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International Journal of Current Research
Vol. 6, Issue, 03, pp.5514-5517, March, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

HISTOLOGICAL EFFECTS OF *AFRAMOMUM MELEGUETA* ON THE SPLEEN OF ADULT WISTAR RATS

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ARTICLE INFO

Article History:
Received 25th December, 2013
Received in revised form
30th January, 2014
Accepted 19th February, 2014
Published online 25th March, 2014

Key words:
Aframomum melegueta,
Body weight,
Liver weight,
Wistar rats.

ABSTRACT

This work focuses primarily on investigating histological effects of *Aframomum melegueta* aqueous extract on the spleen of adult wistar rats following oral administration. Twenty wistar rats of weights 180 – 215kg were divided into four groups designated as A,B,C and D. Group A served as the control and were orally administered with 0.35ml of distilled water daily; the experimental groups B,C & D were orally administered with 0.55ml, 0.65ml and 0.75ml of *Aframomum melegueta* aqueous extract for twenty eight days respectively. Twenty four hours after the last administration, the animals were weighed, anasthetized under chloroform vapour and dissected. Spleen tissues were removed, weighed and trimmed down for histological studies. The final body weight of the experimental groups (B, C & D) increased significantly ($P<0.001$) with the control. The relative spleen weight of the experimental groups B,C & D statistically increased ($P<0.001$) with the control (A). Histological results showed normal spleen architecture in the experimental groups B, C, & D relative to the control (A). This study therefore suggests that consumption of *Aframomum melegueta* aqueous extract at different doses did not induce spleen of adult wistar rats.

INTRODUCTION

Aframomum melegueta (of the family *Zingiberaceae*) is a spice in the Ginger family with the common name of 'Grains of Paradise' or 'Alligator Pepper'. The spice is used in West Africa for the purposes of alleviating stomachache and diarrhea (Ilic *et al*., 2010) as well as hypertension (Gbolade 2012) with some limited reports on it being used for Tuberculosis (Ogbole and Ajaiyeoba 2009) and a remedy for snakebites and scorpion stings. (Lans *et al*., 2001) These seeds are also used for culinary reasons (due to the pungency of the seeds, it is commoly used as seasoning on food products. (Ilic *et al*., 2010; Gbolade 2012; Ogbole and Ajaiyeoba 2009; Lans *et al*., 2001; Van Andel *et al*., 2012) The seeds also tend to have general anti-microbial properties similar to many spices, (Van Andel *et al*., 2012; Konning *et al*., 2004) and has some molluscicidal, (Ndamukong *et al*., 2006) and repellant (Ukeh *et al*., 2009; Ukeh *et al*., 2010) properties as well. It is one of many pungent herbs said to aid in sexuality and aphrodisia (Kamtchouing *et al*., 2002) (although the class of 'pungent herb' appears to be mentioned more than this particular seed). Grains of Paradise are a spice botanically related to Ginger, and have usage for gastrointestinal/digestive health as well as being used to season foods. *Aframomum melegueta* is widely used by many cultures in Nigeria for various purposes. It is served along with Kola nuts to guests for entertainment, members of the Iyayi (Faith) Society of Nigeria as communion and used for religious rites by diviners for invoking spirits. It is a common ingredient in pepper soup, a spicy delight in most parts of West Africa. Concoctions made of *Aframomum melegueta* are often used by traditional doctors as medications for various ailments. (Personal observation) Pregnant women are not excluded from eating this widely used substance. The constituents of an essential oil, extractable by hydro-distillation from the seeds of *Aframomum melegueta* include two sesquiterpene hydrocarbons, humelene and caryophyllene, their oxides and a few non-terpenoids. (Ajaiyieoba and Ekundayo1999).

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MATERIALS AND METHODS

Breeding of Animals

Twenty wistar rats were purchased from animal house of Anatomy Department, University of Calabar, Cross State, Nigeria. They were bred in the Animal house of University of Nnamdi Azikiwe Nnewi Campus, Anambra State. They were allowed for a period of seven days for acclimatization under normal temperature ($27°C$ - $30°C$) before their weights were taken. They were fed ad libitum with water and guinea feed pallets from Agro feed mill Nigeria Ltd.

Drug Preparation

Aframomum melegueta, obtained in Nkwo market, Nnewi, Anambra State, Nigeria and grounded into powder with a clean grinding machine. 200mg of the extract/kg body weight were dissolved in 5mls of distilled water and administered to the animals.

Experimental Protocols

The twenty animals were weighed and allocated into four groups of five animals each. The groups were designated as A,B,C & D. Group A served as the control and received 0.35ml of distilled water. Experimental groups B,C & D received 0.55ml, 0.65ml and 0.75ml of *Aframomum melegueta* aqueous extract respectively for twenty eight days. On the 29th day, the animals were weighed and recorded. Twenty four hours after the last administration, the animals were anaesthetized under chloroform vapour and dissected. Spleen tissues were removed from the animals and weighed. The tissues were trimmed down to a size of 3mm x 3mm thick and fixed in zenkers fluid for four hours for histological studies.

