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Original Research Article

Extract from the Seeds of *Aframomum melegueta* Alters Acetaminophen Oral Bioavailability in Sprague Dawley Rats

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ABSTRACT

Alterations to relevant pharmacokinetic processes by co-administered drugs are common phenomena of primary importance in drug treatment. These may impact therapeutic objectives positively or negatively; depending on whether the effects are desirable or not. The effect of methanol extract of the seeds of *Aframomum melegueta* (AMSE) on the pharmacokinetics of acetaminophen (N-acetyl-p-aminophenol; APAP) was investigated in Sprague-Dawley rats. Rats weighing 200-300 g were divided into two groups, baseline blood samples were taken, and rats in the test group were administered 25 mg/kg AMSE orally while the other group, serving as control, was administered normal saline (2 mL/kg). 30 minutes post-exposure, rats were administered 100 mg/kg APAP orally; blood samples were taken at times between 5 and 150 minutes, centrifuged, and APAP plasma concentrations were assayed using a colorimetric method for pharmacokinetic parameter determinations. AMSE resulted in an 83% reduction in the bioavailability of APAP. Relevant bioavailability indices such as C_{max} (APAP+AMSE, 2.92±0.51 µg/mL; APAP, 15.6±4.9 µg/mL; P<0.05) and AUC (APAP+AMSE, 226±79.9; APAP, 1,320±405; P<0.05), were significantly reduced by AMSE. Although there was a slight improvement in the rate of APAP absorption in the presence of the AMSE ($T_{max,}$ 5 min) compared to its absence ($T_{max,}$ 15 min), bioavailability in the presence of AMSE was only 17% (F, 0.17) of the control value. Results showed the ability of the extract of the seeds to severely reduce the bioavailability of APAP. The factors responsible for this drastic effect are not known, but interactions between APAP and Nitric oxide (NO) signaling molecules cannot be ruled out.

Keywords: acetaminophen, paracetamol, bioavailability, *Aframomum melegueta*, *nitric oxide*

Introduction

Aframomum melegueta (AM) (Rose) K. *Schum* is of the genus *Aframomum* and family Zingiberceae. It is also a plant widely distributed in West Africa and renowned for quite a long time, for its medicinal properties and ubiquitous use; both as spice and folkloric remedy.[1] In Africa, Aframomum species have been traditionally used to treat illnesses such as inflammation,[2] hypertension, diarrhea, stomachache, bacterial infection,[3,4,] and obesity.[5,2,22] Interest in this plant in recent times has brought attention to its various potential health benefits which are attributed to both its little known class of arylalkanoids and the widely known phenolic components.[6, 3]
Acetaminophen (N-acetyl-p-aminophenol; APAP) is the most commonly used drug for the treatment of pain and fever around the world and as an over-the-counter and prescription product.[7,8]
Since previous studies have reported AMSE to have very potent actions on smooth muscles[9,10,11] , also because of the reported analgesic effect attributed to AMSE,[12] we decided to explore the possible pharmacokinetic interactions between the AMSE and APAP. The choice of APAP for this study was due to its ubiquitous over-the-counter use in mild pain, headache relief, and fever reduction, and also the fact that most patient prescriptions following Doctor Visits contain APAP.

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The aim of this study therefore was to see if pharmacokinetic interaction could result in either synergistic or antagonistic analgesic effects of the two medications.

Methods

Apparatus
Absorbance measurements in this study were made with Spectrovis® Plus spectrophotometer (range: 380-950 nm; resolution: ~2.5 nm optical resolution, 570 wavelengths, 1 nm reporting intervals), with Logger Pro ® Software (Vernier International, 5026 Calle Minorga, Sarasota, FL 34242 U.S.A.) running on an Intel Pentium® PC.

Drugs, Chemicals, and Reagents
Acetaminophen (N-acetyl-p-aminophenol; APAP), Trichloroacetic acid (TCA), concentrated hydrochloric acid, sodium nitrite, sulphamic acid, sodium hydroxide, methanol, heparin, and thiopental sodium were analytical grade reagents sourced from Sigma Chemical Company (Sigma-Aldrich Laborchemikalien GmbH. D-30926, Seelze, Germany).

