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A 90-DAY ORAL SAFETY ASSESSMENT OF AQUEOUS GINGER EXTRACT ON SOME VITAL ORGANS IN RATS

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ABSTRACT

Zingiber officinale (Ginger), similar to other herbs is generally believed to be non-toxic because is natural. However, several studies have revealed that some herbal medicines are toxic to vital organs. This study was conducted to investigate the potential toxic effect of aqueous extract of ginger on vital organs in Wistar rats. Eighty Wistar rats divided into four groups of 20 rats each (10 females and 10 males) were used. The rats in the treatment groups were administered ginger extract daily at doses of 500, 1,000 and 2,000 mg/kg for 90 days, while those in the control group were given distilled water. On the 91st day, eight rats per group (four males and four females) were euthanized and blood samples collected for biochemical analysis. The rats were dissected and their vital organs removed, weighed and fixed in 10% formalin for histopathological assessment. The remaining rats were observed for 14 days for any persistence or reversibility of toxic effects. The ginger extract did not produce any significant changes in the relative organ weights of the heart, liver, kidney, lungs, brain, thyroid glands, ovaries and testes of the rats. However, significant increase in blood urea; and serum electrolytes (sodium, potassium, and bicarbonate) were observed. Histopathological assessment showed various pathological lesions in most of the organs at doses of 1,000 and 2,000 mg/kg. However, after a 14-day recovery period, the observed changes were reversed. The findings showed that repeated administration of aqueous ginger extract for 90 days at high doses could induce organ toxicities that are reversible after 14 days.

Keywords: Ginger, Histopathological assessment, Vital organs, Organ toxicities

INTRODUCTION

Traditional medicine continues to be an essential constituent of the primary health care in most developing countries, where it provides the basic healthcare needs for majority of people due to its acceptability, accessibility and affordability (Jităreanu *et al*., 2023; Anne and Gerhard, 2020). Herbs, spices and other naturally derived medicines are generally believed to be safer than orthodox or synthetic medicines. They are thought to be devoid of any health risk because they are considered 'natural' (Sahoo *et al*., 2010). However, scientists have consistently advocated for sufficient toxicological studies on natural medicines to ensure their safe use (Asif, 2015). In addition, studies have shown that not all medicinal plants or natural products are safe or completely free from side effects (Boukandou *et al*., 2015; Ansari and Inamdar, 2010). Drug or medicinal plant can be harmful to human at certain dose (Isah et al., 2023). Some medicinal plants have been reported to cause serious adverse effects such as allergy, liver and kidney dysfunction, cancer and even death (Schultz *et al*. 2020; Ekeanyanwu, 2011). Moreover, the harmful effects of these medicinal plants might be due to overdose or chronic exposure to their toxic principles (Anywar *et al*., 2021; Ferreira-Machado *et al*. 2004).

Zingiber officinale (Ginger) has been a well-known and widely used herb, spice and food additive for thousands of years (Ugbabe *et al*., 2019). It is generally believed to be safe and free of health risk (Hosseini and Mirazi 2014). In fact, ginger is one of the most common herbal remedies recommended for treating nausea and vomiting in pregnancy (Stanisiere *et al*., 2018). It is also used as flavour in confectionaries, tea and carbonated drinks (Hossein *et al*., 2014). In Nigeria, ginger is commonly consumed in different food products, herbal preparations or as dietary supplements. It is freely available as condiment in food store, as an over-the-counter herbal medicine or dietary supplement in drug stores and super markets. Consequently, there is a high possibility of ginger consumption from multiple sources over a long period of time and there is a genuine concern about its potential adverse effects or toxicity from such exposure.

Despite the public promotion of ginger as a safe herbal medicine or spice, there are limited scientific data on the safety of its long term consumption at high doses. A recent safety study on ginger oil recommended caution on long term consumption (Idang *et al*., 2019). Thus, the possible toxicity of ginger or its ingredients cannot be totally ruled out, especially when it is ingested in large quantities or doses over a lengthy period of time. Therefore, it is necessary to evaluate the safety profile of high dose ginger consumption in rats to provide toxicological data on its effects on vital organs. Chronic toxicity studies on changes in biochemical parameters, relative organ weight and histological assessment of vital organs have long been used to evaluate possible toxicity by medicinal and chemical agents (Salawu *et al*., 2010; Sunday *et al*., 2016). The present study is a 90-day oral toxicity study aimed at investigating the long-term effect of repeated oral administration of high dose aqueous ginger extract on vital organs of rats.

MATERIALS AND METHODS

Preparation of plant material

Fresh ginger rhizomes were purchased from a farm in Kaduna, Nigeria in October, 2021. It was identified by Namadi Sanusi, a taxonomist at the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria. Voucher specimen (Number 2099) has been deposited in the herbarium section of the Department. The ginger rhizomes were properly washed, sliced, dried under shade and ground to powder. The pulverized ginger was extracted with water by cold maceration for 24 hours with occasional shaking. The extract was filtered 24 hours later,

and the filtrate was evaporated to dryness in a water bath at 50ºC. The dried extract was stored in a closed airtight container and labelled ginger extract.

Experimental Animals

Male and female Wistar rats (150-170 g) used for this study were procured and housed at the Animal House Unit of the Department of Pharmacology and Toxicology, Kaduna State University, Kaduna. The animals were acclimatized for 14 days; and kept in standard cages (male and female rats separated); to provide sufficient spacing, five rats were housed per cage and maintained in a well-ventilated room. They had unlimited access to water and regular rodent food. All experiments were carried out following the protocols provided by Animal Research Reporting of In Vivo Experiments (ARRIVE).

