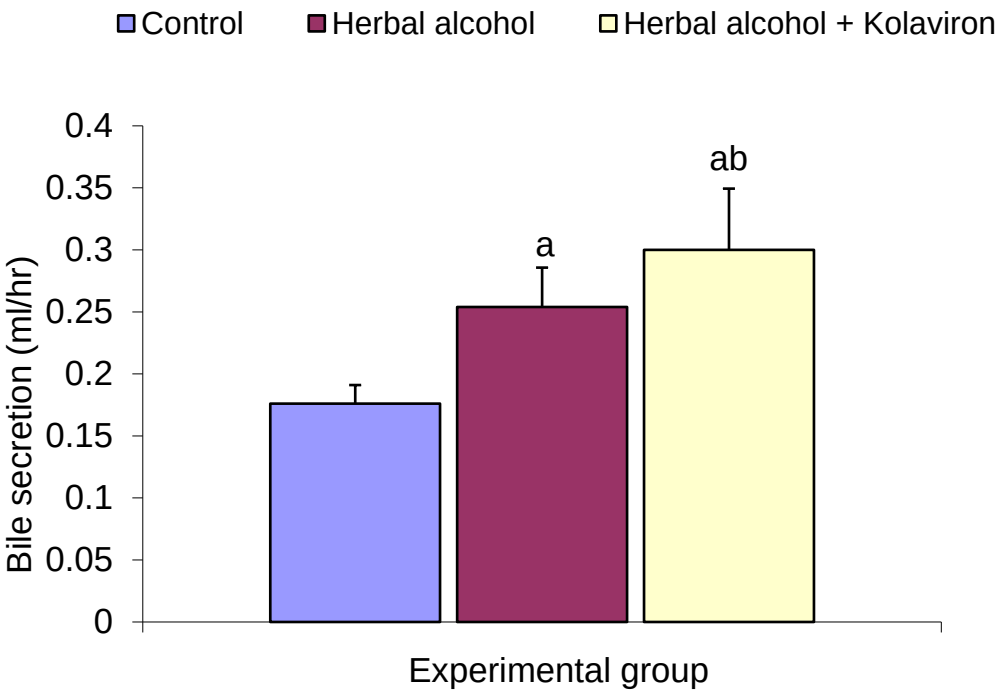

Figure 1: Comparison of rate of bile secretion in the different experimental groups.

Values are expressed as mean + SEM,
a= p<0.05 vs control
b = p<0.05 vs herbal alcohol + kolaviron

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4.4: RESULT FOR CHOLESTEROL

The mean cholesterol value for control was 2.20 ±0.12mmol/L while the herbal alcohol dose and kolaviron+herbal alcohol dose were 0.96±0.01mmol/L and 1.40±0.06mmol/L respectively. The herbal alcohol dose was significantly lower ($P<0.05$) compared to the control. Significant difference was noted between herbal alcohol dose vs herbal alcohol +kolaviron.it was significantly increase ($P<0.05$).

4.3: RESULT FOR CONJUGATED BILIRUBIN

The mean conjugated bilirubin value for control was 3.80±0.23mmol/L while the herbal alcohol dose and kolaviron+herbal alcohol dose were 4.53±0.15mmol/L and 5.50±0.12mmol/L respectively. The herbal alcohol dose was significantly increased ($P<0.05$) compared to the control. Herbal alcohol +kolaviron dose was also significantly increased ($P<0.05$) compared to the control, and herbal alcohol dose.

4.2: THE RESULT OF TOTAL BILIRUBIN

The mean total bilirubin value for control was 6.25±0.14mmol/L while the herbal alcohol dose and kolaviron+herbal alcohol dose were 8.47±0.9mmol/L and 9.30±0.05mmol/L respectively. The herbal alcohol dose was significantly increased ($P<0.05$) compared to the control. Herbal alcohol +kolaviron dose was also significantly increased ($P<0.05$) compared to the control, and herbal alcohol dose.

