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The Effect of Ethanolic Extract of Garcinia Kola on the Spleen of Adult Wistar Rats

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Abstract
This study was designed to determine the effects of ethanolic extract of *Garcinia kola* on the spleen of the adult wistar rats. Twenty four (24) adults wistar rats weighing between 180-270kg were divided into four groups A, B, C & D; each consisting of six animals each. . Group A served as the control and were orally administered 0.2ml of distilled water; the experimental groups received 0.3ml, 0.6ml and 0.9ml of ethanolic extract of *Garcinia kola* respectively for twenty eight days. Twenty four hours after the last administration, the animals were weighed, sacrificed under the influence of chloroform vapour. The spleen were harvested, weighed and fixed in 10% formalin for histological studies. The final body weight of groups C & D decreased significantly ($P<0.05$) compare with the control A. The mean relative organ weight of groups C & D increased significantly ($P<0.05$) when compare with the control group A while group B had a similar mean weight with the control group A. Histological findings revealed distortion of the spleen cells of the experimental groups. The result of this study therefore suggests that consumption of the ethanolic extract of the Garcinia kola in high doses may put the spleen at risk of adverse histopathological conditions.
Keywords: Wistar rats, Organ weight, Garcinia kola, Spleen, Distilled water.

Introduction

In the seventh century AD the Slavic people used *Rosarinus Officinalis*, *Ocimum basilicum*, *Iris germanica, and Mentha Viridis* in cosmetics, *Alium Sativum* as a remedy and *veratrum album, Cucumis sativus, Urtica dioica, Achilea millefolium, Artemisia maritime L., Lavandula officinalis, Sambuci flos* against several injurios insects, i.e. louses, fleas, moths, mosquitos, and spiders and Aconitum napellus as a poison in hunting (Bojadzievski 1992).

In the middle Ages, the skills of healing, cultivation of medicinal plants, and preparation of drugs moved to monasteries. Therapy was based on 16 medicinal plants, which the physicians-monks commonly grew within the monasteries as follows: sage, anise, mint, Greek seed, savory, tansy, etc. Charles the Great (742 AD–814), the founder of the reputed medical school in Salerno, in his “Capitularies” ordered which medicinal plants were to be grown on the state-owned lands. Around 100 different plants were quoted, which have been used till present days such as sage, sea onion, iris, mint, common centaury, poppy, marsh mallow, etc. The great emperor especially appreciated the sage (*Salvia officinalis L.*). The Latin name of sage originates from the old Latins, who called it a salvation plant (*salvare* meaning “save, cure”). Even today sage is a mandatory plant in all Catholic monasteries (Tucakov 1990, and Tucakov 1991).

Garcinia kola is a medium sized forest tree found throughout West and Central Africa. The seed are eating as refreshing past time in Nigeria and are known to contain high content of biflavonoid compound. The toxicity is very low, the oral 50% lethal dose being above 500mg/kg body weight (Nwankwo *et al*., 2000).

Garcinia kola Heckel (*G. Kola; Guttiferae*) is a dicotyledonous plant found in the rain forests of Central and West Africa. It is grown on homesteads in Southern Nigeria; a detailed description and distribution of the plant have been documented (Iwu 1993).

The seed of *Garcinia kola* (bitter kola) is an important component in traditional herbal medicine (Dalziel 1937). G*arcinia kola* enjoys a folk reputation in the management of sickle cell disease (SCD), as poison antidole (Kabangu *et al* 1987; Egunyomi *et al* 2009).

Because of the extensive consumption of *G.kola* nut in Nigeria, this study aimed at investigating the effects of ethanolic extract of *Garcinia kola* on the spleen of adult wistar rats.

Materials and Method

Breeding of Animals

Twenty four adult wistar rats weighing between 180-270kg were used for the experiment. The animals were purchased from the Animal House of Biochemistry Department, Nnamdi Azikiwe University, Awka, Anambra State. They were allowed to acclimatize in the Animal House of Department of Anatomy, Nnamdi Azikiwe University, Nnewi Campus under normal temperature (27-30°C). They were fed ad-libitum with water and guinea feed from Agro feed mill Nigeria Ltd.