Ninety-day toxicity study

The toxicity study was performed according to OECD 408 guidelines (OECD 2018). Eighty Wistar rats were randomly grouped into 4 groups of 20 rats each (of separately 10 females and 10 males). The rats in the first group served as controls and were given distilled water. The other groups received ginger extract orally at doses of 500, 1,000 and 2,000 mg/kg, respectively, daily for 90 days. All animals were observed daily for behavioural changes, clinical signs of toxicity, mortality and general condition throughout the treatment period. Before administering the extract, each animal was weighed every day, and this practice continued until the day of sacrifice. Prior to the day of sacrifice, all animals were fasted overnight. Eight rats per group (4 males and 4 females) were euthanized under ketamine anaesthesia and sacrificed on the 91st day. Blood samples were taken by cardiac puncture from each rat into different sample bottles and used for biochemical analysis. Some vital organs of the rats were removed, weighed and preserved in 10% formalin for histopathological assessment, and the relative organ weights were estimated. Eight (8) rats per group were assigned as satellite groups, kept for 14 days without further treatment with extract and observed for any persistence of or reversibility/recovery from toxic effects.

Reversibility study

At the end of the 90-day treatment period, the remaining animals (at least 8 rats per group) were kept for 14 days without further treatment with ginger extract but allowed free access to food and water. After the 14-day observation period, the animals were euthanized under ketamine anaesthesia, blood samples were collected for biochemical analysis, and vital organs were removed for histopathological examinations (Idang et al. 2019).

Relative Organ Weight (ROW) measurement and Histopathological analysis

Following sacrifice, the rats were dissected, and their vital organs (liver, heart, brain, lungs, kidneys, thyroids, testes and ovaries) was isolated, dried and weighed on a sensitive balance. The tissues of these organs were sectioned into 5 µm slices, stained with hematoxylin and eosin, and examined with the aid of a microscope (x 250) for any histopathological lesions. The relative organ weight (ROW) of the organs were calculated using the formula below:

$$ROW = \frac{Absolute\ organ\ weight\ (g)}{Body\ weight\ of\ the\ animal\ on\ the\ day\ of\ scarifice} \times 100$$

Serum biochemistry analysis

Serum biochemistry analysis was performed according to the manufacturer's instructions using kits obtained from (Shangai Coon Koon Biotech Co. Ltd. China). These included: liver function tests using measurements of the enzymes alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), kidney function tests using serum concentrations of urea and creatinine, and serum concentrations of albumin (ALB) and total protein (TP).

Statistical Analysis

The results were analyzed statistically using SPSS (version 23). One-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test was used to identify significant differences between the experimental groups. All data are presented as the mean ± standard error of the mean (S.E.M), and a p value ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

Relative organ weight

The ginger extract did not produce any significant change in the relative organ weight of the selected organs of the Wistar rats (male and female) at all doses tested, as shown in Tables 1 & 2.

Table 1: Effect of aqueous rhizome extract of ginger extract on relative organ weights after a 90-day oral subchronic toxicity study in female Wistar rats

	Treatment groups				Satellite groups			
Organs	**Control**	**GE 500**	**GE 1000**	**GE 2000**	**Control**	**GE 500**	**GE 1000**	**GE 2000**
Liver	3.70 ±0.26	3.88 ± 0.34	4.28 ±0.18	4.12 ±0.26	3.70 ±0.22	3.78 ±0.03	3.73 ±0.02	4.08 ±0.15
Lungs	0.68 ±0.07	0.65 ±0.08	0.80 ± 0.06	0.70 ± 0.07	0.60 ± 0.08	0.70 ± 0.04	0.75 ± 0.06	0.81 ± 0.02
Kidney	0.92 ± 0.09	0.89 ± 0.08	1.04 ± 0.06	1.08 ± 0.09	0.87 ± 0.08	0.88 ± 0.05	0.85 ± 0.3	0.94 ± 0.08
Brain	0.73 ± 0.01	0.72 ± 0.03	0.72 ± 0.02	0.77 ± 0.09	0.63 ± 0.03	0.68 ± 0.08	0.71 ± 0.04	0.80 ± 0.08
Heart	0.46 ± 0.01	0.55 ± 0.06	0.59 ± 0.08	0.52 ± 0.06	0.39 ± 0.05	0.52 ± 0.50	0.58 ± 0.06	0.70 ± 0.08
Thyroids	0.70 ± 0.09	0.62 ± 0.10	0.61 ± 0.03	0.60 ± 0.10	0.61 ± 0.04	0.64 ± 0.01	0.58 ± 0.02	0.62 ± 0.02
Ovaries	0.23 ± 0.04	0.25 ± 0.01	0.27 ± 0.05	0.32 ± 0.05	0.26 ± 0.03	0.25 ± 0.03	0.20 ± 0.01	0.27 ± 0.06

GE= ginger extract, data are expressed as the mean ± SEM, n=4

Table 2: Effect of aqueous rhizome extract of ginger extract on relative organ weights after a 90-day oral subchronic toxicity study in male Wistar rats