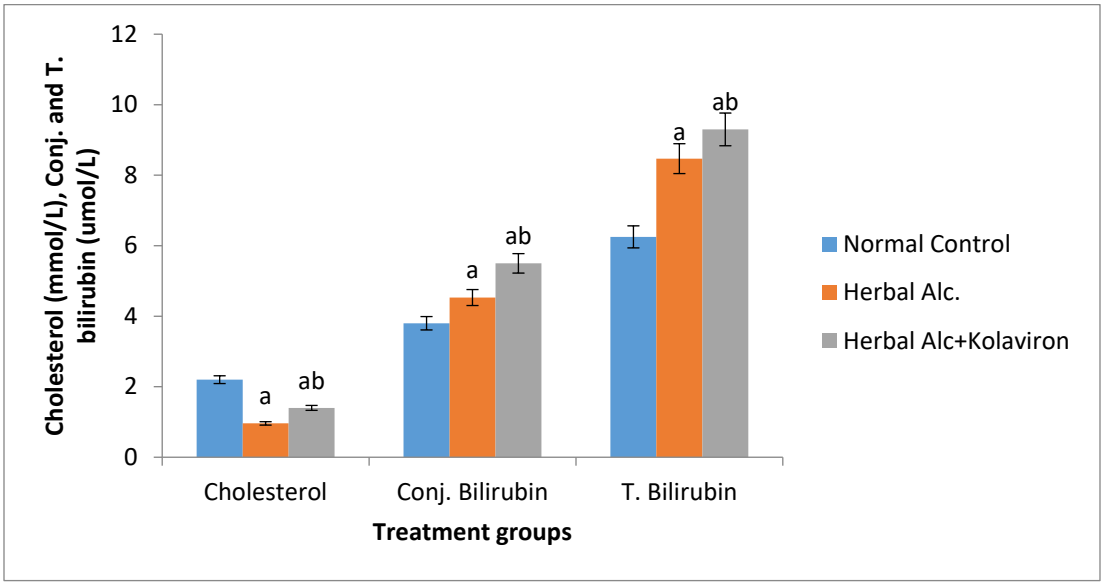

Figure 2: Effect of administration of Herbal alcohol beverages and Kolaviron on bile cholesterol (mmol/L), Conjugated bilirubin and Total bilirubin (µmol/L)

Values are expressed as Mean ± SEM. Normal control = group administered normal saline; Herbal Alc. = group administered herbal alcohol (0.3ml/g) and Herbal Alc+Kolaviron = group administered herbal alcohol (0.3ml/g) and kolaviron extract (100ml/kg). a = significantly different from normal control group ($P<0.05$) and b = significantly different from herbal alcohol group ($P<0.05$).

4.6: RESULTS FOR POTASSIUM

The mean potassium value for control was 5.20±0.23mmol/L while the herbal alcohol dose and kolaviron+herbal alcohol dose were 4.60±0.12mmol/L and 4.50±0.058mmol/L respectively. The herbal alcohol dose and kolaviron + herbal alcohol does were significantly decreased ($P<0.05$) compared to control.

4.7: THE RESULT OF CHLORIDE

The mean chloride value for control was 106.00±1.16mmol/L while the herbal alcohol dose and kolaviron+herbal alcohol dose were 102.33±0.33mmol/L and 98.67±0.67mmol/L respectively. The herbal alcohol dose was significantly decrease ($P<0.05$) compared to control. Herbal alcohol +kolaviron dose significantly decreased ($P<0.05$) compared to the control and herbal alcohol alone.

4.5 THE RESULT OF SODIUM

The mean sodium value for control was 140.00±0.00mmol/L while the herbal alcohol dose and kolaviron+herbal alcohol dose were 142.67±0.67mmol/L and 139.00±0.58mmol/L respectively. Herbal alcohol test group was significantly increased ($P<0.05$) compared to control.

