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Toxicological evaluation of Grains of Paradise (*Aframomum melegueta*) [Roscoe] K. Schum

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Abstract

Ethnopharmacological relevance—Grains of Paradise (*Aframomum melegueta* [Roscoe] K. Schum.) seeds are used in West Africa as a remedy for variety of ailments such as stomachache, snakebite, diarrhea and they have reported anti-inflammatory properties. Additionally, the seeds contain gingerols and related compounds that may be useful against cardiovascular disease, diabetes, and inflammation.

Aim of study—A 28-day sub-chronic toxicity study in male and female Sprague Dawley rats was conducted to evaluate the safety of a Grains of Paradise extract.

Materials and methods—An ethanolic extract of the seeds was evaluated for toxicological effect on rats.

Results—A dose-related increase in absolute and relative liver weights was observed in males and females dosed with 450 and 1500 mg/kg. There was a corresponding increase in alkaline phosphatase with no signs of steatosis or cirrhosis. At the same doses, there was a significant decrease in blood glucose in male rats.

Conclusions—This study shows that Grains of Paradise extract may be useful as a treatment for diabetes, however liver toxicity should be considered.

Keywords

Aframomum melegueta; gingerols; liver toxicity; alkaline phosphatase; dietary supplement

1. Introduction

Grains of Paradise (*Aframomum melegueta* [Roscoe] K. Schum.), also known as Guinea pepper or Alligator pepper, is a member of the Zingiberaceae family native to West Africa. It is a herbaceous perennial plant that grows up to 1.5 meters height, with purple flowers that develop into 5 to 7 cm long pods containing small, reddish-brown aromatic and pungent seeds. In the region of West Africa the seeds are used as a spice for flavoring food and have a wide range of ethnobotanical uses. They are used as a remedy for treating stomachache,

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diarrhea, and snakebite (Akendengue and Louis, 1994; Umukoro and Ashorobi, 2007). Also it has been shown that aqueous seed extract had anti-inflammatory and peripheral analgesic activity (Umukoro and Ashorobi, 2001). Grains of Paradise are very rich in the non-volatile pungent compounds gingerols, shogaols, paradols, and related compounds (Connell, 1970; Connell and McLachlan, 1972; Tackie et al., 1975). Similar compounds are found in many species of the Zingiberaceae family including ginger (*Zingiber officinale* Roscoe), turmeric (*Curcuma longa* Linnaeus), and cardamom (*Amomum cardamomum* Linnaeus). Gingerols have reported blood glucose lowering (Al-Amin et al., 2006) and anti-inflammatory (Tjendraputra et al., 2001; Jolad et al., 2005) activities, which may be useful for treating diabetes and cardiovascular disease. Therefore, toxicology studies were initiated to evaluate the safety of using Grains of Paradise extract as a botanical therapeutic.

2. Materials and methods

2.1 Plant material and extraction

Seeds of *Aframomum melegueta* [Roscoe] K. Schum. were purchased from a commercial supplier from Abidjan, Ivory Coast and identified by Dr. L. Struwe, Rutgers University. A voucher specimen (Struwe 1424 CHRB) was deposited at the Chrysler Herbarium, Rutgers University, New Brunswick, USA.

A single batch of dry Grains of Paradise seeds were extracted in 95% ethanol (1:10 w/v) for 24 h at room temperature (24 °C) with continuous agitation provided by a platform shaker. The resulting ethanolic extract was filtered and the solvent was removed under vacuum, using a rotary evaporator (yield of the extract: 2%). The extract was stored at 4 °C.

2.2 LC-MS analysis

To determine the chemical constituents, Grains of Paradise extract was separated and analyzed with a Waters (Milford, MA) LC-MS Integrity system consisting of a solvent delivery system including a W616 pump and W600S controller, W717 plus auto-sampler, W996 PDA detector and Waters TMD Thermabeam electron impact (EI) single quadrupole mass detector. Data were collected and analyzed with the Waters Millennium v. 3.2 software, linked with the 6th edition of the Wiley Registry of Mass Spectral Data, containing 229,119 EI spectra of 200,500 compounds. Substances were separated on a Phenomenex Luna C-8 reverse phase column, size 150 × 2 mm, particle size 3 μm, pore size 100 Å, equipped with a Phenomenex Security Guard pre-column. The mobile phase consisted of 2 components: solvent A (0.5% acetic acid in double distilled de-ionized water, pH 3–3.5), and solvent B (100% acetonitrile). The mobile phase flow was adjusted at 0.25 ml/min, and generally a gradient mode was used for all analyses. The gradient points were for time 0.0 min – 95% A and 5% B; for time 35.0 min – 5% A and 95% B; held isocratic for 5 minutes and from 40.0 min to 45.0 min. – back to initial conditions of 95% A and 5% B. A column equilibration time of 15 min was set between subsequent injections.

