British Journal of Pharmaceutical Research
4(3): 352-361, 2014

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Reduction in Serum Bilirubin Concentration Following Administration of Crude Leaf Extract of *Viscum album* (Mistletoe) in High Salt Fed Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OOE and NVU designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ENM managed the analyses and literature search. All authors read and approved the final manuscript.

Original Research Article

Received 4th October 2013
Accepted 31st October 2013
Published 3rd December 2013

ABSTRACT

Aims: This study was designed to determine the effect of a high salt diet on serum bilirubin concentration and to ascertain the impact of treatment with *Viscum album* on same.
Methodology: Twenty male albino wistar rats weighing 150 - 200g were used for this study. After seven days of habituation, the animals were randomly divided into four (4) groups of five rats each. Group 1 (NC) served as control and were fed with normal rat pellet and water; group 2(NT) served as the control treated group (administered 150 mg/kg *Viscum album* orally, in addition to rat pallet and water); group 3 (SF) served as the high salt diet fed group (without treatment), while group 4 (ST) served as the high salt diet fed group, treated orally with 150 mg/kg *Viscum album*. The feeding regimen lasted for six weeks, after which the animals were sacrificed and blood samples collected for analysis.
Results: Mean serum total bilirubin concentration was significantly ($p<0.001$) higher in the SF group compared to control, NT and ST group. It was also significantly ($p<0.001$) lower

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in the ST group compared to SF group. Serum conjugated bilirubin concentration was significantly ($p<0.001$) increased in the SF group compared to NC, NT and ST group. It was also significantly ($p<0.001$) reduced in the ST group compared to the SF group. Serum unconjugated bilirubin concentration was significantly ($p<0.001$) increased in the SF group compared to NC, NT and ST group. Serum unconjugated bilirubin concentration was significantly ($p<0.01$) lower in the ST group compared to SF group.
Conclusion: Our results are indicative of the fact that oral administration of *Viscum album* reduces serum bilirubin concentration in high salt fed animals.

Keywords: Bilirubin; serum; sodium chloride; Viscum album.

1. INTRODUCTION

Viscum album, also called mistletoe, belonging to family Loranthaceae, is an evergreen semi-parasitic plant that grows primarily on the branches of deciduous trees. It is widely distributed throughout Europe, North Africa, Austria, Asia and also in Nigeria. It is used traditionally in the treatment of diabetes mellitus, epilepsy, cholera, wounds, asthma, cancer, tumor, anxiety, amenorrhea, atherosclerosis and headache associated with hypertension [1,2,3].

Although the leaf extracts of *Viscum album* have been reported to be beneficial, Ben et al. [4] had reported that the aqueous leaf extract of *Viscum album* may increase plasma total cholesterol in rats. Sjur et al. [5] in another study reported the presence of some toxic phytoconstituents of the plant extract, like viscumin (lectin) and ricin. Lectins are cytotoxic, and act by inhibiting protein synthesis at the ribosomal level. These findings call for close monitoring of biochemical and physiological indices in people who are exposed to leaf extracts of *Viscum album*.

Salt is a vital constituent of our daily diet. Most often referred to as sodium chloride, it gives food a unique taste. There are increasing evidences that high and persistent consumption of salt through diets may contribute to elevated blood pressure [6,7,8], stroke volume, bone demineralization [9], asthma and fluid retention [10].

High salt loading in experimental animals has been reported to cause endothelial dysfunction [6,11], increase in plasma brain natriuretic peptide concentration, perivascular inflammation [12,13], down regulation of cytochrome P-450 in the brain of stroke-prone hypertensive rats, deactivation of ATP-sensitive potassium channels and Na^+- K^+ ATPase pump on the vascular smooth muscle membrane [14]. High salt diet has been associated with increased red blood cell count, packed cell volume and haemoglobin concentration [15].

