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Effect of *Garcinia kola*, Heckel on bioavailability of two commonly used drugs (Sulphamethazine and Paracetamol)

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ABSTRACT
The effects of concurrent administration of Garcinia kola Heckel (Guttiferae) seed suspension were investigated on the bioavailability of two commonly used drugs: sulphomethazine and paracetamol in albino rabbits. Two groups of rabbits (n=4) were treated by gavage with a concentration of 0.5g/kg body weight of seed suspension given concurrently with 150mg/kg body weight of sulphonamide and paracetamol respectively. Control groups were given equivalent doses of either drug alone. Blood was withdrawn from the left ear at one hour intervals for five hours. Results showed that G. kola seeds decreased significantly ($p < 0.05$) the bioavailability of the two drugs. Relative bioavailability was calculated to be 77.56% for sulphonamide and 75.39% for paracetamol. The time of peak and the peak concentration were also reduced while the concentration at one hour was only significantly different for paracetamol at $p < 0.05$. These results suggest that G. kola seeds may reduce bioavailability by interfering with drug absorption across the gastrointestinal mucosa.

INTRODUCTION

Garcinia kola Heckel (Guttiferae), is a large forest tree found throughout Western and Central Africa. Widely known in commerce as 'bitter kola, it is used extensively in African traditional medicine for the treatment of various diseases, especially for cough, mouth and respiratory tract infections (Huchkinson and Dalziel, 1954). The seeds are not just grown for pharmaceutical purposes. They are generally harvested and used as adjunct to the true kola nut as social masticatory agent and for esoteric protection against witchcraft and reptiles and scorpion repellency (Esimone et al, 2007).

Studies have reported the use of G. kola seeds as aphrodisiac and for the treatment of diarrhoea and dysentery; and extracts of the seeds have been reported to possess a wide range of pharmacological activities which include anti-inflammatory (Adegoke *et al*., 1998); antihepatotoxic (Iwu, 1985, Iwu *et al*.,1987; Braide 1991a; Madubunyi, 2010; Akintowa and Essien, 1990); antidiabetic (Iwu *et al*., 1990); antimicrobial (Madubunyi, 1995; Hussain *et al*, 1982; Iwu and Igboko, 1982); adaptogenic (Esimone et al, 2007); molluscidal (Okunji and Iwu, 1991); antiashthmatic (Ore and Ekon, 1993) and inhibition of drug metabolism (Braide,1991b). Most of these pharmacological activities are believed to be related to its antioxidative and immune-enhancing properties (Okunji et al, 1999).

One drawback in the use of G. kola seeds is the acclaimed reduction in the effectiveness of drugs in general. This present study is aimed at determining the effect of concurrent ingestion of G. kola seeds on the bioavailability of two commonly used drugs, namely, sulphamethazine, a sulphonamide antibiotic, and paracetamol, an analgesic and antipyretic with no anti-inflammatory properties, in albino rabbits.

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MATERIALS AND METHODS

Materials:

Animals

The animals used for this work were albino rabbits of average weight 1.2 kg obtained from a Rabbitory at Amawbia in Anambra State, Nigeria. The rabbits were randomly assigned to four groups of four animals each, housed in metal cages, and allowed an acclimatization

period lasting 7 days, prior to use for any experiments. The animals had *ad libitum* access to water and standard laboratory animal feed

Plant material
The G. kola seeds were purchased fresh from Eke market, Awka, Anambra State. The seeds were dried, pulverized and stored at $4^{o}C$, until use.

Chemicals
All drugs used were bought from Gauze Pharmaceuticals, Nnamdi Azikiwe University, Awka. All chemicals used were of analytical grade and products of Sigma, South Africa, BDH, Poole, England and Merck, Germany.

METHODS
Administration of Garcinia kola seed powder and drug
After an 8 hour overnight fast, the animals in the two test groups, received concurrently by gavage 0.5g/kg body weight of *G. kola* seed powder (suspended in distilled water) and 150 mg/kg body weight each of sulphonamide or paracetamol respectively, while the controls received only equivalent doses of the drugs without *G. kola*. The animals were allowed food and water ad libitum.

Collection and handling of blood samples
Blood samples were collected at one hour intervals for five hours and allowed to clot for paracetamol while whole blood was used for sulphonamide estimation.. The clotted blood samples were centrifuged at 5,000g for 10 minutes to obtain the sera which were then used for the estimation of serum paracetamol.