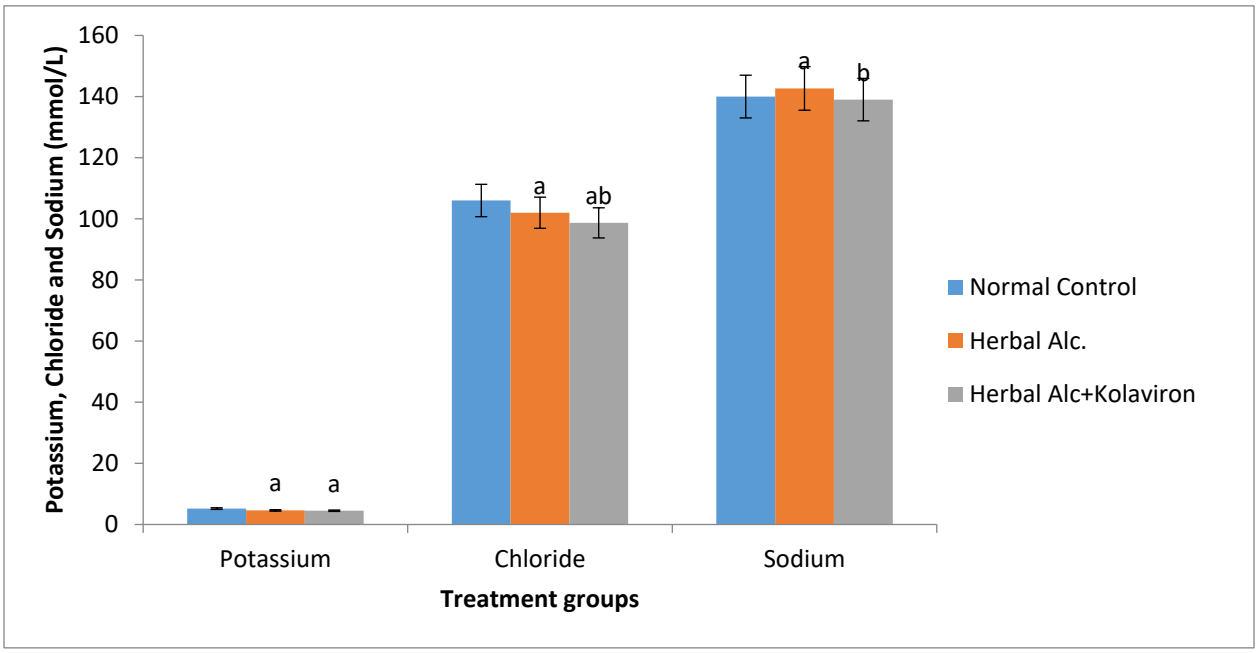

Figure 3: Effect of administration of Herbal alcohol beverages and Kolaviron on bile Potassium, Chloride and Sodium (mmol/L)

Values are expressed as Mean ± SEM. Normal control = group administered normal saline; Herbal Alc. = group administered herbal alcohol (0.3ml/g) and Herbal Alc+Kolaviron = group administered herbal alcohol (0.3ml/g) and kolaviron extract (100ml/kg). a = significantly different from normal control group ($P<0.05$) and b = significantly different from herbal alcohol group ($P<0.05$).

4.8: THE RESULT OF BICARBONATE

The mean bicarbonate value for control was 21.00±0.58mmol/L while the herbal alcohol dose and kolaviron+herbal alcohol dose were 24.33±0.33mmol/L and 26.33±0.33mmol/L respectively. The herbal alcohol dose was significantly higher ($P<0.05$) compared to control. Herbal alcohol +kolaviron dose significantly higher ($P<0.05$) compared to control.

