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THE EFFECT OF THE MIXTURE OF GARCINIA KOLA AND HONEY ON THE LIVER OF WISTAR RATS

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Abstract
Aim: A mixture of honey and Garcinia kola has been used for the treatment of certain dry coughs and diseases. The effect of the recommended dosage of the mixture on the liver was investigated.
Methods: Twenty five Wistar albino rats weighing between 170g and 190g were grouped into 5. There were 5 rats each group. The rats were fed twice daily at 8.00am and 6.00pm with a mixture of G. kola and honey 0.16g/kg + 0.5ml, 0.20g/kg + 0.5ml, 0.24g/kg + 0.5ml, 0.28g/kg + 0.5ml and normal feeds respectively for 14 days. The rats were sacrificed on the 15th day and blood and liver tissues were taken for biochemical analysis and histology respectively.
Results: Aspartate aminotransferase, alanine aminotransferase, bilirubin, alkaline phosphatase and total protein as well as the histology of the liver were not significantly different from the control animals.
Conclusion: Oral intake of one nut G. kola along with 5 ml honey twice daily for 14 days does not have adverse effects on the liver of Wistar rats.

Key words: Medicinal plant, Honey, Garcinia kola, Liver, Biochemistry

INTRODUCTION

The presence of several chemicals of pharmacological relevance in various parts of plants makes them important in the production of drugs as several drugs have been developed from them. Garcinia kola commonly known as bitter kola is found in the rain forest and swamps and grows to about 12m in height. It is a perennial crop that grows in West and Central Africa (Vivien et al., 1985) and valued for its medicinal nuts which has led to its exploitation in the natural forests in recent times (Farombi et al., 2005). The nut is chewed extensively in Southern Nigeria as a masticatory to cause nervous alertness and for the treatment of coughs and throat infections (Farombi et al., 2005). G. kola stem bark contains a complex mixture of phenolic compounds such as tannins, guttiferin (Etkin, 1981), biflavonoids, xanthenes, benzophenone, kolaflavanone and garcinia flavanone (Iwu and Igboko, 1982) all of which have antimicrobial activity. The seed of G. kola also contains 1-3, 8-11 benzophenones, Garcinia biflavonones and kola flavonone (Cotterih et al., 1978). Apigenin based flavonoids represent 60% of the flavonoids present in the diethyl ether fraction of G. kola seeds (Iwu and Igboko, 1982). The seed of G. kola has similarly been used to prevent and treat colic and headache (Ayensu, 1978), high fever, jaundice and liver disorders (Iwu, 1991), skin infections, sexual dysfunction and stomach pain (Braid, 1991) and as a spermatogenic agent (Adesanya et al., 2007). G. kola has purgative, antiparasitic, anti-inflammatory, anti-bacterial and antiviral properties (Akoachere et al., 2002). The roots and bark are taken for sexual dysfunction and stomach pain (Braid, 1991). Adesanya et al., (2007) stated that it could be used as a spermatogenic agent. It has also been used to relax muscles (Braid, 1991). Honey is a sweet viscous substance made by bees from the nectar of flowers, transformed and stored in honeycombs. The specific composition of a batch of honey depends largely on the type of flowers available to the bees that produced the honey. Typically, honey contains Fructose:

38.0%, Glucose: 31.0%, Sucrose: 1.0%, Water: 17.0%, other sugars (maltose, melezitose): 9.0%, Ash: 0.17%. Others: 3.38% (Erguder et al., 2008). Honey also contains tiny amounts of several compounds thought to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase, and pinocembrin (Martos et al., 2000). Honey has long been used as a remedy for certain ailments such as gastric disturbances, ulcers, asthma, as an antibiotic and as an antiseptic (Grotte, 1998). Honey promotes wound healing and circulatory system (Pand, 2014) and for the treatment of chronic rhino sinusitis (Philip, 2014), for sore throat and cough (Chris, 2014) as well as an ointment for rashes and burns (Vanghn, 2001). Honey is used as an additive to a variety of food and beverages not only for its sweetness but because it counteracts microbial spoilage of food. Recently, traditional medical practitioners have treated dry coughs and certain ailments with a combination of honey and Garcinia kola. The side effects of the mixture on the liver have not been investigated. This work therefore investigated the effect of the mixture of G. kola and honey on the liver at a dosage of one Garcinia kola (7±3g) mixed with about 5ml honey when taken twice daily for 14 days.

