Asian Journal of Biochemistry, Genetics and Molecular Biology

2(3): 1-8, 2019; Article no.AJBGMB.50637

Therapeutic Effects of *Viscum album* Combined with *Garcinia kola* against CCL_4 Induced Liver Injury in Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author RE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TEDG and BN managed the analyses of the study. Author BN managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2019/v2i330060
Editor(s):
(1) Dr. Mohammed Rachidi, Director of Research, Molecular Genetics of Human Diseases (MGHD), French Polynesia, University Paris Denis Diderot, Paris, France.
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Reviewers:
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(2) Ahmed S. Ibraheem, Sohag University, Egypt.
(3) Maha Zaki Rizk, Egypt.
Complete Peer review History: https://sdiarticle4.com/review-history/50637

Original Research Article

Received 28 June 2019
Accepted 06 September 2019
Published 19 September 2019

ABSTRACT

This study was aimed at evaluating the efficacy of *Viscum album* (mistletoe) and *Garcinia kola* seed (bitter kola) in treating Rat liver against CCl_4-induced liver injury. Mistletoe and bitter Kola are used in traditional medicine for the treatment of various disorders, including hepatic disorders. Biochemical parameters and histological structure were assessed and used as a measure of the therapeutic potential of the herbs against CCl_4 induced liver injury. The experimental animals (15 male wistar albino rats) weighing between 100-120 g were randomly divided into three (3) groups. Each group comprised 5 rats and was labeled as group 1, 2 or 3. Group 1 (negative control) animals were administered saline orally daily for 6 weeks (1 ml volume per kg body weight) while group 2

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(CCl_4 group) animals were administered CCl_4 mixed with olive oil as vehicle in 1:1 ratio (3 ml/kg body weight). Group 3 represented the treatment group with extracts of the two herbal plants (250 mg/kg daily). The combination of the herbal extracts administered orally for 6weeks showed a significant decrease ($P<0.05$) in serum protein, albumin, total bilirubin and conjugated bilirubin concentrations and in serum activities of AST, ALT, ALP and GGT compared with CCl_4-induced increases in concentrations or activities of the aforementioned biochemical parameters. Histological examination of the liver of CCl_4–treated rats with the combined herbal extracts showed less destruction of liver architecture in comparison to the group induced with CCl_4 only. The results indicated that the combination herbal extracts investigated (mistletoe and bitter kola) had therapeutic effect against CCL_4-induced liver injury when used either in combination and this effect could be due to the phytochemicals present in the herbs.

Keywords: Therapeutic effects; Viscum album; Garcinia kola; CCL_4 induced; liver injury.

1. INTRODUCTION

The liver, as a vital organ in the body, one of its role in metabolism of endogenous and exogenous agents. It plays an important role in drug elimination and detoxification. Liver damage may be caused by Xenobiotics, alcohol consumption, malnutrition, infection, anemia and medications [1]. Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug, or another non-infectious agent [2]. Carbon tetrachloride (CCl_4) is an occupational chemical agent widely used as a solvent in insecticide. The hepatotoxicity of halogenated hydrocarbons, particularly CCl_4, has been the subject of numerous investigations in experimental animals [3]. Over 50% of modern clinical drugs are of natural product origin and natural products play important roles in drugs development in the pharmaceutical industry [4]. Studies have shown that plant extracts having antioxidant activity protect against CCl_4 hepatotoxicity by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity. The major use of mistletoe (*Viscum album*) is as a palliative cancer therapy. The seeds are also used in folk medicine, many herbal formulations and have potential therapeutic benefits due to the activity of their flavonoids and other bioactive compounds [5,6,7,8,9,10,11]. The potential utilization of *Garcinia kola* in brewing operations as hop substitutes in lager beer brewing has also been reported [12] Dosunmu & Johnson, 1995, [13,14,15]. These applications require only the pulp while the hull is discarded. The presence of beneficial bioactive compounds have been reported in the seed coats of almonds, peanuts (*Arachis hypogea*), lotus seeds (*Nelumbo nucifera*) and African yam bean (*Sphenostylis stenocarpa*) [16,17] thereby opening up the possibility of the presence of potentially beneficial compounds in the seed coat of other plant materials with bioactive components in their pulp.