As red blood cells attempt to squeeze through the capillaries of the reticuloendothelial system, the membranes of the aged cells (cells over 120 days old) become ruptured, leading to the release of hemoglobin in the process [16,17]. The released haemoglobin undergo a series of reaction leading to the formation of bilirubin. In addition to red blood cell rupture, bilirubin is also formed by breakdown of catalase, myoglobin, tryptophan pyrrolase, peroxidase and cytochromes [17,18,19]. About 20 percent of the daily bilirubin production is contributed by hemoproteins and a rapidly turning-over small pool of free heme [20] while the remaining 80 percent of the daily bilirubin production is derived from hemoglobin [20].

Increased bilirubin formation is observed in conditions associated with liver damage, presence of immature erythrocytes in circulation, and in all conditions associated with increased red blood cell turnover [18].

The liver conjugates bilirubin and excretes it as bile pigment through its detoxification and excretory function [19,20]. However, not all the bilirubin molecules are conjugated by the liver. The unconjugated fraction forms unconjugated bilirubin [20]. Under normal conditions, this process is highly efficient, thus plasma unconjugated bilirubin concentrations remain at low levels. Intestinal bacteria degrades bilirubin into urobilinogen, most of which is absorbed from the intestine and undergoes enterohepatic recirculation [20].

Some studies have proposed that increased serum levels of bilirubin may be beneficial in treatment of some form of cancer and gastric ulcer by virtue of its antioxidant effect [19,21]. Despite these acclaimed benefits of mild hyper bilirubinemia, several hazardous effects still exist. Bilirubin is toxic to the central nervous system and may cause a sequence of neurological symptoms called acute bilirubin encephalopathy [22]. Although hyperbilirubinemia is most frequently reported in infants, as seen in jaundice and kernicterus, it is becoming pronounced in adults, with the likely causes being, but not limited to liver damage, hemolysis and Gilbert syndrome [22,23].

Following wide range of reports outlining the detrimental effects of increased dietary intake of salt on red blood cell count, packed cell volume, haemoglobin concentration and liver histology, it became important to ascertain its effect on serum bilirubin concentration, which is directly related to the above mentioned parameters, and the impact of treatment of possible derangements with *Viscum album* leaf extract which is widely used in Nigeria as a natural medication for a wide range of medical conditions.

2. MATERIAL AND METHODS

2.1 Plant Material and Preparation of *Viscum album* Extract

Fresh leaves of *Viscum album* (mistletoe; Fig. 1a and b) were obtained from a host plant (citrus) in Odukpani local government area of Cross River state, Nigeria on the 20th of March, 2013, and were authenticated by the Chief herbarium officer of Botany department, University of Calabar. The voucher number assigned to the specimen was UCDB1243.

The leaves were rinsed to remove debris and sand. They were air dried and subsequently transferred into the Astell Hearson oven where it was dried at temperature range of 40-45ºC. The dried leaves were ground to powder using an electric blender to obtain a gram weight of 1000g. The dry sample was peculated in 5000 ml distilled water for 24 hours. The mixture was then filtered with size 1 Whatman's filter paper. The filtrate was oven dried at 45ºC. The pasty filtrate obtained after drying was weighed using a mettler P163 electronic weighing balance. The stock solution of the extract was prepared by dissolving 15 g of extract in 10ml of distil water to give a concentration of 1500 mg/ml. The stock solution was labeled appropriately and refrigerated at 4ºC until required for use. The median lethal dose (LD_{50}) of the plant extract was determined by method of Lorke (1983) [24] and found to be 420.70 mg/kg (i.p). The dose of 150 mg/kg was adopted for this study.

Fig. 1a. Mistletoe plant on an orange (Citrus) tree

Fig. 1b. Rear and front view of Mistletoe leaf obtained from an orange tree

2.2 Preparation of High Salt Diet

High salt diet was prepared by mixing 80 g NaCl with 1 kg of diet to obtain a concentration of 80 g/kg [14,25]. Their drinking water contained 1 % NaCl. This was achieved by mixing 10 g of NaCl with 1 L distilled water.