Determination of serum paracetamol
Serum paracetamol was determined by a modified method of Glynn and Kendall (1975). The principle is based on the fact that nitrous acid introduces a nitro group into the paracetamol nucleus. The resulting nitrophenol is coloured deep yellow .in alkaline solution.
1 ml of serum was placed in a 25ml centrifuge tube containing 2ml TCA (10%), mixed thoroughly and centrifuged for 2minutes.1ml of supernatant fluid was transferred to a test tube to which 0.5ml of HCL (6mol/L), followed by 1ml sodium nitrite (100g/L). This was allowed to stand for 2minutes after which1.0ml of sulphamic acid solution was added. After the mixture had stopped frothing, 1.0ml of sodium hydroxide (100g/L) was added. A standard paracetamol solution (300mg/ml) was likewise treated. Readings were taken at 430nm against a reagent blank

Calculations:

Serum paracetamol =

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 323$$

Determination of serum sulphonamide
Serum sulphonamide determination was carried out by the method of Bratton and Marshall (1939). The micromodification principle is based on the estimation of free drug in blood. After precipitating the proteins with TCA, the sulphonamide is diazotized and then coupled with N-(1-Naphthyl)-ethylenediamine dihydrochloride after destroying excess nitrate with sulphamic acid. The resulting colour was read at 540nm.
Working standard solution of sulphonamide (2.5mg/L) was obtained by adding 40ml of TCA to 5ml of stock standard solution of sulphonamide (100mg/L) and made up to 299ml with distilled water.

100µl of whole blood was added to 3.0ml of water in a test tube, mixed and 0.9ml of TCA (200mg/l) added. This was allowed to stand for 5 minutes and centrifuged. 2.0ml of the supernatant, standard solution or water were pipetted into test tubes as test, standard or blank respectively to which 0.5ml of HCL (6mol/L), followed by 1ml sodium nitrite (100g/L). This was allowed to stand for 2 minutes after which 2.0ml of ammonium sulphamate (5g/l) was added to each tube. The mixture was left for 2minutes and 1.0ml of coupling agent (500mg/l) added, allowed to stand for 10 minutes and read against the blank at 540nm.

Calculations

Plasma sulphonamide (µg/ml) =

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 100$$

The mean values (SEM) of the serum/plasma concentrations of the drugs were plotted against time Figs 1 and 2), and the pharmacokinetic parameters were determined and shown in table 1.

Relative bioavailability for both drugs was calculated by comparing the AUC in the presence and absence of G. kola seeds respectively, calculated by using the trapezoid rule. The data were analyzed using the student t test (Graph pad Prism software version 4). $P < 0.05$ was considered significant.

Phytochemical tests

Phytochemical analysis was carried out on the crude G. Kola seeds, according to established methods (Evans, 1996, Harborne, 1998).

RESULTS AND DISCUSSION

The effect of *Garcinia kola* seed extract (100 mg/kg) on the bioavailability of two drugs, paracetamol and sulphamethazine was studied. The results (mean ± SEM) indicated that concurrent administration of both drugs and G. kola seeds significantly ($P < 0.05$) decreased average serum concentration, peak serum concentration and time of peak, which occurred at 2 hours for sulphonamide and at 1 hour for paracetamol, in the groups which received concurrent administration of both drugs and G. kola seeds. No peaks were recorded in the groups which received only the drugs (Figs 1 and 2). The initial concentrations at 1 hour were however not affected significantly ($p < 0.05$) for sulphonamide (19.10± 0.07 µg/ml for test and 20.00± µg/ml for the control). However, for paracetamol, the concentrations at one hour for test and control were 71.2±µg/ml and 78.00±µg/ml respectively, showing a more enhanced interaction. The peak plasma concentration of paracetamol and sulphonamide were 71.2 ± 0.00 µg/mL at one hour and 21.4 ± 0.07 µg/mL at two hours, respectively, in the group that received both drug and G. kola seeds. The bioavailability of concurrent administration of drug and G. kola relative to drug alone within five hours was calculated to be 77.56% for sulphonamide and 75.39% for paracetamol (Table 1).

Bioavailability, which is the fraction of an administered drug that reaches the systemic circulation, is dependent on the extent of drug absorption, and is thus crucial to its therapeutic value. Whereas for intravenously administered drugs the bioavailability is 100% or 1.0, drugs administered by other routes have bioavailability values relative to the IV route. Orally administered drugs reach the systemic circulation amidst various adverse factors such as solubility, pH of the gastrointestinal tract; first pass metabolism, etc., which interfere with absorption within the gastrointestinal tract. The test drugs, sulphonamide and paracetamol are both well absorbed in the gastrointestinal tract. Interactions may however occur between the drugs and food or other stomach contents, which would reduce their absorption and hence their bioavailability. Concurrent administration of G. kola seeds with the two drugs reduced their bioavailability most likely by interfering with absorption across the gastrointestinal mucosa. Esimone et al (2002) reported similar in vivo interaction between aqueous seed extracts of G. kola and ciprofloxacin, a broad spectrum antibiotic.

The notion that G. kola seeds can be used to neutralize poisons and to reduce the efficacy of drugs has been of long time ethnobotanical knowledge in the South – Eastern part of Nigeria. Patients undergoing ethnotherapy other than those requiring G. kola are normally advised to avoid chewing the seeds during the duration of therapy. On the other hand, people who have accidentally taken poison are advised to chew the seeds as initial first aid. Phytochemical analyses of G. kola seeds in this work revealed mainly the presence of flavonoids (Table 2), similar to reports by previous workers, consisting mainly of bioflavonoids, xanthones and triterpenes which have been implicated for most of the properties (Iwu, 1985; Monago and Akhidue, 2002). The freshly cut seeds were however observed to release resinous exudates and a suspension of the dried seeds was colloidal. It is not apparent at this stage which of these components, singly or collectively, might be responsible for these observed effects. However, it is suggested that the colloidal suspension may have formed an entrapment for

the drug molecules, thus reducing their absorption across the gastrointestinal tract.