Preparation of Reagents
15 % trichloroacetic acid, sodium nitrite, and sulphamic acid were prepared by dissolving 15 g of each substance in 100 mL of distilled water, respectively. Heparinized saline was prepared by mixing 1 mL of heparin with 50 mL of normal saline.

Preparation of Plant Extract
Dried *A. melegueta* fruits were bought in June 2019 from a local market in Abia State, Nigeria, authenticated by a match against the University of Uyo Botanical Gardens Herbarium Species Collection; Voucher No: MIA2011, and further dried to a consistent weight in a 40^{o}C oven. The pods were opened to release the seeds which were

ground into fine power using a laboratory manual grinder. 100 g of the powder was weighed and extracted in a Soxhlet Extractor of 500 mL capacity. The material was sequentially extracted with petroleum ether followed by methanol. The methanol extract was evaporated to dryness at a reduced temperature of 40^0C and stored in a desiccator until further use. The percent yield of the methanol extract was calculated to be 6.7%.

Standard Curve for Acetaminophen

1,000 µg/mL stock solution of APAP was prepared by dissolving APAP powder in warm distilled water and then, a serial dilution of the stock solution was performed to give eight (8) concentration series ranging from 25 µg/mL to 500 µg/mL.

The colorimetric assay of acetaminophen in plasma was based on the Glynn and Kendall method as reported by Shihana and co-workers.[13,14] Using a 1 mL syringe, 0.5 mL was withdrawn from each of the samples obtained from the serial dilution and put into labeled 10 mL centrifuge tubes containing 1.0 mL TCA. After vortex mixing, the solutions were centrifuged for five minutes at 4,000 rpm. The clear supernatant was decanted into 10 mL test tubes containing 0.5 mL 6N HCl following which 0.4 mL of 15 % sodium nitrite was added to each test tube and the resulting solution was left to stand for two minutes. 1.0 mL of 15 % sulphamic acid was then added followed by the addition of 2.5 mL of 15 % NaOH. The absorbances of the samples were read at 430 nm against reagent blank and a graph of absorbance versus concentration was plotted to obtain the standard curve for acetaminophen.

Assessment of the Effect of Aframomum melegueta on the Pharmacokinetics of Acetaminophen in vivo.

Eight rats weighing 200-300 g were used for this study; four serving as controls while the other four were experimental rats. They were fasted for twelve hours before the study and subsequently anesthetized using thiopental sodium given intraperitoneally at a dose of 1.6 mg/kg and maintained with ether inhalation where necessary. Following anesthesia, the femoral artery of the rats were cannulated using catheters for rat femoral vein (PU 3Fr 19 cm, collars @ 4.5, purchased from INSTECH Laboratories Inc. 5209, Militia Hill Road, Plymouth Meeting PA 19462-1216, USA), neatly fitted unto a 23-gauge needle attached to a 1 mL syringe.

The control rats received APAP orally at a dose of 100 mg/kg while the experimental rats received the AMSE at a dose of 25 mg/kg orally, thirty minutes before the oral administration of 100 mg/kg APAP.

At time intervals 5, 10, 15, 30, 45, 60, 90, 120, and 150 minutes respectively, 0.3 mL of blood was withdrawn from the rat via the femoral artery cannula into an EDTA sample bottle. The volume of blood sampled from the rats at each sampling time was replaced with an equal volume of normal saline to maintain plasma volume. The period for blood sampling lasted for two hours and the schedule was selected to obtain enough samples during the absorption phase of APAP. The restriction to 2-hour duration of sampling was to avoid anemia and depletion of blood in the rats. At the end of sampling, the contents in the EDTA bottles were transferred into microcentrifuge tubes and centrifuged for fifteen minutes to obtain the plasma.

APAP concentrations in these samples were determined with the Glynn and Kendall method,[13] modified for small sample volume considering the volume of blood obtainable from a rat.

Statistical Analysis

Relevant bioavailability parameters were determined for the control and test groups, data was recorded as Mean ± SEM and statistical comparison was performed using the Student's *t-test*. All calculated probability values were two-tailed and *P*-values of 0.05 or less were considered significant. All statistical tests were carried out using the GraphPad Prism (GraphPad Prism Five for Windows, version 5.01. GraphPad Software Inc.).