Drug Preparation

Garcinia kola when ripened, the fruits of *Garcinia kola* was collected from Okofia, Nnewi Anambra State and it was identified at the Department of Botany Nnamdi Azikiwe University Awka. They were kept in an open cool place till the pericarp and the pulpy mesocarp become soft. After softened, the fruits are threshed to release the nuts, which are thoroughly washed to remove the sticky mucilaginous materials that sheath the nuts. The plants material was sun-dried. The dried *Garcinia kola* was milled to a powder. Extraction was done using ethanol. 250mg of this extract/kg body weight was dissolved in 10ml of distilled water and administered to the animals.

Experimental Protocols

The twenty four animals were weighed and allocated into four groups of six animals each. The groups are designated as A, B, C & D. Group A serve as the control and administered 0.2ml of distilled water; the experimental groups B, C & D received 0.3ml, 0.6ml & 0.9ml of ethanolic extract of *Garcinia kola* orally respectively for sixty days. Twenty four hours (24 hours) after the last administration, the animals were weighed and weights were recorded. They were anaesthetized using chloroform vapour inhalation method and dissected. Spleen tissues were trimmed down to a size of 3mm×3mm and fixed in zenkers fluid for histological studies.

Tissues Processing

For easy study of sections under microscope, the tissues passed through several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. The fixed tissues were kept in zenkers fluid for four hours. After fixation, the tissues were washed over night under a stream tap water. Dehydration of the fixed tissues was carried out in different percentages of 50%, 70% and 90% and absolute. The tissues were cleared in xylene for hours after which infiltration was due in molten paraffin wax at a temperature of 60°C for two hours each in two changes and eosin method was used.

Results

Morphometric Analysis of Body Weight

Table 1: Comparison of the mean initial, final body weight and weight changes in all the groups (A, B, C & D) before and after the administration of the extract.

(Mean± SEM given for each measurement)

	Group A	Group B	Group C	Group D	F-Ratio	Prob. of Sig
Initial body weight	182.40±2.50	184.60±2.10	187.20±1.90	192.30±2.50	64.130	≤0.001
Final body weight	202.60±2.60	198.10±2.40	174.10±1.10	170.20±2.30	49.116	≤0.001
Weight change	20.20±0.10	13.50±0.30	-13.10±0.80	-14.10±0.20	9.240	≤0.001

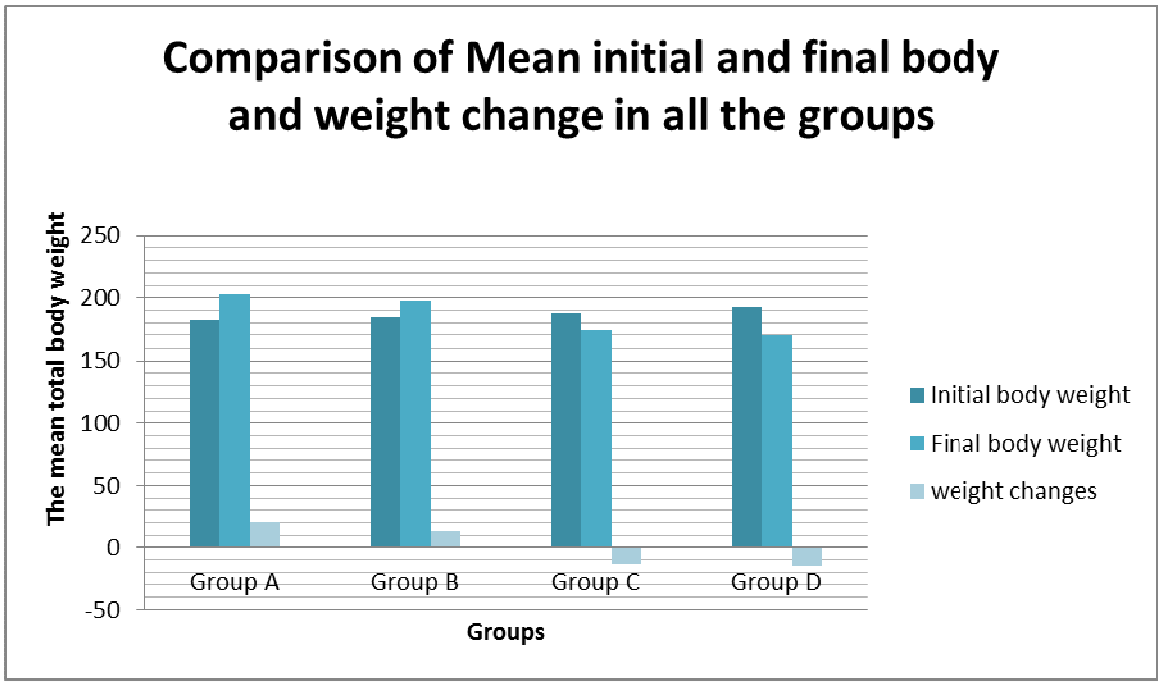

Figure 1: Bar chart showing the mean initial body weight, final body weight and weight changes in all the groups.