Tissue Processing

For easy study of sections under microscope the tissues passed through several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in 10% formaldehyde. The tissues remained in the 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 alcohol 50%, 70% and 90% absolute. After dehydration, tissue were cleared in xylene for two hours after which infiltration was done in molten paraffin wax at a temperature of 60°C for two hours each in two changes and then sectioned. Haematoxylene and eosine method was used.

respective EC_{50} values of 6 Ginger shogaol (11.2µM and 0.2µM) and 6-paradol (71µM and 0.7µM) (Riera *et al*., 2009) suggesting they are weaker than Evodiamine from Evodia rutaecarpa (856nM) and Capsaicin (45nM). (Pearce *et al*., 2004) Appears to activate TRPV1 recpetors, with less potency relative to other TRPV1 agonists; also appears to activate TRPA1 receptors. A toxicological study in rats feeding *Aframomum melegueta* dailt for 28 days noted that, in male rats only, a dose-dependent decrease in blood glucose was observed at 450mg/kg (7.3%) and 1500mg/kg (20%) of the ethanolic extract.[(1)] In rats given a large amount of Alcohol (4.8g/kg) for 15 days, coingestion of *Aframomum melegueta* (100-200mg/kg water extracts) noted that the higher dose was able to prevent an increase in liver weight and fully abolish lipid peroxidation as assessed by MDA while preserving both GSH and GST; hepatic superoxide dismutase (SOD) was not significantly influenced by *Aframomum melegueta* despite it being reduced with ethanol. (Nwozo and Oyinloye 2011) The increase in serum AST and ALT was also fully normalized. (Nwozo and Oyinloye 2011). One study has noted that *Aframomum melegueta* methanolic and chloroform extracts held cytotoxic potential against PANC-1 pancreatic cancer cells *in vitro* with IC_{50} values of 13.8µg/mL and 47.8µg/mL, respectively (Dibwe *et al*., 2012) In the present study, the mean initial and final body weight change indicated that the body weight of the experimental groups treated with *Aframomum melegueta* aqueous extract increased significantly($P<0.001$) relative to the control. The aqueous extract of *Aframomum melegueta* in this

RESULTS

Morphometric Analysis of Body Weight

Table 1. Comparison of mean initial and final body and weight change in all groups (A, B, C & D) (Mean ± SEM given for each measurement)

	Group A	Group B	Group C	Group D	F-Ratio	PROB. OF SIG
Initial Body Weight	190.20 ± 2.30	192.20± 3.60	193.40 ± 4.10	194.10 ± 2.70	64.240	<0.001
Final Body Weight	215.30 ± 4.20	219.40± 3.50	220.70 ± 3.20	221.60 ± 2.40	40.180	<0.001
Weight Change	25.10± 3.70	27.20± 4.10	27.30± 3.90	27.50 ± 3.40	19.155	<0.001

The final body weight for the experimental groups B, C & D increased significantly (<0.001) relative to the control (A).

Morphometric Analysis of Liver Weights

Table 2. Comparison of mean relative spleen weight of group A (control) and experimental groups B, C & D. (Mean ± SEM given for each measurement)

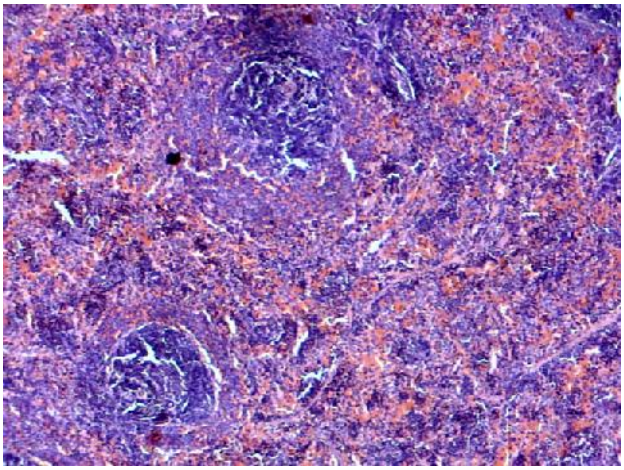
	Group A	Group B	Group C	Group D	F-Ratio	PROB. OF SIG
Spleen Weight	4.60 ± 0.150	4.61 ± 0.220	4.66 ± 0.400	4.69 ± 0.170	40.60	<0.001

The relative spleen weights for the experimental groups B, C, & D increased significantly ($P<0.001$) relative to the control (A).