2. MATERIALS AND METHODS

2.1 Study Area/Population

The study was conducted at Department of Human physiology, University of Port Harcourt. It was a biological trial with Albino Wistar rats which were considered the choicest animals for this experiment because of their availability, cost, genetic makeup, handling technique and nature of the study. Fifteen (15) healthy mature male albino wistar rats of 12weeks old weighing between 100-120 g were used in this study. The rats were obtained from the Experimental Animal Unit of the University. The rats were housed in conventional wire mesh cages under standard laboratory conditions and were allowed free access to water and feed throughout the experiment.

2.2 Preparation of Ethanolic Extract of *Garcinia Kola* and Aqueous Leaf Extract of Mistletoe *(Viscous album)*

Two kilograms (2 kg) of powdery form of the *Garcinia Kola* was processed at the Department of Pharmacology Laboratory of University of Port Harcourt for extraction using Soxhlet extraction method. During the extraction, 70% of ethanol and 30% of water were used for the maceration. Two kilograms (2 kg) of the seeds was macerated with ethanol and water then allowed to stand at room temperature for 3 days with frequent stirring until the soluble matter dissolved. The mixture then was sieved, the damp solid material was pressed, and the solvent was clarified by filtration. The solvent was then placed in the reservoir of soxhlet for extraction. The

liquid extract in the reservoir was subjected to heat for several minutes to vaporize the moisture. The sample was evaporated over the water bath at a temperature of 45°C and was constantly monitored until a gelatinous extract was formed.

Two kilograms (2 kg) of powdery form of the Mistletoe leaves was taken to the Department of Pharmacology Laboratory of University of Port Harcourt for extraction. During the extraction water was used for the maceration. Two kilograms (2 kg) of the leaf was macerated with water then allowed to stand at room temperature for a period of 3 days with frequent stirring until the soluble matter dissolved. The mixture then was sieved, the damp solid material was pressed, and the solvent was clarified by filtration. The extract was then placed in the reservoir of soxhlet for extraction. The liquid extract in the reservoir was subjected to heat for several minutes in order to vapourize the moisture. The sample was evaporated over the water bath at a temperature of 45°C and was constantly monitored until a gelatinous extract was formed [18].

2.3 Grouping and Treatment of Animals

Fifteen (15) male Albino Wistar Rats were used for this research and were divided according to their body weight into 3 groups with each group containing five (5) Rats.

Group 1: This was the negative control group; they received 1ml of distilled water daily for six (6) weeks.

Group 2: This group was induced with Carbon tetrachloride (CCl_4) causing hepatotoxicity using 3 ml/kg body weight and served as a positive Control.

Group 3: 24 hours after inducing with CCl_4 this group received 250 mg/kg body weight each of both Mistletoe and bitter kola daily for six (6) weeks.

In the studied animals, all groups except standard control was induced by single oral administration of CCl_4 mixed with olive oil as vehicle in 1:1 ratio (3 ml/kg of rat body weight. A pilot study was first carried out using 10 Albino Wistar Rats for each of the two (2) herbs extracts used (Mistletoe, Bitter Kola). The results obtained showed that the lethal dose was estimated at 1,500 mg/kg.

2.4 Procedures for Administration of Extracts

Administration of extract was by oral gavage route. The rat was held at the skin over the head and turned so that the mouth was faced upward and the body lowered towards the holder. The syringe needle knob was then placed into the mouth of the rat a bit laterally to avoid the teeth which are centrally located. The syringe content was then gradually emptied drop by drop into the mouth of the rat.

2.5 Sample Collection

The blood samples were collected from the animal via cardiac puncture and sacrificed under 70% chloroform anesthesia into the plain specimen bottle. The samples were allowed to clot, then centrifuged at 3000 revolutions per minute for 3 minutes. Then sera obtained were stored in a freezer until required for use for analysis for liver function.

2.6 Histological Studies

After 24 hours of induction and after 6 weeks of the experiment (for both controls and treatment), an animal in each group were dissected and their liver tissues were histologically studied.