Morphometric Analysis of the Spleen Weight

Table 2: Comparison of Mean relative Spleen weight of all the groups (A, B, C & D)

(Mean ± SEM given for each measurement)

Groups	Group A	Group B	Group C	Group D	F-Ratio	Prob. of Sig
spleen weight	6.00±0.140	6.10±0.210	6.31±0.430	6.40±0.131	58.70	<0.001

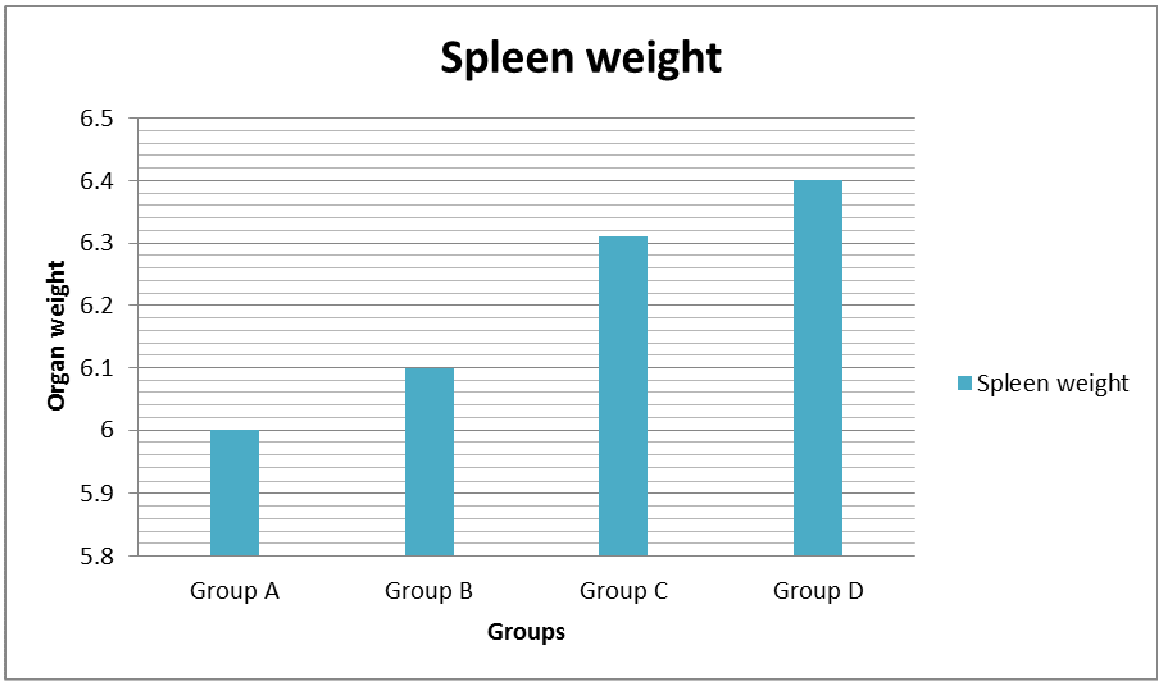

Figure 2: Bar chart showing the organ weights of all the groups