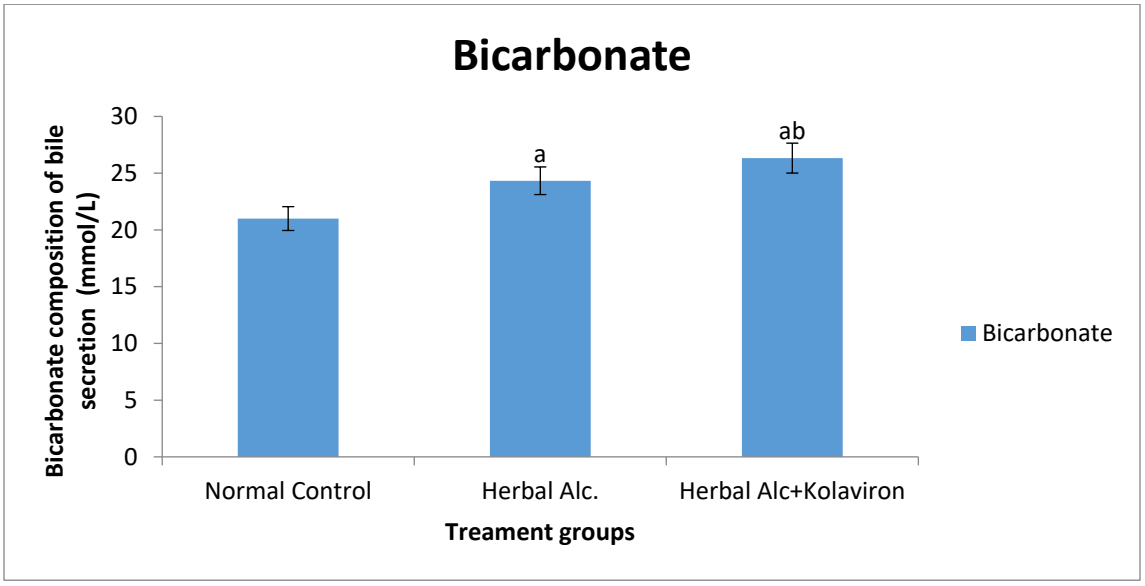

Figure 4: Effect of administration of Herbal alcohol beverages and Kolaviron on bile Bicarbonate, Chloride and Sodium (mmol/L)

Values are expressed as Mean ± SEM. Normal control = group administered normal saline; Herbal Alc. = group administered herbal alcohol (0.3ml/g) and Herbal Alc+Kolaviron = group administered herbal alcohol (0.3ml/g) and kolaviron extract (100ml/kg). a = significantly different from normal control group ($P<0.05$) and b = significantly different from herbal alcohol group ($P<0.05$).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1: DISCUSSION

This study was aimed at investigating the effect of long term administration of kolaviron and herbal alcohol on the rate of bile secretion and biliary composition in albino Wistar rats. After 21 days of administration, the result of the study showed that treatment with kolaviron and herbal alcohol increased bile secretion and bile composition of Wistar rats.

Kolaviron (an extract of *Garcina kola*) has been shown to have beneficial effects on biliary composition like, total bilirubin (TB), conjugated bilirubin (CB), cholesterol, sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3) (Rotimi *et al*., 2022). Kolaviron has also been reported to have antitussive effects and to attenuate hypercholesterolemia in rodents (Ozolua *et al*., 2016). Alcohol has also been shown to have effect on cholesterol. Bitter kola increases bile secretion (Okwu, 2004)**.** The rate of bile secretion in group II (herbal alcohol) and group III (herbal alcohol +kolaviron) significantly increased when compared to control. Bile acts as a detergent to emulsify fats. Treatment with kolaviron further increased bile secretion. This result is biologically plausible because kolaviron improves hepatic function and protects the hepatocytes, which may enhance bile formation (Farombi, 2000), but direct evidence of the choleretic effect has not previously been established.

The result of this study shows that bilirubin increased in the treated groups. Bilirubin is formed in the body mostly by the breakdown haemoglobin (Hall & Hall, 2021). Total bilirubin was significantly higher in the test groups II (herbal alcohol) and III (herbal alcohol +kolaviron) compared to control. Treatment may have altered the physiological state of the rats, leading to an increased degradation of the hemoglobin to form bilirubin. Conjugated bilirubin was also

significantly increased in the test groups compared to control. It follows that an increase in total bilirubin should also lead to an increased level of the level of conjugated bilirubin. It can be opined that herbal alcohol (1960 rootz) may lead to an increase in the formation of bilirubin, further study is required to elucidate the complete relationship. Cholesterol concentration, Potassium and chloride were significantly reduced in the test groups compared to control. Sodium and bicarbonate were significantly higher in the test groups compared to control. The increase in concentration of sodium and bicarbonate may be attributed to the high rate of bile secretion from the gall bladder, which did not allow time for reabsorption of this electrolytes. Increase in bicarbonate concentration is important to neutralize acidic chyme entering the duodenum from the stomach, thereby increasing intestinal pH, which is optimal for fats digestion (Barrett et al., 2023). This results indicates that kolaviron and herbal alcohol extract may afterall be beneficial.

5.2: CONCLUSION

In conclusion, extract of kolaviron and herbal alcohol may boost biliary composition especially sodium and bicarbonate, and may increase bile secretion rate, reduce the conjugated bilirubin and total bilirubin levels. Hence, the acclaimed use of the extract in our domestic homes as spices in food may be beneficial as it helps maintain or increase bile secretion.

DECLARATION OF CONFLICT OF INTEREST

Authors hereby declare that there is no conflict of interest.

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