Results and Discussion

Standard Curve of APAP

Figure 1A shows the standard curve for APAP; a plot of absorbance against the concentrations obtained from the serial dilution of the stock APAP solution. The standard curve was fitted to a linear equation of the type; Y = m*X + C where Y = absorbance; m = slope X = concentration and C = intercept. Following a linear regression, the parameters obtained for the slope and Y-axis intercept were 0.01635 and 0.01532 respectively (n = 6).

Effect of AMSE on the In-Vivo Absorption and Bioavailability of Acetaminophen

Plasma concentration versus time values for orally administered APAP alone, as well as in the presence of prior administration of AMSE, are shown in Table 1; the corresponding graphic relationship is depicted in Figure 2. In the group given APAP alone, the mean plasma concentrations of APAP peaked (C_{max} = 15.6 ±4.94 µg/mL) in the first fifteen minutes (T_{max} = 15 min.) followed by a gradual decrease to 4.43 ±4.43 µg/mL by 150 minutes. In the presence of AMSE however, the APAP concentration peaked (C_{max} = 2.92 ± 0.51 µg/mL) in the first five minutes (T_{max} = 5 min.), the concentration remains relatively flat up to 150 minutes; fluctuating between 1.58 ± 0.64 µg/mL and 0.74 ± 0.40 µg/mL.

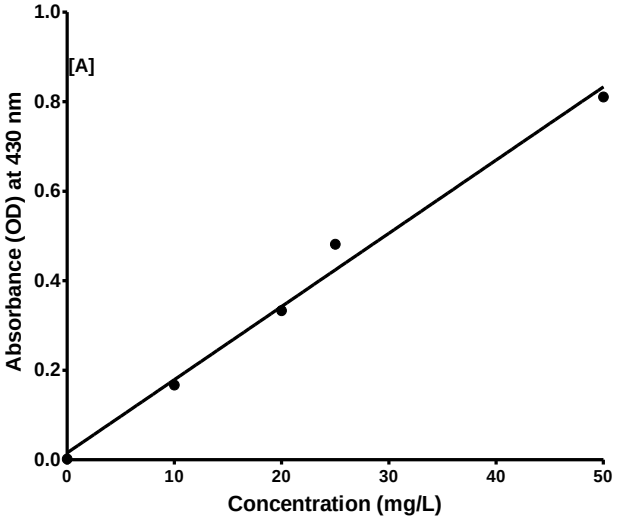

Figure 1: Standard curve for paracetamol

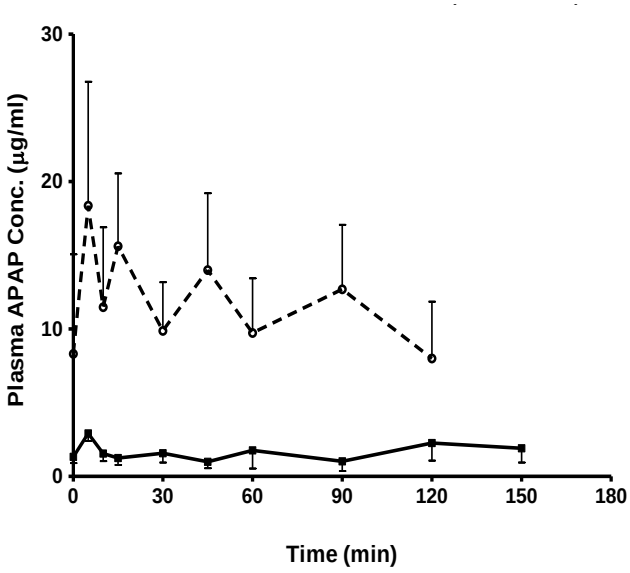

Figure 2: AMSE effect on the oral absorption of APAP

Table 2 lists the relevant bioavailability parameters for the two groups. The area under the curve (AUC) for APAP alone was found to be 1,320 ± 405 µg.min/mL (n=4) while the AUC in the presence of AMSE was significantly lower at 226 ± 79.9 µg.min/mL (n=4) ($p<0.001$) (Table 2 and Figure 3). The relative bioavailability in the presence of AMSE was calculated as 0.17 (17%).