Histological Findings

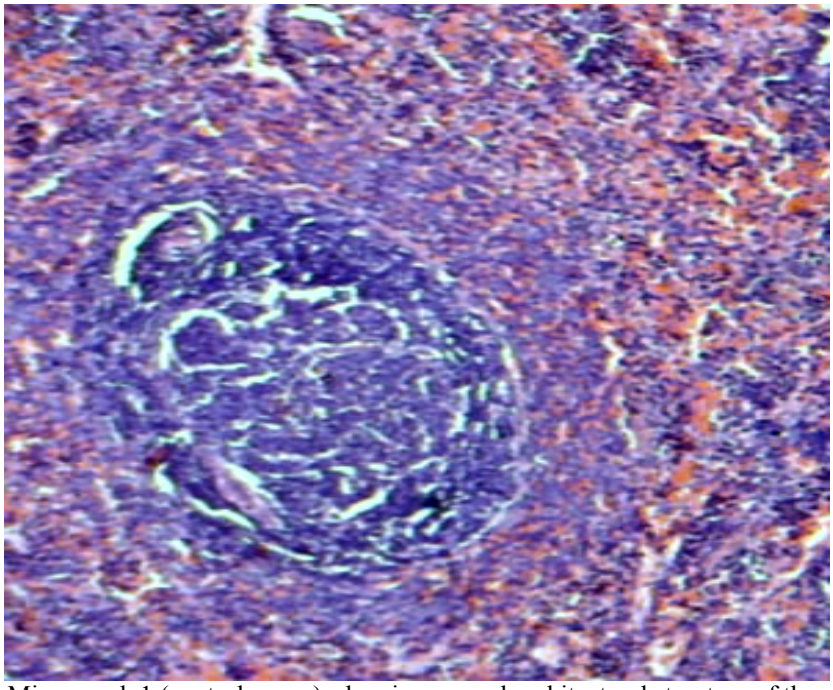

Fig 1: Micrograph 1 (control group). showing normal architectural structure of the spleen.

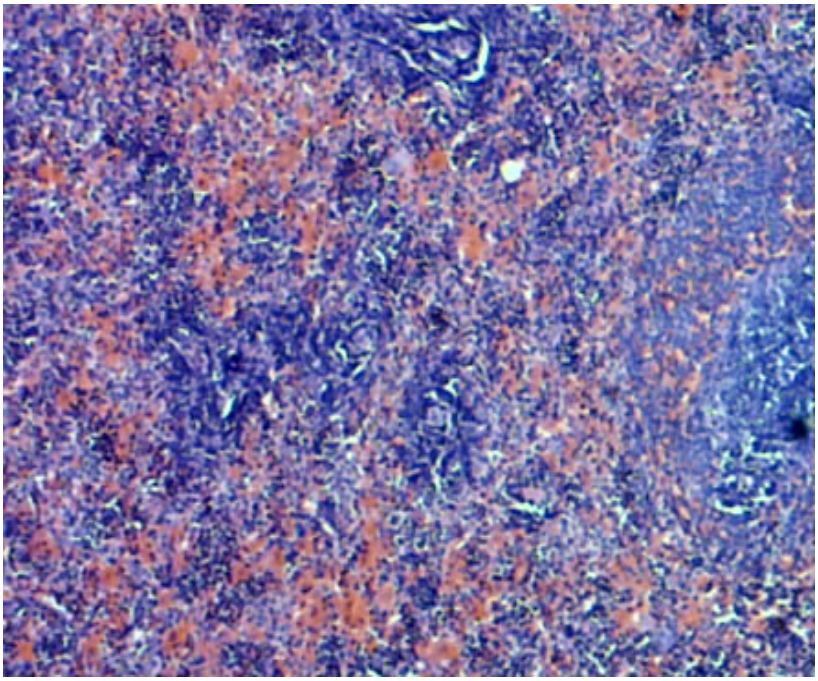

Fig 2: Micrograph 2 of group B (treated with 0.3ml of ethanolic extract of *Garcinia kola*) showing normal architectural structure of the spleen.