2.7 Statistical Analysis

The data were evaluated statistically by SPSS version 20. Using one-way analysis of variance (one way ANOVA) and subjected to Fischer LSD post Hoc. Results were expressed as mean $\pm$SD. Difference between means was considered significant at $P<0.05$.

3. RESULTS

3.1 Comparison of Parameters for Rats Treated with Combined Extracts of Mistletoe and Bitter Kola with Negative and Positive Controls

The Table 1 showed ANOVA results and Post hoc results. ANOVA results showed a significant difference in the means of the three groups (Group 1, Group 2, and Group 3) while Post hoc result showed a significant difference between the means of the groups being compared.

4. DISCUSSION

This study showed the combined effect of mistletoe and bitter kola extracts and it was

observed to show potency by significantly reducing the levels of the liver enzymes (ALT, AST, ALP and GGT) as seen in Table 1. In the present study, acute hepatic injury was induced in Albino Wistar Rats exposed to CCl_4, leading to high levels of serum aminotransferases and considerable perivenular necrosis. Hepatic injury was reduced significantly by the administration of the combination herbal therapy with a reduction in plasma levels of hepatocellular enzymes ALT and AST as well as the cell membrane enzyme ALP and with marked improvement in liver morphology as seen in Fig 3. These results pointed to a therapeutic potential of mistletoe and bitter kola extracts when used together. Little information is available in the literature on the mechanism of the herbal extracts in recovering hepatic injury. The plants, however, have been widely investigated for their anticancer and antioxidant properties [19]. Also, *Viscum album* extracts have both immunomodulatory (induces TNF-α and IL-12) and apoptosis-inducing properties, which are likely to be dose-dependent. Findings in the present study suggest that the use of the extracts can have an additive beneficial effect in lessening liver inflammation and necrosis caused by CCl_4. This observation is corroborated by Nyblom et al. [20] who explained that the perioperative administration of the extract attenuated the immuno-suppressive effects of surgery, increasing the number of NK cells, the T and B cells, complement, and IgA, IgG, and IgM values.

Another Study also suggested that European mistletoe possesses insulin-secreting [21], antihyperglycemic, antioxidant activity [22] and cholinomimetic activities [23]. The release of aminotransferases into the plasma was markedly reduced, indicating a reduction in the severity of liver damage by the combination. Aminotransferases are sensitive indicators of liver-cell injury and are released into the blood in increasing amounts whenever the liver cell membrane is damaged [24].

A study by Alade and Ani [25] demonstrated the protective effects of *Garcinia kola* seed extract against paracetamol-induced hepatotoxicity in rats. This study demonstrated a significant reduction in liver enzymes. The hepatoprotective effect of the extract was attributed to the inhibition of cytochrome P-450 which normally converts paracetamol to the toxic intermediate metabolite N-acetyl-p-benzo-quinoneimine. In a related study, Osifo et al. [26] and Galam et al. [27] demonstrated no observational histopathological effects by *Garcinia kola* on the histology of the liver reflecting its hepatic safety in healthy condition.