	Treatment groups				Satellite groups			
Organs	**Control**	**GE 500**	**GE 1000**	**GE 2000**	**Control**	**GE 500**	**GE 1000**	**GE 2000**
Liver	3.14 ± 0.11	3.14 ± 0.10	3.21 ± 0.13	3.17 ± 0.30	3.38 ± 0.20	3.59 ± 0.09	3.90 ± 0.29	3.54 ± 0.14
Lungs	0.57 ± 0.03	0.58 ± 0.03	0.59 ± 0.02	0.60 ± 0.01	0.57 ± 0.06	0.70 ± 0.06	0.79 ± 0.12	0.70 ± 0.06
Kidney	0.79 ± 0.04	0.78 ± 0.04	0.98 ± 0.08	0.97 ± 0.04	0.82 ± 0.05	0.81 ± 0.04	0.79 ± 0.06	0.74 ± 0.03
Brain	0.59 ± 0.01	0.60 ± 0.01	0.59 ± 0.03	0.60 ± 0.03	0.60 ± 0.01	0.61 ± 0.08	0.77 ± 0.12	0.65 ± 0.03
Heart	0.47 ± 0.02	0.47 ± 0.01	0.43 ± 0.01	0.47 ± 0.01	0.38 ± 0.06	0.45 ± 0.03	0.62 ± 0.1	0.47 ± 0.03
Thyroids	0.59 ± 0.01	0.59 ± 0.01	0.38 ± 0.04	0.55 ± 0.08	0.58 ± 0.06	0.55 ± 0.01	0.64 ± 0.03	0.55 ± 0.02
Testes	1.32 ± 0.04	1.33 ± 0.02	1.29 ± 0.14	1.14 ± 0.05	1.20 ± 0.09	1.50 ± 0.06	1.41 ± 0.06	1.28 ± 0.1

GE= ginger extract, data are expressed as the mean ± SEM, n=4

Liver Parameters

The results of the liver function test showed that 90-day subchronic administration of ginger extract did not produce any significant changes in liver transaminases (ALT, AST, ALP), total protein, and albumin when compared with the control group as shown in Table 3. However, at the dose of 2,000 mg/kg, there was a significant decrease in the ALP levels compared to the groups that received 500 and 1,000 mg/kg of the extract (Table 3).

Table 3: Effect of aqueous ginger rhizome extract on liver parameters after a 90-day oral subchronic toxicity study in Wistar rats

Group	Liver Parameters				
	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dl)	ALB (g/dl)
Control	26.50 ± 4.02	115.83 ± 12.28	32.95 ± 4.3	13.12 ± 0.7	8.25 ± 0.8
GE 500	29.67 ± 3.11	89.17 ± 10.52	52.78 ± 4.9	16.52 ± 2.3	8.13 ± 0.9
GE 1000	24.50 ± 3.59	100.83 ± 2.71	52.73 ± 8.9	15.78 ± 1.0	10.62 ± 1.2
GE 2000	28.67 ± 4.00	125.83 ± 15.41	14.72 ± 1.5*	14.72 ± 1.2	8.97 ± 0.8
Sat. Control	18.40 ± 3.08	98.00 ± 6.04	27.30 ± 5.8	21.50 ± 4.2	7.98 ± 0.8
Sat. GE 500	18.50 ± 2.77	121.67 ± 12.56	38.95 ± 4.7	13.37 ± 2.0	10.52 ± 1.2
Sat. GE 1000	17.33 ± 2.39	126.50 ± 4.19	55.75 ± 10.0	13.78 ± 1.2	7.68 ± 0.3
Sat. GE 2000	19.33 ± 1.23	130.83 ± 15.41	37.07 ± 4.8	13.83 ± 1.0	9.25 ± 1.3

GE= ginger extract, Sat. = Satellite, Data are expressed as the mean ± SEM. *$P \leq 0.001$ in relation to the GE 500 and GE 1000 groups (one-way ANOVA followed by Bonferroni's post hoc test), n=8. No significant differences in liver parameters between male and female rats

Kidney Parameters

Analysis of kidney function parameters such as blood urea and serum creatinine showed a significant increase in the blood urea of the rats that received 2,000 mg/kg ginger extract, as shown in Table 4. However, after 14-day recovery period, the blood urea level normalized (see table 5). Additionally, the extract significantly increased the serum levels of sodium, potassium, and bicarbonate at different doses, as shown in Table 4. However, after 14-day recovery period, the sodium level significantly decreased in the animals that had received 1,000 and 2,000 mg/kg ginger extract (see table 5). There were no significant differences in kidney parameters of male and female rats thus, they were both combined (Tables 4 and 5).

Table 4: Effect of ginger extract on kidney parameters in Wistar rats after a 90-day subchronic toxicity study

Parameters	Treatment group (mg/kg)			
	Control	GE 500	GE 1000	GE 2000
Urea (mEq/L)	53.93±4.7	56.14 ± 11.0	84.27 ± 11.5	89.50 ± 4.9*
Creatinine (mg/dL)	0.85±0.1	1.07±0.1	0.83±0.2	0.93±0.1
Sodium (mmol/L)	139.55±6.7	186.86±21.5	199.57±5.3*	117.54±20.7
Potassium (mmol/L)	8.08±1.3	42.70±13.5*	49.65±4.2*	49.93±9.1*
Chloride (mg/dL)	40.33±2.5	34.17±3.8	32.67±4.1	30.83±3.4
Bicarbonate (mg/dL)	99.50±9.8	134.0±5.9*	101.83±8.3	90.0±4.3

The data are presented as the mean±SEM, *$p \leq 0.05$ in relation to the control group (one-way ANOVA followed by Bonferroni's post hoc test), n=8. No significant differences in kidney parameters between male and female rats

Table 5: Effect of ginger extract on kidney parameters in Wistar rats after recovery period

Parameters	Treatment group (mg/kg)			
	Satellite Control	Satellite GE 500	Satellite GE 1000	Satellite GE 2000
Urea (mEq/L)	67.77 ± 7.7	82.28 ±8.5	69.78±7.0	72.32±8.0
Creatinine (mg/dL)	1.10±0.1	1.03±0.1	1.15±0.1	0.87±0.1
Sodium (mmol/L)	90.30±7.7	68.48±9.4	25.83±5.9**	48.06±5.1*
Potassium (mmol/L)	25.08±5.7	20.25±3.2	30.03±4.6	37.42±5.0
Chloride (mg/dL)	31.83±4.6	38.17±4.0	35.67±5.1	27.83±5.1
Bicarbonate (mg/dL)	89.83±5.3	96.17±9.2	84.50±7.3	93.0±7.7

The data are presented as the mean±SEM, *$p \leq 0.05$, ** $p \leq 0.01$ in relation to the satellite control group (one-way ANOVA followed by Bonferroni's post hoc test), n=8.