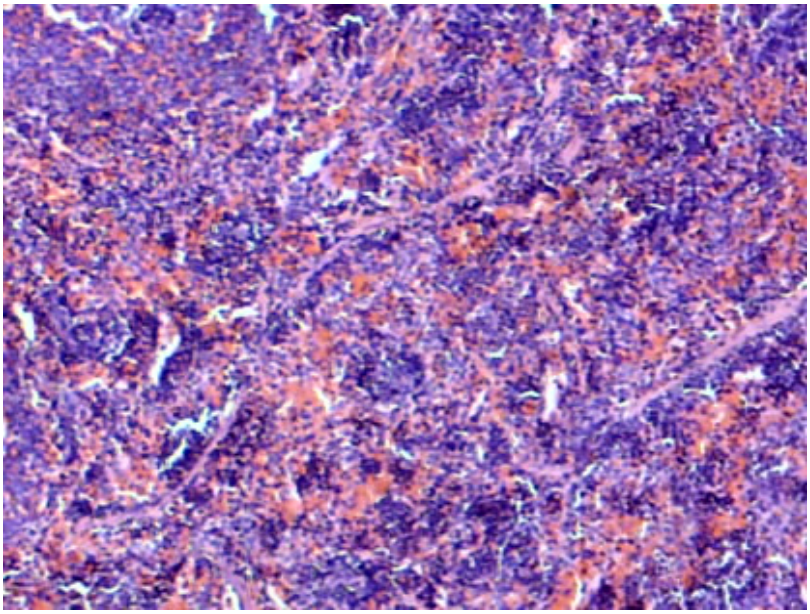

Fig 3: Micrograph 3 of group B (treated with 0.6ml of ethanolic extract of *Garcinia kola)* showing mild degeneration of the spleen cells.

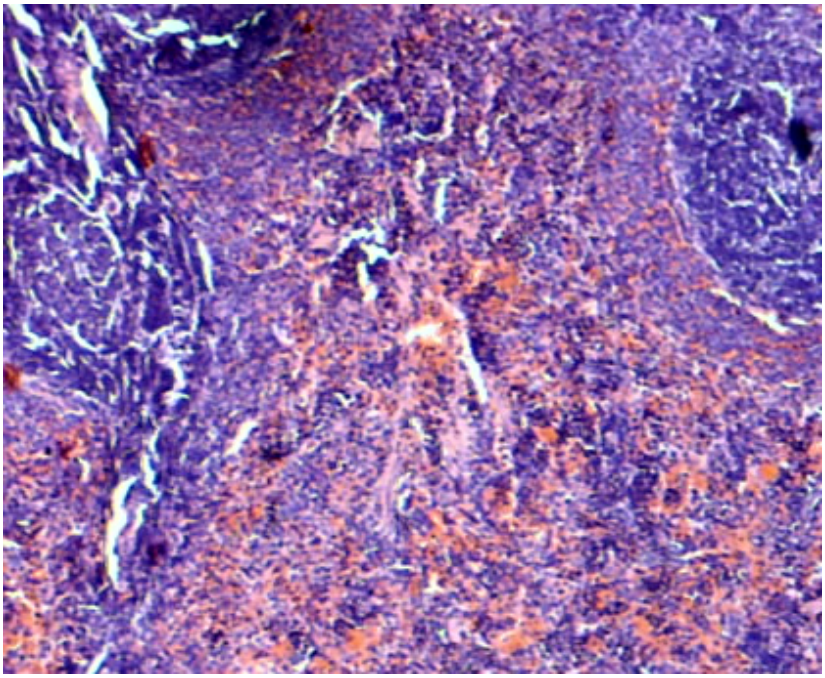

Fig 4: Micrograph 4 of group B (treated with 0.9ml of ethanolic extract of *Garcinia kola)* showing distortion of the spleen cells.

Discussion

Garcinia kola seed is a social masticatorys agent used at ceremonies and presented to guests in several communities of South – Eastern Nigeria. *Garcinia kola* seed is rich in bioflavonoid and has been speculated to stimulate the immune system because of its antioxidant and other related activities demonstrated by the seed extracts (Okonji *et al*., 1991).

Garcinia kola contains antibacterial properties, according to clinical data. They are effective in treatment of infectious disease while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu 1993).

Recently, two new chromanols, Garcinnoic acids, Garcinial, together with 8- tocotrienol were reported, Cardiac glycosides, Saponins, Alkaloids, Hydroxymethyl anthraquinones, phlobatannins, Polyphenols, Glucosides and reducing compounds (Ebanu *et al.,* 1991).

In this study, the final body weight for group C and D decreased significantly (P<0.001) when compared with the control group.

The final body weight of group B animals increased significantly with the experimental control. The comparison of the mean relative organ weight of groups C and D increased significantly (P<0.001) when compare with the control while group B mean relative organ weight was statistically similar with the experimental control group A.

Histological findings showed histological lesions in group C and D treated with high doses of ethanolic extract of *Garcinia kola*.

Conclusion

The finding of this study suggest that consumption of ethanolic extract of *Garcinia kola* in low doses may not put the spleen at risk but when administered in high doses could cause alteration in the cytoarchitecture of the spleen.

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