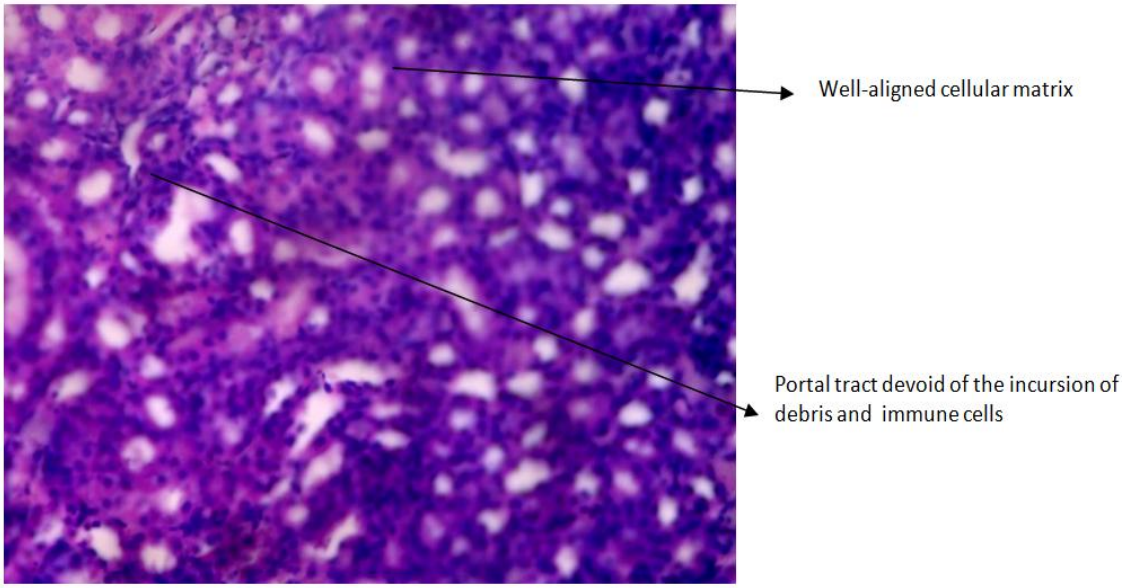

Fig. 1. Photo micrographic slide of the liver organ of group 1 (negative control saline) H & E X400

Table 1. Comparison of parameters for rats treated with extracts of mistletoe and bitter kola

Groups	Protein (g/L)	Albumin (g/L)	Total Bilirubin (mmol/L)	Conj. Bilirubin (mmol/L)	ALT (u/l)	ALP (u/l)	GGT (u/l)	AST (u/L)
Group 1 (Negative control)	30.46± 5.44	25.96±5.01	10.41±6.09	6.78±0.64	7.60±0.89	86.0±1.00	27.98±0.78	28.40±11.50
Group 2 (positive control)	81.34±10.08	78.08±3.94	91.23±1.42	48.99±1.95	19.00±2.12	387.80±4.82	88.0±4.69	144.4±18.62
Group 3 mistletoe+kola+CCl_4	73.34±5.74	34.60±3.56	29.23±2.78	14.25±1.34	13.20±1.09	206.80±176.3	39.2±0.84	38.8±7.12
P-Value	<0.0001	<0.0002	<0.0006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F-Values	15.38	14.36	39.77	34.59	13.56	20.72	11.98	20.08
Post Hoc								
Group 1 vs Group 2	S	S	S	S	S	S	S	S
Group 1 vs Group 3	S	S	S	S	S	S	S	S
Group 2 vs Group 3	S	S	S	S	S	S	S	S

Values are presented in mean ±SD, n=5 per group, S=significantly different when compared