Histopathology

Daily repeated oral administration of aqueous ginger extract for 90 days caused slight myocardial necrosis in groups of rats that received 1,000 and 2,000 mg/kg of the extract (Figure 1). However, the slight necrosis was reversed after 14-day recovery period (Figure 2). Similarly, there was slight hepatic necrosis and Kupfer cell hyperplasia in the livers of the rats that received 2,000 mg/kg extract (Figure 3) but the lesions observed in the study were reversed during a 14-day recovery period (Figure 4). Photomicrograph sections of the kidney tissues showed moderate tubular necrosis and slight glomeruli necrosis in the groups administered 1,000 and 2,000 mg/kg of the extract respectively (Figure 5). However, during recovery period, the pathological changes observed were reversed (Figure 6). Additionally, some form of pathological changes (hyperplasia of inflammatory cells and slight alveoli congestion) were observed in the lungs of the groups that received 1,000 and 2,000 mg/kg of the extract (Figure 7). However, these effects were reversed after a 14-day recovery period (Figure 8).

There was slight necrosis of neurons in the brain sections of the experimental group that was administered 2,000 mg/kg ginger extract at the end of the 90-day treatment (Figure 9). However, the animals recovered from slight necrosis after a

14-day recovery period (Figure 10). There was no observable pathological change in the histology of the thyroid glands of all experimental groups compared with the control group (Figure 11). Moreover, no lesions were observed in the thyroid gland sections of rats in all experimental groups after the 14-day recovery period (Figure 12). Histopathological examinations of the uterus showed glandular hyperplasia and endometrial necrosis at doses of 1,000 and 2,000 mg/kg (Figure 13). These pathological lesions were reversed after a 14-day recovery period, as no pathological change was observed (Figure 14).

The histopathological analysis of the testes after the 90-day subchronic toxicity study showed some form of pathological lesions in all rats, including the treatment and control groups. Spermatogenic and Leydig cell necrosis were observed in the testes (Figure 15). There was persistence of the pathological lesions in all the groups (treatment and control groups) even after the 14-day recovery period (Figure 16).

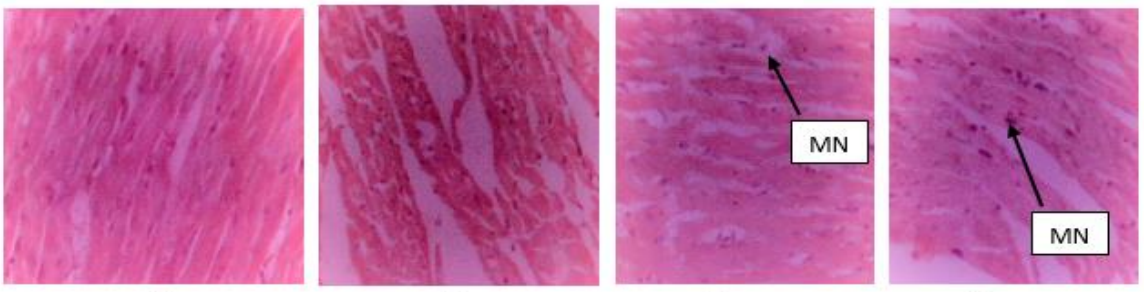

Figure 1: Photomicrographs of heart sections of rats after a 90-day subchronic toxicity study with aqueous ginger extract (GE) (stained with hematoxylin and eosin at ×250 magnification). A= Control (distilled water); B = GE 500 mg/kg; C= GE 1,000 mg/kg; D= GE 2,000 mg/kg; A & B (normal cardiac muscles), C & D (slight myocardial necrosis)

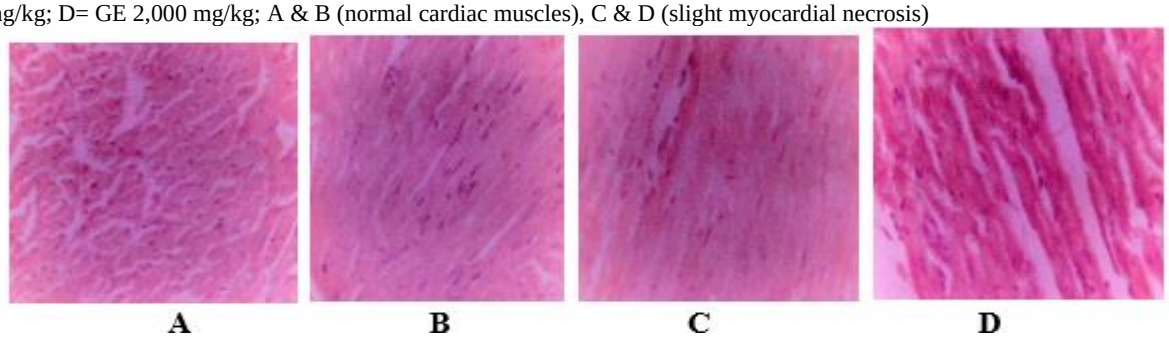

Figure 2: Photomicrographs of heart sections of rats after 14 days' recovery period (stained with hematoxylin and eosin at ×250 magnification). A= Satellite control; B = Satellite GE 500; C= Satellite GE 1000; D= Satellite GE 2000; A, B, C & D (normal cardiac muscles)