Results from this study revealed that after prior oral administration of AMSE, the bioavailability of orally administered APAP was severely altered. Relevant parameters, such as the maximum concentration C_{max}, were significantly reduced ($P<0.05$) in the group given AMSE compared to the control group. In contrast, however, the meantime for achieving this maximum concentration (T_{max}) was reduced in the AMSE group (5 min.) than in the control group (15 min.), an important pointer to favorable absorption. The overall indicator of drug delivery; the area under the plasma concentration-time curve (AUC), was however significantly reduced ($P<0.05$) in the AMSE group compared to the control. The reason for this severe effect of AMSE on APAP bioavailability is not immediately apparent, but if considered in the context of the reported ability of the extract to decrease gastrointestinal motility, it all begins to offer some hints. Lawal and co-workers had reported the blood pressure lowering effect of seeds of *A. melegueta* in both normotensive and hypertensive patients,[10] an effect they attributed to the relaxation of blood vessel smooth muscles. Umukoro and Ashorobi attributed the antidiarrhoeal activity of the seed extracts to their ability to relax the GIT smooth muscles and decrease the intense peristaltic activity produced by castor oil.[15]

The dependence of paracetamol on the rate of gastric emptying has also been noted for some time and the majority of APAP absorption occurs at the upper GIT, the jejunum, and the ileum; meaning that transit from the stomach to the small intestines constitutes the rate-limiting step in APAP absorption.[16,17] This phenomenon has been utilized as a marker for the measurement and determination of the rate of gastric emptying.[18] The stomach is considerably smaller than the surface area of the main drug absorption site; the duodenum and ileum and considering that the intestinal surface area (30 40 m^2) is far larger than the gastric surface area (1 m^2),[19] it appears logical that anything that will impede the transit of APAP into the jejunum and ileum will reduce its bioavailability.[20] In addition to this also is the reported ability of APAP itself or its metabolite to inhibit the L-type calcium channel by blocking the release of Ca^{2+} from the sarcoplasmic reticulum and therefore delaying transit into the intestine;[7] this effect by APAP however is expected to be cancelled out by comparison with the control group. It can also be reasonably argued that the relaxing effect on the gastrointestinal tract will afford APAP enough resident time as well as increase blood flow and surface area to allow enhanced total absorption from oral administration, which probably is responsible for the reduced t_{max} in the group given AMSE+APAP. This effect is minimal given the greater impediment to the total amount of APAP in the presence of AMSE. It may seem logical that since the extract slows down peristalsis along the whole length of the tract, and since the stomach is the first region of transit by APAP and other orally administered drugs, coupled with the fact that the relative surface area of the stomach is considerably smaller than the surface area of the main drug absorption site; the duodenum and ileum, retention of APAP in the stomach will greatly affect its relative absorption and therefore bioavailability. This would be a consequence of the fact that the drug will not get to the intestine at a rate fast enough for optimal absorption; by overwhelming the first-pass metabolism of APAP, and the intestine is by far a better absorption site, in terms of surface area than the stomach. It is not immediately obvious what the specific mechanisms of the GIT relaxant effect of the extract are, and to what extent the suggested inhibition of peristalsis impacted on GIT absorption; particularly that of APAP. Consideration of the relative contributions of the physical characteristics and the anatomical differences between the stomach and the small intestine may not necessarily be the sole cause of the 83% reduction of APAP absorption by AMSE. Components of AMSE have also been shown to be able to stimulate the estrogen receptor thereby leading to the release of NO and subsequently smooth muscle dilation. These components referred to as the phytoestrogens, are structurally arylalkanoids and their relaxant action will explain the ability of AMSE to inhibit gastric emptying. However, a direct chemical interaction between the NO molecule produced through phytoestrogen-membrane estrogen receptor interaction and APAP itself is an emerging subject in the hitherto unknown biochemical interaction involving endogenous APAP. In the presence of phytochemicals capable of producing copious amounts of NO in the intestinal tract; which is the site of APAP absorption, this potential direct interaction will severely affect the bioavailability of APAP, regardless of the hepatic first-pass metabolism. This may be a substantial contributor to the 83% depression of APAP bioavailability. While this phenomenon of herbal impact on orthodox drug kinetics is becoming more commonplace,[23] the possibility that modulation of the NO metabolism is responsible for the observed effect in this study is currently under investigation.