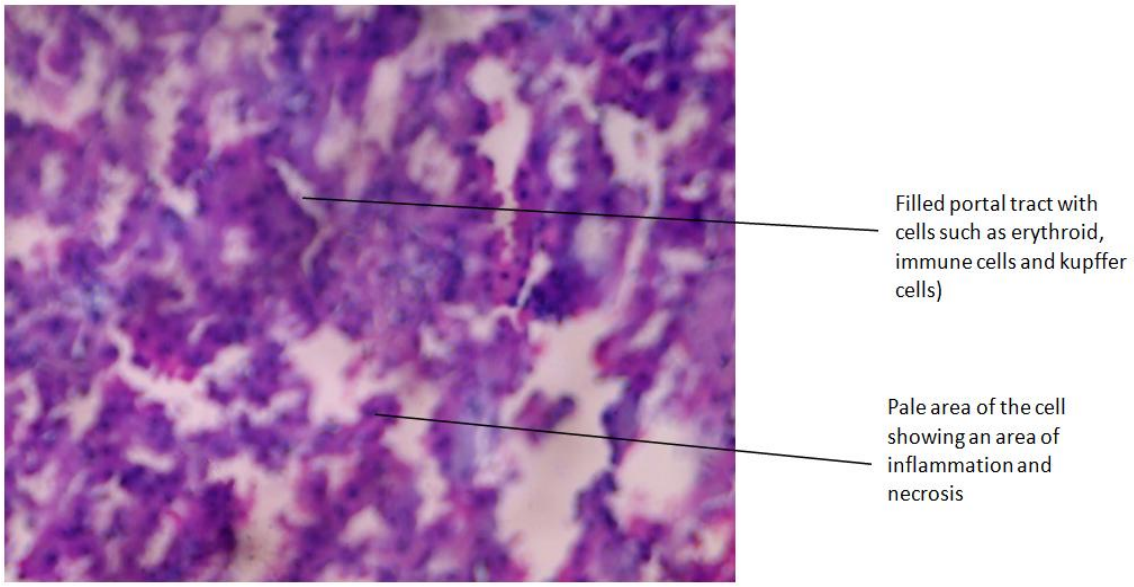

Fig. 2. Photo micrographic slide of liver organ of group 2 (Positive control CCl4-induced hepatotoxicity) H & E X400

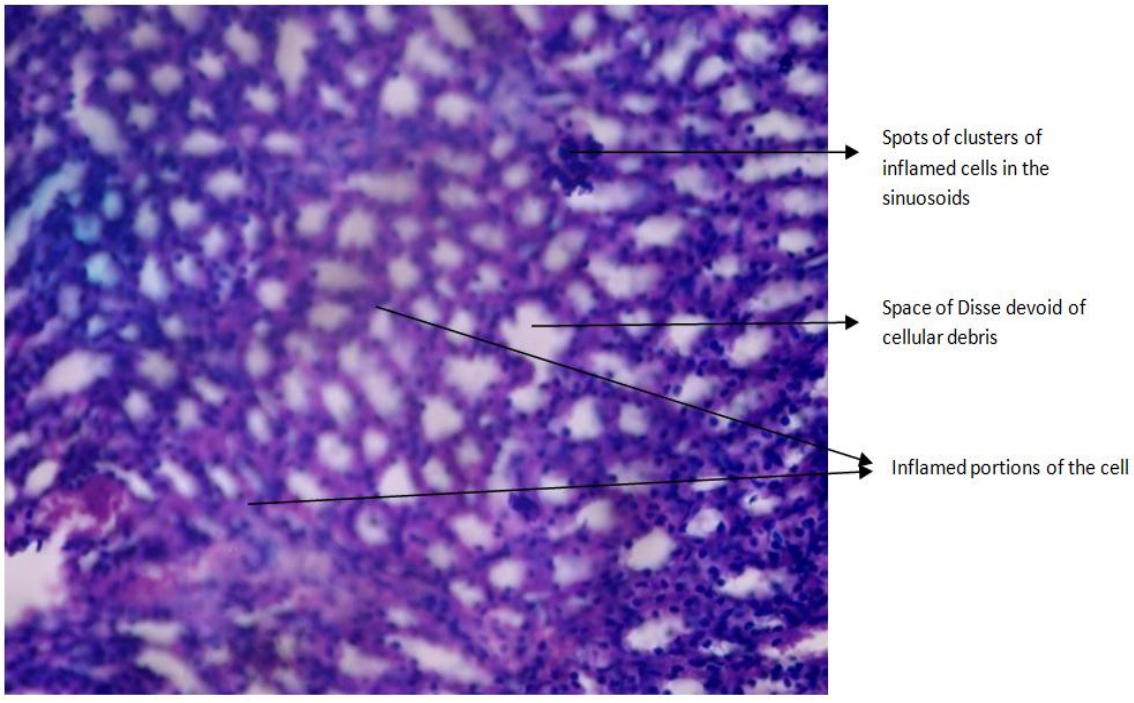

Fig. 3. Photo micrographic slide of the treated liver organ of group 3 using extracts of mistletoe and kola (250 mg/kg) H & E X400

5. CONCLUSION

The results of this study suggest that the consumption of mistletoe combined with bitter kola exhibited a therapeutic effect against liver injury. This therapeutic effect may be due to the mineral, antioxidant and anti-inflammatory natural elements present in the herbs.

ETHICAL APPROVAL

Formal approval was obtained from the Department of Biochemistry University of Port Harcourt for the biochemical analysis of the specimen for toxicity studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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