2.3 Animals

The Sprague-Dawley derived rat was the system of choice because, historically, it has been the preferred and commonly used species for oral toxicity tests. Fifty Hsd: Sprague Dawley (SD) rats (25 males and 25 females) were obtained from Harlan, Haslett, Michigan and were designated by the supplier to be 6–7 weeks old. The rats were individually housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent National Institute of Health Guide for the Care and Use of Laboratory Animals. Animal room temperature and relative humidity were 19–22 °C and 30–70 %, respectively, and there was a 12 h light/dark cycle. Animals were allowed to acclimate to the environment for 1 week and were supplied with PMI Lab Diet Purina

Certified Rodent Meal #5002 and filtered tap water *ad libitum* during the acclimation and study periods. All experimental procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 80–23 revised 1996). This study was performed by Eurofins / Product Safety Laboratories (Dayton, NJ), study #20684, protocol #P712.02 PHY.

2.4 Dose preparation and administration

From the original 50 rats, 40 healthy rats (20 males and 20 females) seven to eight weeks of age, weighing 193–207 g (males) and 150–175 g (females) were randomly assigned (5/sex/group) to each of the test groups and administered Grains of Paradise extract or vehicle control. The dose levels were assigned as low dose (120 mg of extract/kg/day), intermediate dose (450 mg of extract/kg/day) and 1500 mg of extract/kg/day). The dose selection was based on the planned human exposure in the potential nutritional supplement. Individual doses were calculated based on the most recent weekly body weights of each animal. Extract concentrations were adjusted each week to maintain the targeted dose level for each animal (i.e., mg/kg/day) so that all doses were administered at a constant volume of 5 ml/kg. The control animals received the vehicle only at the same volume as the test groups. The test article was administered as a 2.4%, 9.0%, or 30.0% w/v solution in 10% ethanol in corn oil. On each day of dosing, for each concentration, an appropriate amount of the test article was weighed into a 50 ml volumetric flask. To facilitate the mixing process, approximately 5 g of ethanol was added to the flask and vortexed. The mixture was diluted to 50 ml with corn oil and vortexed again. Dose preparations were used within approximately 2 h and maintained on a magnetic stir plate during administration. Each animal was dosed by oral gavage (using a stainless steel ball-tipped gavage needle attached to an appropriate syringe) daily at approximately the same time ± 2 h for 28 consecutive days. The first day of administration was considered day 1 of the study.

2.5 Clinical observations

All animals were observed twice daily for mortality. Cage-side observations were made daily during the study and any abnormal findings recorded. Detailed observations were recorded on day 1 (prior to administration of test article) and weekly thereafter on all animals. These observations were conducted both while handling the animal and with the animal placed in an open field. Observations included, but were not limited to: changes in skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypes (e.g., excessive grooming, repetitive circling), or aberrant behavior (e.g., self-mutilation, walking backwards) were also recorded.

2.6 Food consumption and food efficiency

Animals were allowed *ad libitum* access to food throughout the study. Animals were fasted overnight prior to blood collection on day 1, 15, 29 and prior to terminal sacrifice on day 31. Individual food consumption was measured, adjusting for spillage, and recorded weekly to coincide with body weight measurements. Mean food consumption was calculated for each sex/dose level during each weekly interval and overall testing interval. Mean food efficiency was also calculated for each sex/dose level based on body weight gain and food consumption data.

2.7 Body weight and body weight gain

Individual body weights were recorded twice during the acclimation period, at study initiation (day 1), and weekly thereafter. Mean body weight gains were calculated for each

group at each interval and for the overall (days 1–28) testing interval. Animals were also weighed immediately prior to sacrifice (fasted body weight) for calculation of organ to body weight and organ to brain weight ratios.