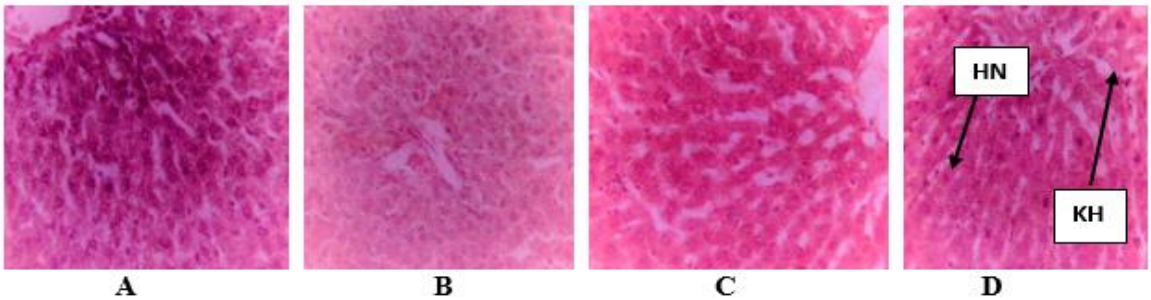

Figure 3: Photomicrographs of liver sections of rats after a 90-day subchronic toxicity study with aqueous ginger extract (GE) (stained with hematoxylin and eosin at ×250 magnification). A= Control (distilled water); B = GE 500 mg/kg; C= GE 1,000 mg/kg; D= GE 2,000 mg/kg; A, B & C (normal hepatocytes), HN= slight hepatic necrosis KH= Kupfer cell hyperplasia

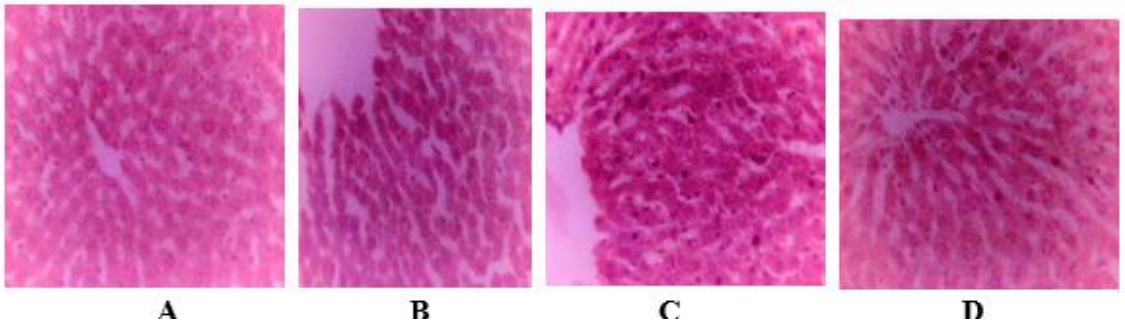

Figure 4: Photomicrographs of liver sections of rats after 14 days' recovery period (stained with hematoxylin and eosin at ×250 magnification). A= Satellite control; B = Satellite GE 500; C= Satellite GE 1000; D= Satellite GE 2000; A, B, C & D (normal hepatocytes)

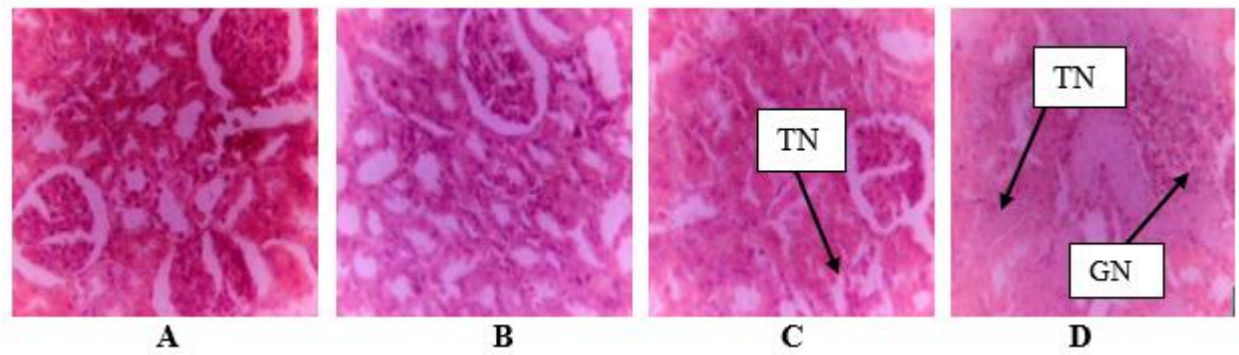

Figure 5: Photomicrographs of kidney sections of rats after a 90-day subchronic toxicity study with aqueous ginger extract (GE) (stained with hematoxylin and eosin at ×250 magnification). A= Control (distilled water); B = GE 500 mg/kg; C= GE 1,000 mg/kg; D= GE 2,000 mg/kg; A & B (normal kidney tubules glomerulus), TN= moderate tubular necrosis and GN= slight glomeruli necrosis