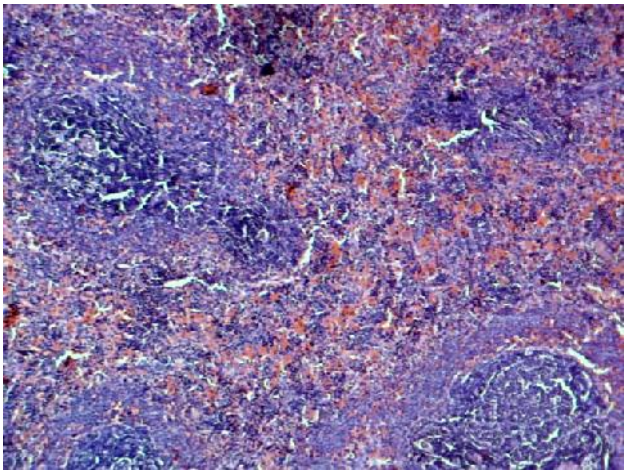
DISCUSSION

Aframomum melegueta (as well as *Aframomum cuspidatum* and *Harrisonia abyssinica*) have been noted to, *in vitro*, inhibit CYP3A enzymes with *Aframomum melegueta* at 10mg/mL concentration inhibiting CYP3A4 (Full inhibition with ethanolic extract, 98.85+/-0.1% with tea), CYP3A5 (99.87+/-0.3%), and CYP3A7 (97.24+/-0.2%) (Agbonon *et al*., 2010) Appears to inhibit CYP3A enzymes, and is remarkably potent at doing so. Although preliminary, there is a high possibility of drug-drug interactions with *Aframomum melegueta* 6-shogaol and 6-paradol, two components of *Aframomum melegueta* as well as, appear to active TRPA1 and TRPV1 receptors with the instance functions primarily as a dietary supplement enhancing growth. Histopathological findings of this study showed that *Aframomum melegueta* consumption in low and high doses did not distort the spleen architecture when compared with the control. Administration of extract of *Aframomum melegueta* did not cause weight loss to the experimental animals compared with the control. By this observation, one may deduce that administration of *Aframomum melegueta* may boost the tolerance capacity against toxic compounds. Thus the protective effect of aqueous extract of *Aframomum melegueta* recorded in the present study is attributed to its antioxidant properties.

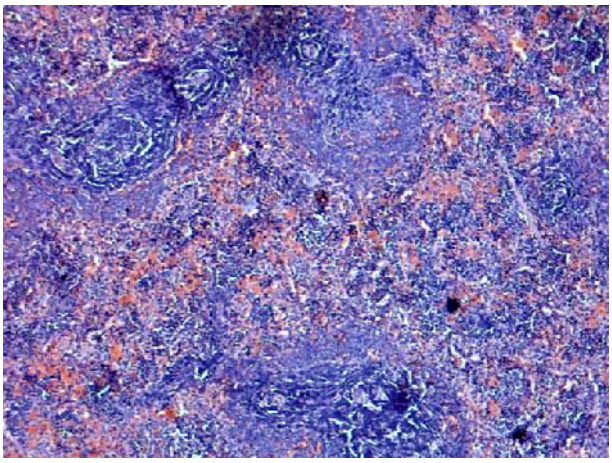
Histopathological findings



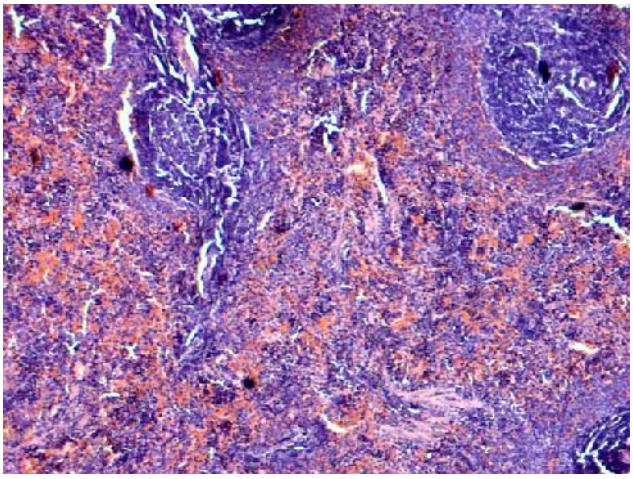
Micrograph 1, (control), shows normal histological structure of spleen, stained by H & E technique, x 200.



Micrograph 2, Group B, (treated with 0.55ml of *Aframomum melegueta* aqueous extract) showing normal histoarchitecture of the spleen, stained by H & E technique, x 200.



Micrograph 3, Group C, (treated with 0.65ml of *Aframomum melegueta* aqueous extract) shows none distortion of the histoarchitecture of the spleen, stained by H & E technique, x 200.



Micrograph 4, Group D, (treated with 0.75ml of *Aframomum melegueta* aqueous extract), showing near normal histological structure of the spleen, stained by H & E technique, x 200.

Conclusion

The aqueous extract of *Aframomum melegueta* did not induce any histopathological lesions in the spleen tissues of the rats. Rats tissues are very similar to those of human. The findings of this study suggests that *Aframomum melegueta* administered to individuals exposed to toxic compounds could provide some protection and perhaps ameliorate its toxic effects on the spleen.

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