2.8 Clinical pathology

All animals were fasted overnight prior to each blood collection. Blood samples for hematology (except prothrombin time and partial thromboplastin time) and clinical chemistry from the test and control groups were collected via orbital sinus bleeding under isoflurane anesthesia on study day 29. Fasting blood glucose was measured on days 1, 15, and 29. Blood samples used to determine the prothrombin time and partial thromboplastin time were collected via the inferior vena cava under isofurane anesthesia for all groups at terminal sacrifice. All blood samples were evaluated for quality by visual examination prior to analysis. Upon completion of clinical chemistry, remaining serum samples were pooled for serology. Pooled serum samples from test animals were evaluated for the absence of viruses near the end of the in-life portion of the study. Examinations were made by Haskell Laboratory for Health and Environmental Sciences.

2.9 Necropsy and histopathology

At scheduled sacrifice, all rats were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia. Isoflurane (5–10ml) was placed on gauze and administered to the rats enclosed in a bell jar. The animals were placed on the perforated surface above the gauze so they were never in contact with the liquid. The animals were monitored until anesthetized by checking for the absence of reflexes (pain response to pinch test) and that respiration was even and unlabored. Blood collection was performed via the vena cava. Once this procedure was done the exsanguination was performed. All rats were sacrificed on day 31. Gross necropsy included an initial examination of external surfaces and orifices, as well as the cranial, thoracic and abdominal cavities and their contents. Rats were examined for gross lesions. Tissues of interest and any gross lesions were retained in neutral buffered 10% formalin (NBF).

The liver, kidneys, adrenals, brain, heart, thymus, spleen, uterus, ovaries, testes, and epididymides (of all animals sacrificed by design) were weighed wet as soon as possible after dissection to avoid drying. The following organs and tissues from all animals were preserved in NBF for possible future histopathological examination: all gross lesions, lungs, trachea, brain- including sections of the medulla/pons, cerebellar cortex and cerebral cortex, spinal cord (3 levels: cervical, mid-thoracic, and lumbar), thymus, heart, sternum with bone marrow, adrenals, liver, spleen, kidneys, thyroid/parathyroid, ovaries, testes, uterus (with attached urinary bladder, cervix and vagina), esophagus, ileum, cecum, accessory genital organs (epididymides, prostate, and seminal vesicles), peripheral nerve (sciatic), stomach, duodenum, jejunum, colon, rectum, representative lymph node (mesenteric and mandibular), and pancreas and salivary glands. Histological examination was performed on the preserved organs and tissues of the animals from the control groups and test high dose groups. The fixed tissues were trimmed, processed, embedded in paraffin, sectioned, placed on glass microscope slides, and stained with hematoxylin and eosin. Slide preparation and histopathological assessments were performed by Histo-Scientific Research Laboratories.

2.10 Statistics

Mean and standard errors were calculated for all quantitative data. Treated and control groups were compared using a one-way analysis of variance (ANOVA), followed by comparison of the treated groups to control by Dunnett’s t-test for multiple comparisons. A 95% confidence level was used to determine statistically significant differences between treated and control groups.

3. Results

3.1 Major compounds in the extract

Analysis of the extract by LC-MS revealed three major peaks. The peaks in the HPLC chromatogram (Fig.1) matched the UV spectra and mass fragmentation patterns of previously reported gingerols (Connell, 1970). They are putatively identified as 6-gingerol, 6-shogaol and 6-paradol. These compounds make up the bulk of extract and were most likely responsible for the biological activity. The most abundant were putatively identified as [6]-gingerol (19.5 % of the total extract), [6]-shogaol (12.5 % of the total extract) and [6]-paradol (30.5 % of the total extract; calculated as a percentage relative to the total peak area at 254 nm).

3.2 Clinical signs

There were no extract related mortalities, however one female (1500 group) and one male (control group) died on test days 5 and 7, respectively, as the result of an intubation error. Additionally, one female (120 group) died on test day 29 during orbital sinus bleeding. Transient clinical signs, most prevalent in the 1500 groups, included soft feces (5/5 males; 2/5 females), facial staining (1/5 males; 4/5 females), ocular and oral discharge (1/5 females), and uro-genital staining (1/5 males; 3/5 females). In addition, one male of the low dose group had red discharge of the penis on test day 3.