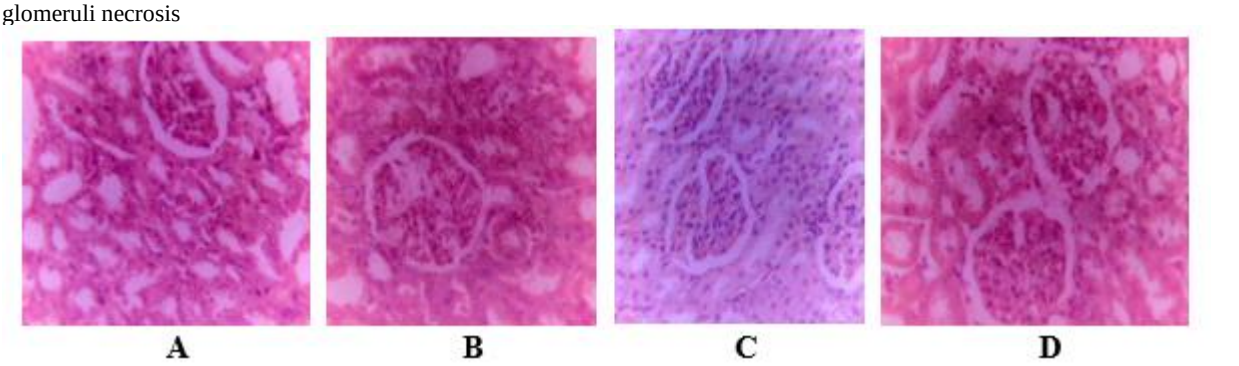

Figure 6: Photomicrographs of kidney sections of rats after 14 days'recovery period (stained with hematoxylin and eosin at ×250 magnification). A= Satellite control; B = Satellite GE 500; C= Satellite GE 1000; D= Satellite GE 2000; A, B, C & D (normal kidney tubules and glomerulus)

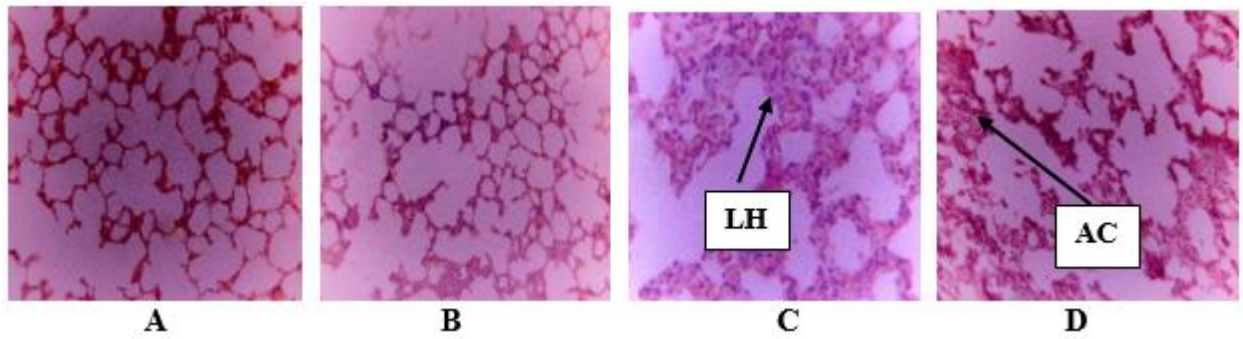

Figure 7: Photomicrographs of lung sections of rats after a 90-day subchronic toxicity study with aqueous ginger extract (GE) (stained with hematoxylin and eosin at ×250 magnification). A= Control (distilled water); B = GE 500 mg/kg; C= GE 1,000 mg/kg; D= GE 2,000 mg/kg; A & B (normal lung alveoli), C (LH= hyperplasia of inflammatory cells) & D (AC= slight alveoli congestion)

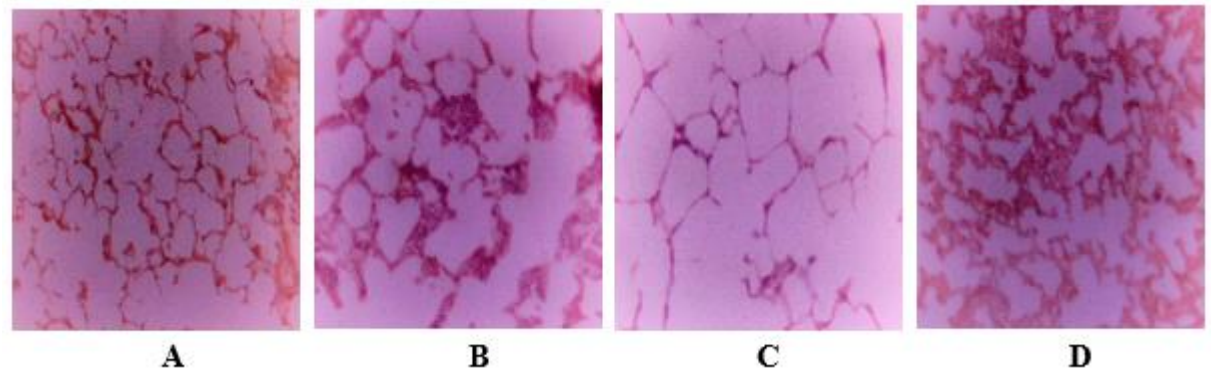

Figure 8: Photomicrographs of lung sections of rats after 14 days' recovery period (stained with hematoxylin and eosin at ×250 magnification). A= Satellite control; B = Satellite GE 500; C= Satellite GE 1000; D= Satellite GE 2000; A, B, C & D (normal lung alveoli)