2.3 Animal Preparation and Protocol

Twenty (20) male albino Wistar rats weighing 150-200 g were randomly divided into 4 groups of 5 rats each. They were fed as follows: Group 1 (control) was fed on normal rat pellet + drinking water. Group 2 (normal treated, NT) was fed with normal rat pellet+ drinking

water + 150 mg/kg of extract, orally, once daily. Group 3 (salt fed, SF) was placed on high salt diet (8 % sodium chloride) + 1 % sodium chloride drinking water. Group 4 (salt treated, ST) received same as the group 3 plus mistletoe extract (150 mg/kg body weight) orally, once daily. The feeding regimen lasted for six (6) weeks. The animal cages were well ventilated and exposed to 12/12 light/dark cycle. At the expiration of six weeks, the animals were sacrificed and blood samples collected for analysis.

2.4 Extract Administration

Extract administration commenced after two weeks of acclimatization. The extract was orally administered to NT and ST groups at a dose of 150 mg/kg body weight, once daily for 6 weeks. Administration was facilitated by the use of a syringe and or gastric tube. All experiments were in line with ethical standards laid down in the 1964 Declaration of Helsinki.

2.5 Collection of Blood Samples

The animals were anesthetized using chloroform anesthesia. Blood sample was then collected via cardiac puncture. Collection of blood samples was facilitated by the use of a 5 mls syringe and 21 G needle. The samples were introduced into plain capped bottles and allowed to stand for 2 hours, after which they were centrifuged at 10,000 rpm for 10 minutes and the serum collected for bilirubin estimation.

2.6 Measurement of Serum Bilirubin Concentration

Serum bilirubin was measured by the method described by Sherlock [26].

2.7 Statistical Analysis

Results are presented as mean $\pm$ standard error of mean. The One – way Analysis of Variance (ANOVA) was used to determine the differences between means, followed by post hoc multiple comparisons (Least Significant Difference test), with P=.05 considered significant. Computer software SPSS version 17.0 and Excel Analyzer were used for the analysis.

3. RESULTS

3.1 Comparison of Mean Total Bilirubin Concentration in the Different Experiment Groups

The mean total bilirubin concentration was 1.00 ± 0.07, 1.08 ± 0.07, 4.72 ± 0.22 and 3.12 ± 0.12 µmol/L for control (NC), NT, SF and ST group respectively. The mean total bilirubin concentration was significantly ($p<0.001$) increased in the SF and ST group compared to NC and NT group. Mean total bilirubin concentration was significantly ($p<0.001$) reduced in the ST group compared to SF group (Fig. 2).

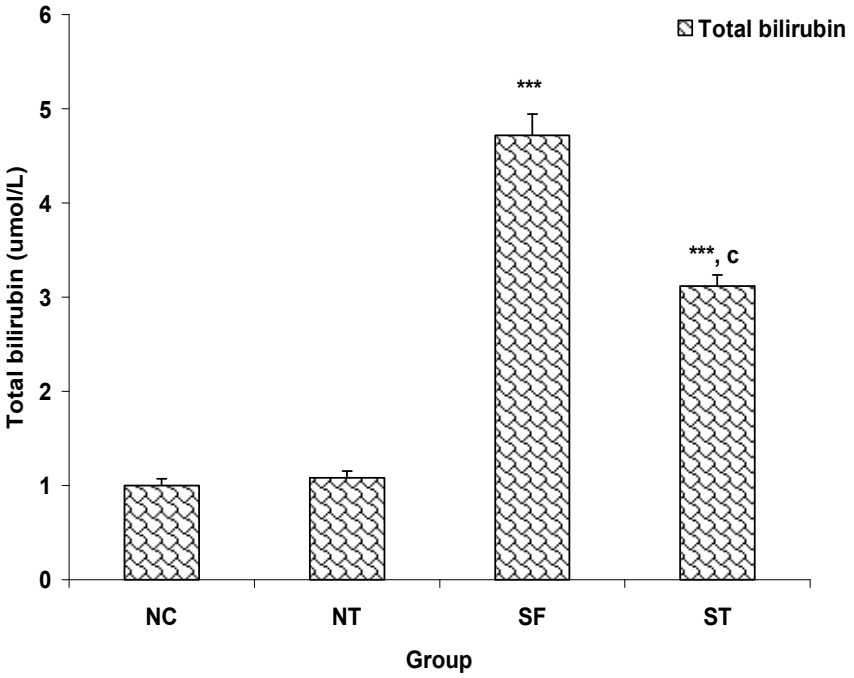

Fig. 2. Comparison of total bilirubin concentration in the different experimental groups