3.3 Body weights and food consumption

Overall (days 1 to 28) body weight, mean daily body weight gain, mean daily food consumption, and daily food efficiency for male and female rats at 120, 450 and 1500 mg/kg/day were comparable with control values.

3.4 Clinical pathology

Red cell mass parameters (red blood cell, hemoglobin, hematocrit) were decreased at test day 29 in male rats dosed with 120, 450 or 1500 mg/kg/day (variable statistical significance; means were 90 to 98% of the respective control group means), however the changes were not dose-dependant (Table 1). Red cell distribution width was increased at test day 29 in male and female rats dosed with 1500 mg/kg/day. Although statistically significant, this increase was not associated with any change in related red cell parameters (reticulocyte counts, mean corpuscular volume or microscopic findings). Prothrombin time was minimally shortened at test day 31 in female rats dosed with 450 or 1500 mg/kg/day (means were 94% and 92% of the control group mean, respectively). This change was considered treatment-related, however, minimally shortened coagulation times have no toxicological significance. Prothrombin time and activated partial thromboplastin time on test day 31 in males dosed with 450 mg/kg/day were increased; however, this increase was not dose-dependant.

There were some treatment-related changes in the clinical chemistry parameters in male and female rats (Table 1). The liver enzyme alkaline phosphatase was mildly increased on test day 29 in male and female rats dosed with 1500 mg/kg/day (means were 207% and 186% of the respective control group means, $p \leq 0.05$). Aspartate aminotransferase was also elevated in male rats in the 450 and 1500 groups, although the changes were not statistically significant.

Glucose was decreased on test days 15 and 29 in male rats dosed with 450 or 1500 mg/kg/day (Table 1). This minimal decrease in glucose is considered to be treatment related as it intensified with dose and prolonged treatment. In contrast, there were no statistically significant changes in glucose in female rats at any dose tested.

Inorganic phosphorous was increased in male rats dosed with 450 or 1500 mg/kg/day (means were 110% and 117% of the control group mean, respectively). Total protein and globulin were increased on test day 29 in female rats dosed with 1500 mg/kg/day (means were 108, and 110% of the respective control group means) but total protein, albumin, and globulin were decreased on test day 29 in males dosed with 120 mg/kg/day. These findings appear to be treatment related in the females, since it only occurred in the 1500 group, however there was no dose relationship in the males.

3.5 Necropsy and histopathology

No gross abnormalities of toxicological significance were noted for any of the euthanized animals during necropsies at the conclusion of the 30-day observation period. In the male (control group) and female (1500 group) that died as a result of intubation error, the thoracic and cervical findings were histomorphologically consistent with acute purulent inflammation secondary to gavage trauma.

Absolute and relative weights for all organs were comparable to controls in both male and female treated animals, with the notable exception of liver weights. There was a dose dependent increase in mean absolute and relative liver weights in both the male and female test animals compared with the control (Fig. 2). Relative liver weights refer to the ratio of liver weight to body weight. Both the absolute and relative liver weights are significantly ($p \leq 0.05$) increased in the 450 and 1500 group females. In the 450 and 1500 group males, only the relative liver weights were significantly different from control ($p \leq 0.05$). These findings did not correlate with any histomorphological changes in the livers of any of the test animals, such as cirrhosis, or steatosis.

4.0 Discussion

This study has demonstrated that a Grains of Paradise extract rich in 6-gingerol, 6-shogaol and 6-paradol is capable of decreasing blood glucose, but at higher doses may cause liver toxicity. Beyond liver enlargement and elevation in alkaline phosphatase, there were few dose-related changes of toxicological significance. Clinical signs were essentially normal except for the facial and ocular staining in the high dose groups, which was likely due to porphyrin staining from the Harderian gland. Along with the soft feces, these symptoms suggest heightened stress levels or ill health. Although the symptoms were likely treatment related, most of the animals recovered to active and healthy status, with normal feces and no staining, by the end of the study (5/5 males; 3/4 females).

Overall, hematology did not reveal any dose-dependant changes that were of toxicological significance. These results are supported by a study evaluating the safety of a similar ginger extract rich in gingerols and shogaols, which found no significant effect on blood coagulation, blood pressure, or heart rate (Weidner and Sigwart, 2000).