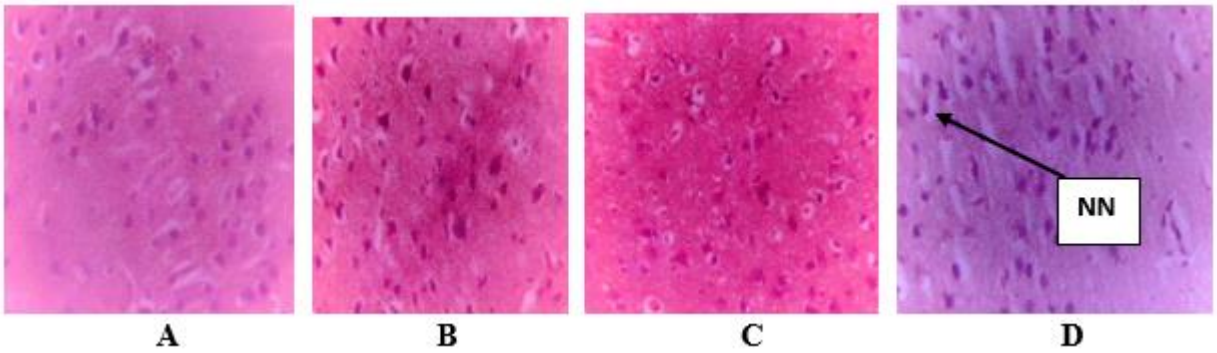

Figure 9: Photomicrographs of brain sections of rats after a 90-day subchronic toxicity study with aqueous ginger extract (GE) (stained with hematoxylin and eosin at ×250 magnification). A= Control (distilled water); B = GE 500 mg/kg; C= GE 1,000 mg/kg; D= GE 2,000 mg/kg; A, B & C (normal neurons), D (NN= slight necrosis of neurons)

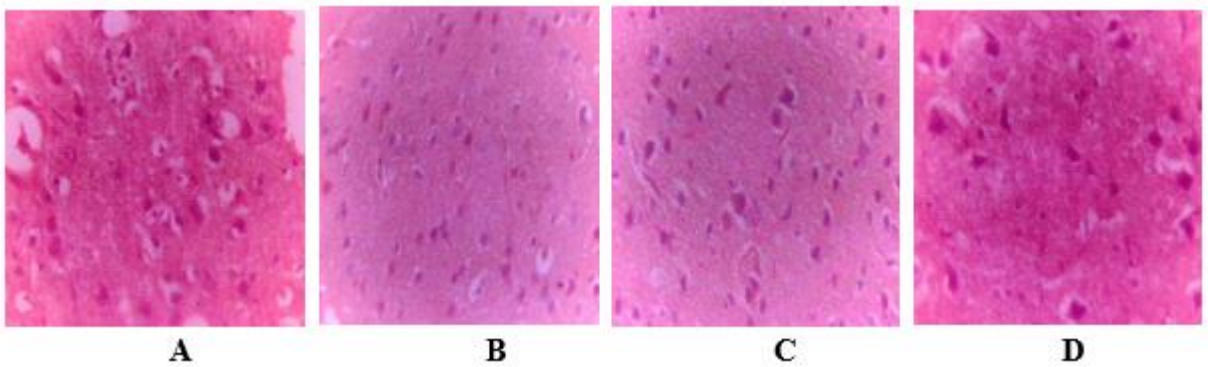

Figure 10: Photomicrographs of brain sections of rats after 14 days' recovery period (stained with hematoxylin and eosin at ×250 magnification). A= Satellite control; B = Satellite GE 500; C= Satellite GE 1000; D= Satellite GE 2000; A, B, C & D (normal neurons)

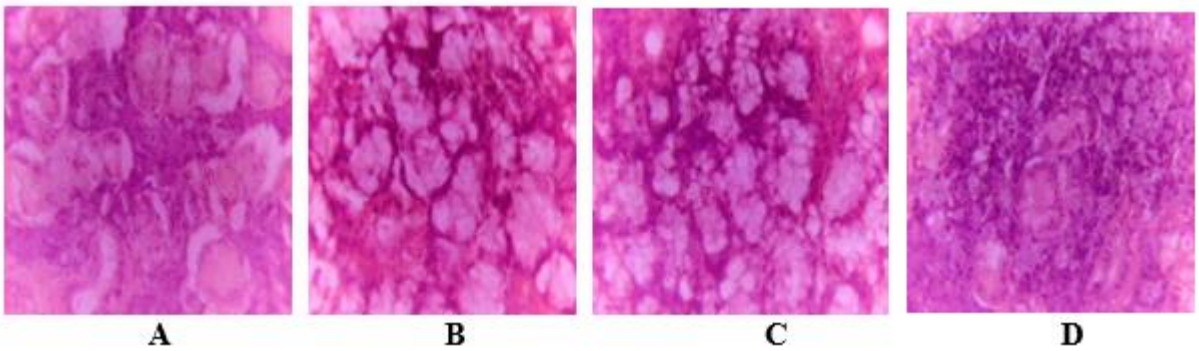

Figure 11: Photomicrographs of thyroid gland sections of rats after a 90-day subchronic toxicity study with aqueous ginger extract (GE) (stained with hematoxylin and eosin at ×250 magnification). A= Control (distilled water); B = GE 500 mg/kg; C= GE 1,000 mg/kg; D= GE 2,000 mg/kg; A, B, C & D (normal gland)

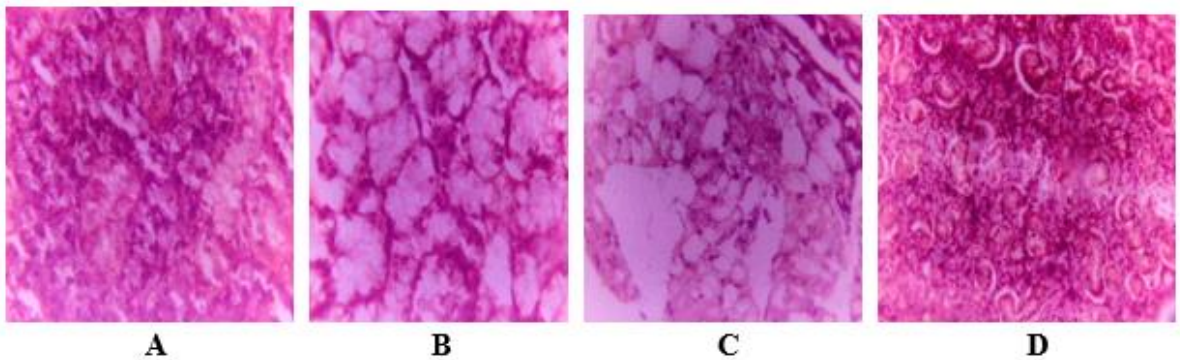

Figure 12: Photomicrographs of thyroid gland sections of rats after 14 days' recovery period (stained with hematoxylin and eosin at ×250 magnification). A= Satellite control; B = Satellite GE 500; C= Satellite GE 1000; D= Satellite GE 2000; A, B, C & D (normal gland)