Values are mean ± SEM, n = 5
****p<0.001 vs control, NT; c = p<0.001 vs SF.*

3.2 Comparison of Mean Conjugated Bilirubin Concentration in the Different Experiment Groups

The mean conjugated bilirubin concentration was 0.3 ± 0.03, 0.38 ± 0.04, 0.88 ± 0.04 and 0.54 ± 0.04 µmol/L for control (NC), NT, SF and ST group respectively. Mean conjugated bilirubin concentration was significantly ($p<0.001$) higher in SF group when compared to NC, NT and ST group; it was significantly ($p<0.001$) reduced in the ST group compared to SF group. Mean conjugated bilirubin concentration was significantly ($p<0.01$) increased in ST group compared to NC group (Fig. 3).

3.3 Comparison of Mean Unconjugated Bilirubin Concentration in the Different Experiment Groups

Values obtained for mean unconjugated bilirubin concentration were 0.70 ± 0.05, 0.70 ± 0.09, 3.84 ± 0.26 and 2.58 ± 0.13 µmol/L for control (NC), NT, SF and ST group respectively. The mean unconjugated bilirubin concentration was significantly ($p<0.001$) higher in the SF and ST group compared to control (NC) and NT group. It was also significantly ($p<0.01$) lower in the ST group compared to SF group (Fig. 4).

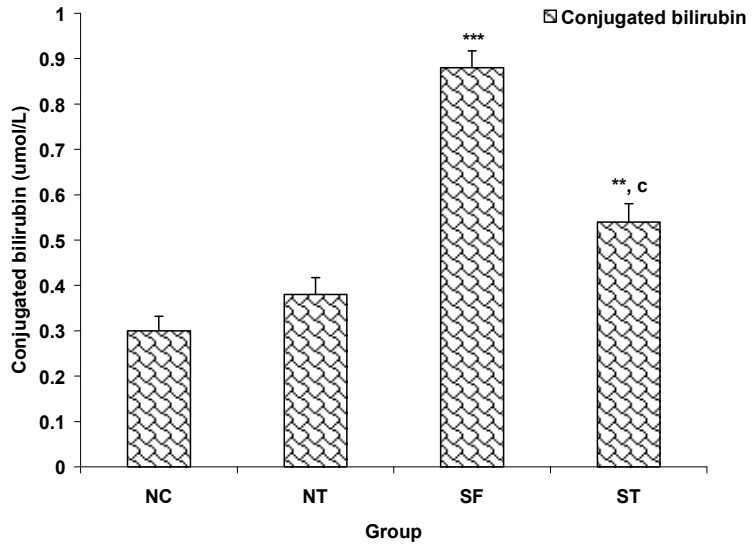

Fig. 3. Comparison of conjugated bilirubin concentration in the different experimental groups