Clinical chemistry revealed decreased blood glucose in the male 450 and 1500 groups, but not in the female groups. Previous studies (Akhani et al., 2004; Al-Amin et al., 2006) showed that gingerol extracts lower blood glucose in male streptozotocin-induced diabetic rats. One study that used both sexes of streptozotocin-induced diabetic rats showed a decrease in blood glucose using a ginger extract (200 mg/kg) however data between the sexes was not distinguished. Further studies are necessary to evaluate the different hypoglycemic effects of gingerol extracts on male verses female rats.

Clinical chemistry also revealed elevated liver enzymes which are toxicologically significant. Increases in alkaline phosphatase or aspartate aminotransferase may occur secondary to induction by a xenobiotic or in association with cholestasis. In this study there

was no evidence of cholestasis, i.e., no increase in bilirubin, but absolute and relative liver weights were increased, suggesting early stages of liver damage. Gingerols and related compounds appear to be metabolized by enzymes in the liver (Nakazawa and Ohsawa, 2002; Naora et al., 1992). Liver toxicity has been reported for some herbal extracts including Kava (*Piper methysticum* G. Forst), Chaparral (*Larrea tridentate* (DC) Coville), Mistletoe (*Phoradendron flavescens* (Pursh) Nutt.), Germander (*Teucrium chamaedrys* Linnaeus), and Comfrey (*Symphytum officinale* Linnaeus) (Dasgupta, 2003), but a literature search revealed no reports of plant extracts containing gingerols causing liver enlargement or toxicity. In fact, several studies showed a protective effect of gingerols on the liver, with lowered liver enzymes (Hikino et al., 1985; Nie et al., 2006; Yemitan and Izegbu, 2006). Therefore, the liver enlargement and corresponding elevation in alkaline phosphatase observed at high doses (450, 1500 mg/kg) are likely a result of the inability of the liver to metabolize the large amount of gingerols and similar compounds present in high doses of the Grains of Paradise extract.

In conclusion, this 28-day toxicology study in rats showed that a Grains of Paradise extract rich in gingerols may be effective in lowering blood glucose, and is worth investigating as a therapy for diabetes. However, the dose-dependant liver enlargement and elevation in alkaline phosphatase observed in the present study suggest liver toxicity should be considered when administering high doses of Grains of Paradise extract. Further studies would be necessary to determine if other plant extracts that are high in gingerols produce the same effects on the liver when administered at high doses for 28 days.

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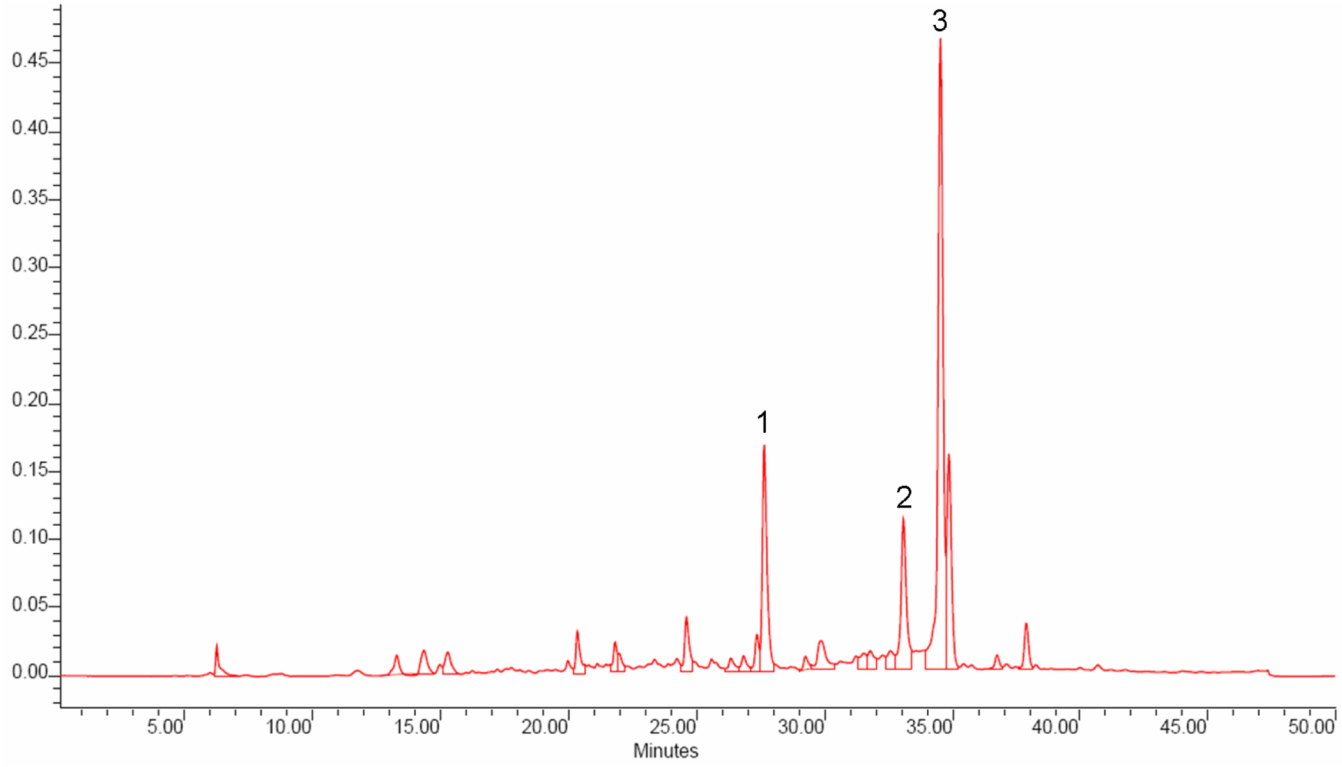