Conclusion

The seed extract of *Aframomum melegueta* caused a massive decrease in the oral bioavailability of APAP. From the available information in this study, it is apparent that the reduced bioavailability of APAP in the presence of AMSE cannot be attributed solely to the inhibition of peristalsis and the reduction of gastric emptying. A direct chemical interaction between NO and APAP may contribute significantly to the reduced availability. AMSE has been known to contain NO-releasing phytoestrogens, acting indirectly through the membrane-bound estrogen receptors

Conflict of Interest

The authors declare no conflict of interest.

Table 1: Plasma concentration-time values of acetaminophen (APAP) in the absence and presence of AMSE

Time	Plasma concentration (µg/mL)	
	APAP	APAP + AMSE
0	8.31 ± 6.76	1.32 ± 0-43
5	14.7 ± 7.47	2.92 ± 0.51
10	9.18 ± 4.79	1.55 ± 0.52
15	15.6 ± 4.94	1.25 ± 0.47
30	7.89 ± 3.23	1.58 ± 0.64
45	11.2 ± 4.91	0.74 ± 0.40
60	9.73 ±3.71	1.32 ± 1.0
90	12.7 ± 4.38	1.03 ± 0.7
120	8.00 ± 3.85	1.13 ± 0.81
150	4.43 ± 4.43	1.91 ± 1.0
180	0.548 ± 0.548	NA

Table 2: Relevant bioavailability parameters following oral acetaminophen with or without AMSE

Bioavailability parameters	**Treatment**	
	APAP	**APAP + AMSE**[†]
T_{max} (min)	15	5
C_{max} (µg/mL)	15.6 ± 4.9	2.92 ± 0.51
AUC (µg.min. ml^{-1})	1,320 ± 405 (N=4)	226 ± 79.9[*] (N=4)
Dose (mg/kg)		
F	0.171212 (17%)	

The F parameter, which is an indication of relative bioavailability, was derived as the ratio of the AUC of acetaminophen alone and the AUC of acetaminophen in the presence of AMSE ($F = AUC_{APAP + AMSE}/AUC_{APAP}$)

† The dose of AMSE administered was 25 mg/kg. * P<0.06

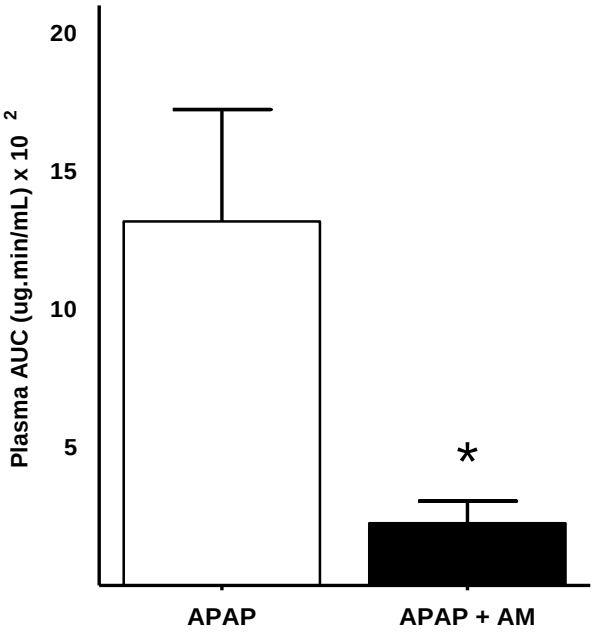

Figure 3: AUC for APAP in the presence and absence of AMSE.

The Mean AUC for acetaminophen plasma concentration in the absence of AMSE was 1,320 ± 405 N=3 and in the presence of AMSE was 226 ± 79.9 N=4. The difference was significant (P<0.05).

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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