MATERIALS AND METHODS

Preparation of Garcinia kola

Nuts of G. kola were obtained from Owo, Ondo State, Nigeria. They were weighed (8±4g) and dried in an open air oven at 56^0C for 48 hours. The coats were removed and the nuts cut into tiny bits of about 0.2mm thick with a knife and further dried for 72 hours at 56^0C and milled to obtain a fine powder which was used for the experiment.

Administration of the Mixture

Twenty five Wistar albino rats weighing between 170g and 190g were obtained from the Animal House, Delta State University, Abraka, Nigeria. They were acclimatized for 14 days and grouped into 5 with 5 rats in each group. Each rat in Group 1 received oral administration of a mixture of G. kola 0.16g/kg and 0.5ml honey at 8.00am and at 6.00pm twice daily for 14 days. Each rat in Group 2 received a mixture of 0.20g/kg G. kola and 0.5ml honey for a similar period as Group 1. Groups 3 and 4 received 0.24g/kg G. kola and 0.5ml honey, 0.28g/kg G. kola and 0.5ml honey respectively. The rats in Group 5 were the control rats and were not given the mixture. All the test and control rats had access to water and feeds ad libitum. The rats were sacrificed on the 15^{th} day and blood was collected into lithium heparin bottles, while the liver tissues were fixed in 10% formol saline for 24 hours.

Biochemistry

The blood samples were spun slowly for 10 minutes and the sera removed from the red blood cells with a Pasteur pipette into clean bottles. The sera were analyzed for aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total and conjugated bilirubin and total protein using standard biochemical techniques.

Histology

Thin sections were cut from the fixed liver tissues and processed by the paraffin wax method using the automatic tissue processor (Histokinette). Sections, 4µm thick were cut with the Rotary microtome (Tissue Tek) and stained with heamatoxylin and eosin. The sections were examined with the light microscope at X100 and X400.

RESULTS

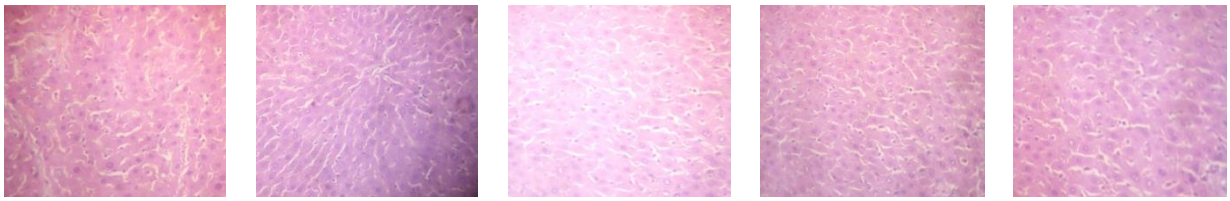
Biochemistry

Table 1: Liver function test

Group	Alk.Phos (units/L)	ALT (units/L)	AST (units/L)	T. Bil (mg/dl)	C. Bil (mg/dl)	TP (g/L)
1	98±15	12±2	55±3	0.23±0.03	0.12±0.01	66±3
2	96±18	12±2	60±3	0.21±0.02	0.11±0.01	65±3
3	95±20	12±2	54±2	0.32±0.02	0.16±0.02	56±2
4	100±18	13±3	55±2	0.31±0.03	0.15±0.01	56±3
Control	96±19	12±2	50±2	0.29±0.03	0.15±0.01	55±3

Key: Alk. Phos (Alkaline phosphatase), AST (Aspartate aminotransferase, ALT (alanine aminotransferase), T. Bil (Total bilirubin), C. Bil (Conjugated bilirubin), TP (Total protein).
Group 1 (0.16g/kg G. kola and 0.5ml honey), Group 2 (0.20g/kg G. kola and 0.5ml honey), Group 3 (0.24g/kg G. kola and 0.5ml honey), Group 4 (0.28g/kg G. kola and 0.5ml honey), Control (Untreated group).
There was no significant difference between the control blood samples and the treated groups.