Fig. 1.
HPLC chromatogram of Grains of Paradise extract showing major compounds present: 1: 6-gingerol; 2: 6-shogaol 3: 6-paradol.

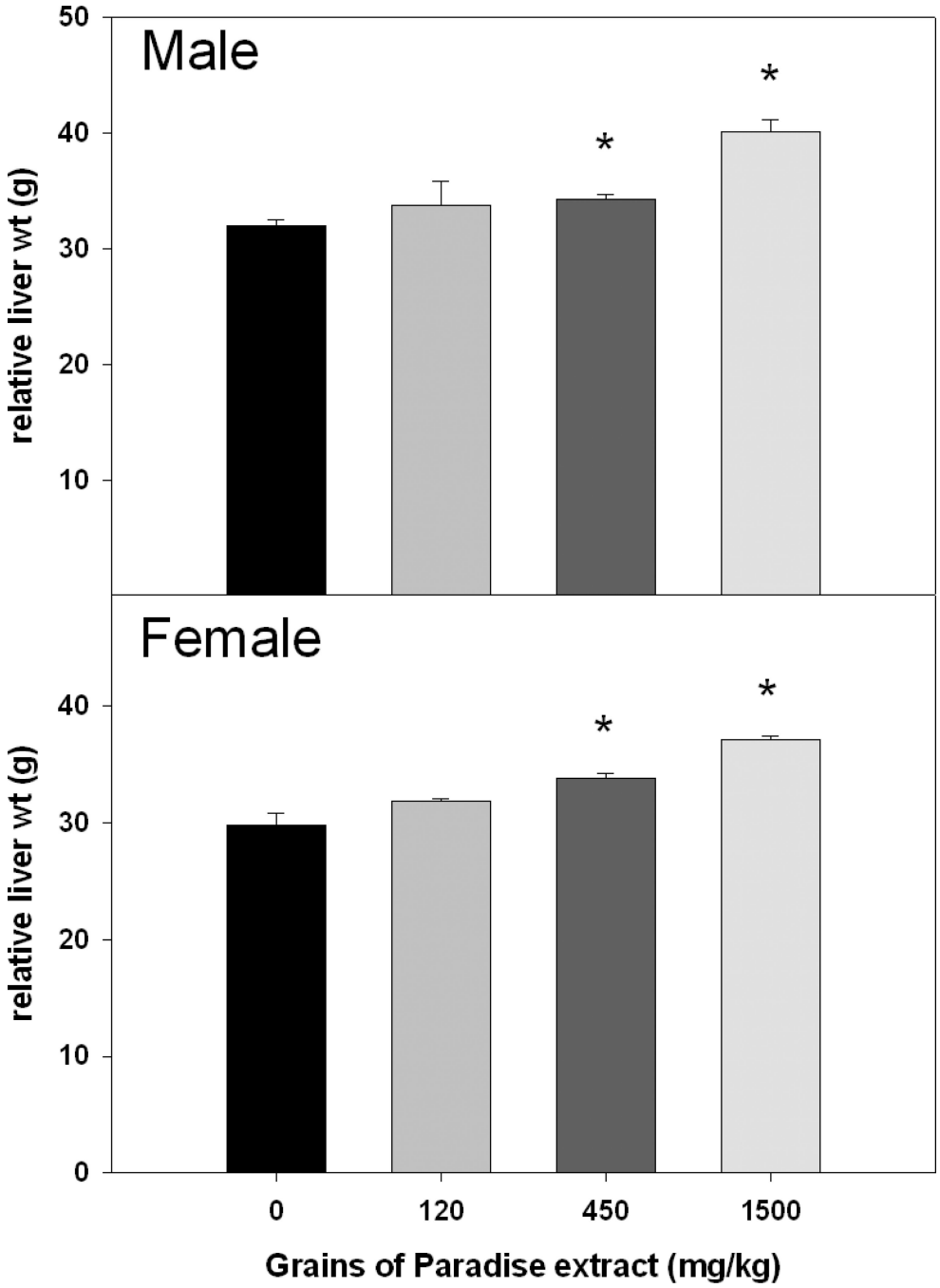

Fig. 2.

Relative liver weights for male and female rats treated for 28 days with 0, 120, 450, or 1500 mg/kg Grains of Paradise extract (n = 5 per group, bars are mean ± SE); *, p≤ 0.05 compared to control (ANOVA).

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Table 1

Clinical pathology parameters

Sex Dose level (mg/kg)	male 0		female 0		male 120		female 120		male 450		Female 450		male 1500		female 1500	
	mean	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**
RBC (×106/uL)	9.07	0.06	8.31	0.10	*8.31	0.18	8.33	0.17	8.66	0.06	8.22	0.08	8.85	0.08	8.58	0.12
HGB (g/dL)	17.9	0.1	16.1	0.3	*16.3	0.2	16.2	0.3	*17.0	0.2	16.3	0.1	16.6	0.1	16.5	0.1
HCT (%)	50.9	0.1	44.6	0.8	*45.6	0.8	44.9	0.8	48.5	0.5	45.2	0.2	48.0	0.4	46.0	0.7
PT (sec)	20.00	0.09	14.60	0.22	14.50	0.27	14.10	0.02	*16.80	0.49	*13.70	0.18	14.60	0.18	*13.40	0.09