Garcinia kola seeds extract was reported to have protective effect against paracetamol induced hepatotoxicity (Akintowa and Essien, 1990), and CCL4 – induced erythrocyte damage (Farombi, 2002). This was attributed to its antioxidant properties (Olatunde et al, 2002). It is also probable that part of its effectiveness might be due to its interference with drug absorption as observed in this work.

Chronic ingestion of Garcinia kola seeds has however caused untoward pharmacological effects in rats (Braide, 1990; Braide and Grill, 1990; Braide, 1991b, Atawodi et al, 1995,). Reports have also shown considerable levels of oxalates, tannins and saponins in the seeds which may be potentially toxic when ingested in high amounts for long periods (Monago and Akhidue, 2002).

This work has validated the claim that G. kola seeds reduce the bioavailability of drugs when taken concurrently and as such by the same mechanism may also be exploited in reducing the absorption of chemicals and poisons..

The seeds of G. kola continue to be significant in traditional medical practices, for both cultural and therapeutic purposes. In view of its numerous benefits, the use plant however should not preclude its use with orthodox healthcare. However, timing is of essence. It is suggested that G. kola seeds should be eaten at least five hours after taking the last dose of a drug to allow enough time for sufficient/adequate absorption of drug to occur.

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Fig 1: Plasma sulphonamide concentrations with and without G. kola administration

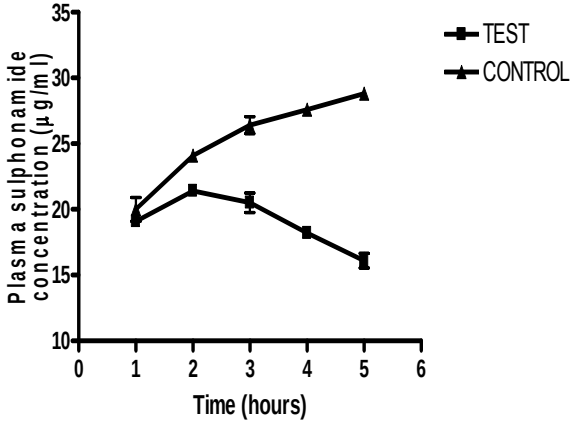

Fig 2: Plasma paracetamol concentrations with and without G. kola administration

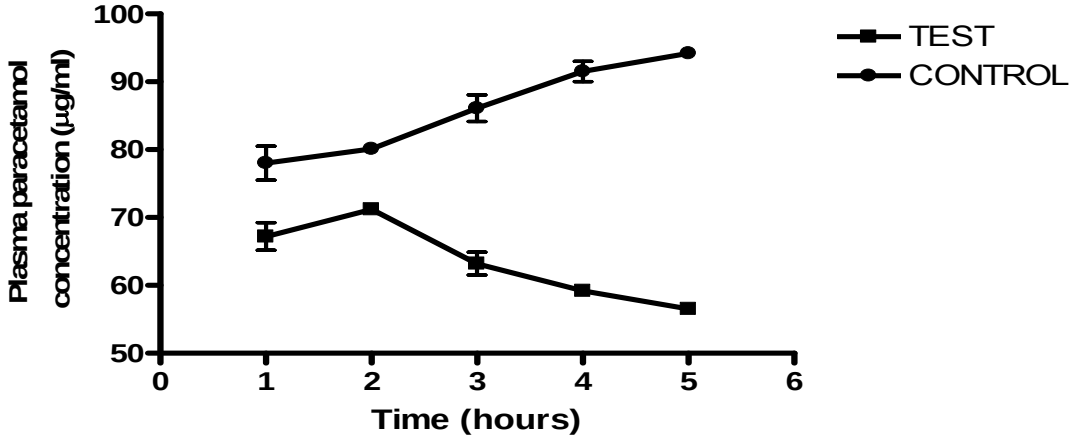

Table 1: Some Pharmacokinetic parameters

Drug	Relative Bioavailability	Concentration at 1hr (µg/ml)	Peak Conc. (µg/ml)	Time of peak
Sulphonamide	77.56%	Test- 19.10 ±0.00 Control- 20.00±2.15	21.4±0.07	2 Hours
Paracetamol	75.39%	Test - 71.20±5.46 Control - 78.00±7.20	71.2±0.00	1 Hour

Table 2: Phytochemical Constituents

Phytochemicals	**Results**
Alkaloids	-
Saponins	+
Tannins	+
Flavonoids	++
Proteins	+
Glycosides:	
Cyanogenic	-
Anthracene	+
Steroid	+
Carbohydrate:	
Reducing sugar	+
starch	+
sterols/Terpenoids	+