Histology



Group A Group B Group C Group D Group E

No evidence of inflammation, degenerative changes or cyto-architectural distortions of the hepatic parenchyma in Groups A, B, C and D when compared with the control tissue (Group E). H&E X100.

DISCUSSION

Nanyak et al., (2013), administered up to 0.5g/kg Garcinia kola to Wistar albino rats and did not find any evidence of degenerative changes or cyto-architectural distortions of the hepatic parenchyma. They concluded that intake of Garcinia kola does not cause any acute morphological changes in the liver. The result of their experiment is similar to our findings where we could not find any biochemical or histological difference between the tests and the control rats although what we administered was about half what was administered by Nanyak et al., (2013). One nut G. kola was administered along with 5ml pure honey for 5 to 7 days by the traditional medical practitioners for the treatment of dry coughs and other ailments. The effect of the mixture of aqueous *G. kola* extract and honey on bacterial growth was investigated by Akinnibosun and Itedjere, (2013). They observed that the synergy of aqueous *G. kola* extract and honey was more effective in inhibiting bacterial growth than the separate use of aqueous *G. kola* extract and honey. We intentionally extended the duration of this study to 14 days in order to determine the effect of the mixture on the liver when its use is prolonged. It is not clear if the honey had a protective effect on the liver which counteracted the effect of G. kola, but it is clear from literatures that honey and G. kola separately have medicinal properties and have been used for the treatment of certain disease conditions including cough. The LD_{50} of Garcinia kola seed powder was found to be 6.74g/kg by Udenze et al., (2012). Essien and Nwafor (2014) found the LD_{50} to be 1±0.067g/kg while Kagbo and Ejebe, (2009) found the 24 hour LD_{50} value to be 358mg/kg.

This sharp difference in the results of experiments performed differently by the three groups of researchers is worrisome. However, we restricted the dosage given to the rats to a maximum of 0.28g/kg, equivalent to the weight of one nut of G. kola per adult human according to the prescription of the traditional medical practitioners. For several centuries, traditional medical practitioners have prescribed honey for the treatment of several ailments as well as a food additive. It has been used by Pand, (2014) for wound healing and to improve the circulatory system, while Philip, (2014) used it for the treatment of chronic rhino sinusitis, Chris, (2014) used it for the treatment of sore throat and cough, as well as an ointment for rashes and burns (Vanghn, 2001). Several experiments have been performed to determine the effects of honey and G. kola on humans and rats. These are well documented. Recently, traditional medical practitioners treated dry coughs with a mixture of 5ml pure honey with one nut of Garcinia kola. The G. kola was eaten over a period of 30 minutes followed by oral administration of about 5ml pure honey. This could be taken twice daily for up to 5 days. Kagbo and Ejebe, (2009) stated that Garcinia kola stem bark was used by traditional medical practitioners in Nigeria to treat dysmenorrhea and burns and that the decoction and infusion of the stem bark was often taken without any standardized measurement which resulted in over dosage because of the large amounts taken. It was for this reason that they investigated the acute toxicity profile of G. kola. There are no literatures on the synergic effect of the mixture of honey and G. kola on animal and human tissues, hence, this research. While there is evidence that the mixture could be used for the treatment of certain disease conditions, its level of toxicity to the liver has not been determined. It is obvious that excessive intake of drugs and other products have adverse effects, sometimes lethal, this work is limited to the recommended dosage by the traditional medicine healers of one nut eaten over a period of about 30 minutes followed with about 5 ml of honey.

CONCLUSION

Intake of the mixture of one nut G. kola and 5ml pure honey does not have adverse effects on the liver when taken twice daily for 14 days.

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