APTT (sec)	20.40	0.49	15.90	1.25	21.00	1.25	16.00	0.94	*24.00	0.54	18.40	1.57	20.00	0.89	17.40	1.61
AST (U/L)	87	1	86	4	84	5	79	3	105	4	76	3	105	11	76	3
ALKP (U/L)	161	2	103	4	158	14	95	5	183	17	122	7	*334	45	*192	17
GLUC (mg/dL) d1	94	4	100	4	93	3	97	2	97	4	103	3	94	1	96	3
GLUC (mg/dL) d15	113	4	101	3	108	3	102	3	100	4	101	4	*90	6	102	1
GLUC (mg/dL) d29	110	4	100	2	96	3	102	5	*90	4	94	1	*75	7	89	4
TP (g/dL)	6.5	0.0	6.3	0.1	*6.1	0.0	6.3	0.1	6.4	0.1	6.4	0.1	6.6	0.0	*6.8	0.1
ALB (g/dL)	3.5	0.0	3.4	0.1	*3.4	0.0	3.4	0.0	3.4	0.0	3.5	0.0	3.6	0.0	3.6	0.0
GLOB(g/dL)	3.0	0.0	2.9	0.0	*2.8	0.0	2.9	0.1	3.0	0.0	2.9	0.1	3.0	0.0	*3.2	0.0
IPHS(mg/dL)	7.6	0.1	7.0	0.2	8.1	0.2	7.3	0.4	*8.4	0.1	7.1	0.1	*8.9	0.2	6.6	0.3

RBC, erythrocyte count; HGB, hemoglobin concentration; HCT, hematocrit; PT, prothrombin time; APTT, activated partial thromboplastin; AST, aspartate aminotransferase; ALKP, alkaline phosphatase; GLUC, glucose (d1,15,29 day 1,15,29); TP, total protein; ALB, albumin; GLOB, globulin; IPHS, inorganic phosphorous