Values are mean ± SEM, n = 5

****p<0.001, **p<0.01 vs control, c = p<0.001 vs* SF

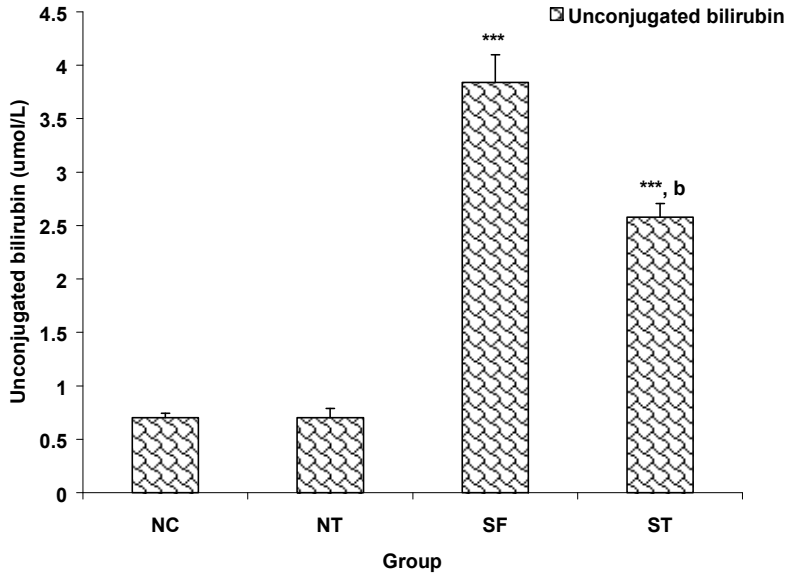

Fig. 4. Comparison of unconjugated bilirubin concentration in the different experimental groups

Values are mean ± SEM, n = 5

****p<0.001 vs control, b = p<0.01 vs SF.*

4. DISCUSSION

Chronic consumption of salt - rich (sodium chloride) diet significantly increased serum total bilirubin, conjugated and unconjugated bilirubin concentrations. This is in line with reports by Ofem et al. [27] that high salt diet increased serum bilirubin concentration. Apart from the fact that chronic consumption of sodium chloride is a risk factor for hypertension, it is evident from our study that chronic consumption of sodium chloride is also a risk factor for hyperbilirubinemia.

Elevated serum unconjugated bilirubin concentration most often indicates hepatic damage. In this condition, the hepatocytes can no longer conjugate bilirubin with glucuronide. Consequently, the unconjugated bilirubin re-enters circulation. High levels of unconjugated bilirubin are also seen in severe haemolytic anaemia, when excessive unconjugated bilirubin overwhelms the liver's conjugating mechanisms. Increased serum conjugated bilirubin concentration on the other hand is indicative of possible biliary obstruction [27,28].

Although administration of crude extract of mistletoe to the salt-fed treated group significantly reduced serum bilirubin levels when compared to the salt-fed untreated group (SF), the normal control (NC) group still presented with lower serum bilirubin concentration compared to salt treated group (Fig. 2, 3, 4).

Administration of crude extract of mistletoe to the control treated (NT) group did not significantly influence the serum bilirubin concentrations when compared to control (NC). This is suggestive of the presence of a negative feedback mechanism that ensures bilirubin concentration does not fall below normal, despite treatment with mistletoe leaf extract.

In the absence of liver damage, increased serum total bilirubin, conjugated bilirubin and unconjugated bilirubin concentration may be caused by increased rate of red blood cell formation (polycythemia), hemolysis; as seen in ineffective erythropoiesis, or from deficient bilirubin transport across the liver as presented in Gilbert's syndrome [28]. In a study conducted by Ofem et al. [27], high salt-diet was associated with liver damage. He further reported that photomicrograph of a section of the liver in high salt fed group showed marked necrotic condition, pyknosis, karyorrhexis, numerous but deranged sinusoids, with many pockets of lipid deposits similar to that of alcoholic syndrome, signifying degeneration of the hepatocytes [27]**.** Previous studies had reported the hepatoprotective property of mistletoe extracts [29]. From our results, it can be deduced that mistletoe leaf extract reversed the liver damage caused by the high salt diet. This probably accounted for the decreased serum bilirubin concentration in the high salt-fed treated group (ST).

Phytochemical screening has revealed that *Viscum album* leaf extract contains alkaloids, carbohydrates, tannins and flavoniods [30]. Further investigation is necessary to further define which phytoconstituent is particularly responsible for this observed anti-hyperbilirubinemia effect and the mechanism by which this effect is propagated.

5. CONCLUSION

Increased salt intake has been proven to be associated with increased risk of hyperbilirubinemia. Although mistletoe leaf extract did not reduce serum bilirubin concentration to levels observed in the control group, the decrease was appreciable and a strong indication of its potential to reverse hyper bilirubinemia.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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