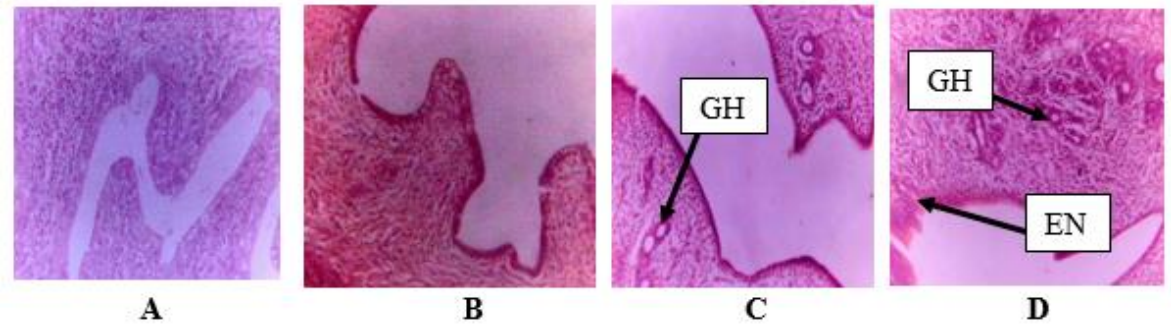

Figure 13: Photomicrographs of ovary sections of rats after a 90-day subchronic toxicity study with aqueous ginger extract (GE) (stained with hematoxylin and eosin at ×250 magnification). A= Control (distilled water); B = GE 500 mg/kg; C= GE 1,000 mg/kg; D= GE 2,000 mg/kg; A& B= (normal features), C = glandular hyperplasia (GH), D = endometrial necrosis (EN) and glandular hyperplasia (GH)

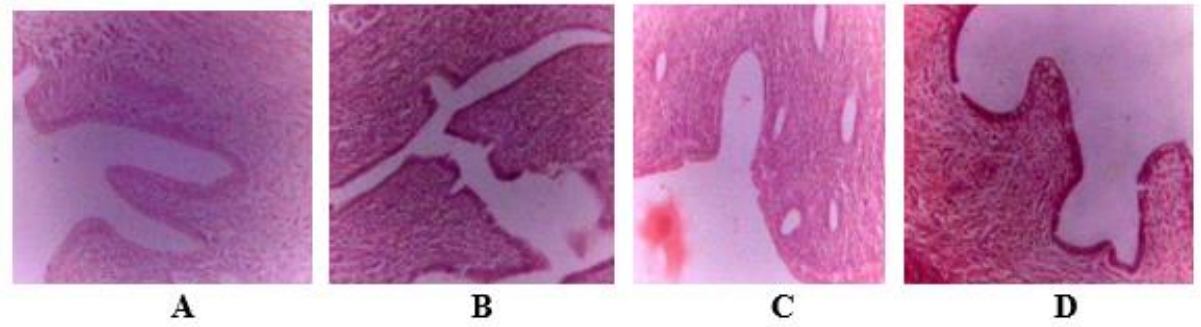

Figure 14: Photomicrographs of ovary sections of rats after 14 days' recovery period (stained with hematoxylin and eosin at ×250 magnification). A= Control (distilled water); B = GE 500 mg/kg; C= GE 1,000 mg/kg; D= GE 2,000 mg/kg; A, B, C & D (normal features)

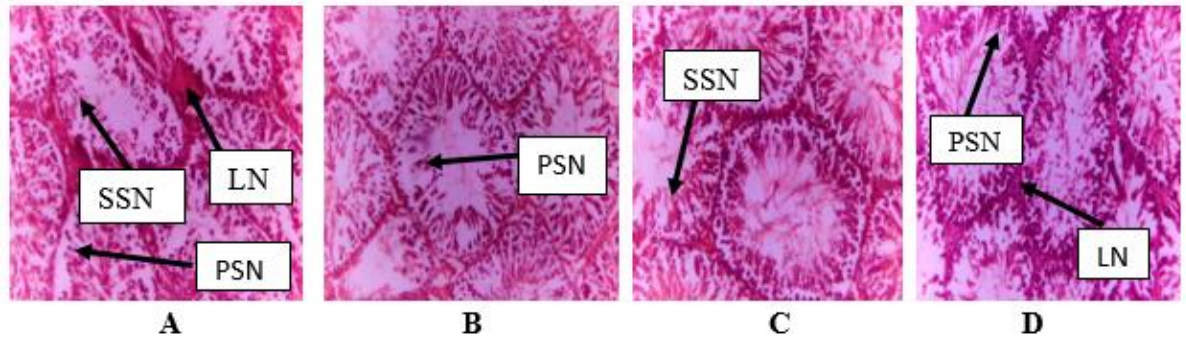

Figure 15: Photomicrographs of testes sections of rats after a 90-day subchronic toxicity study with aqueous ginger extract (GE) (stained with hematoxylin and eosin at ×250 magnification). A= Control (distilled water); B = GE 500 mg/kg; C= GE 1,000 mg/kg; D= GE 2,000 mg/kg; PSN= primary spermatogenic cell necrosis, SSN= secondary spermatogenic cell necrosis and LN= Leydig cell necrosis

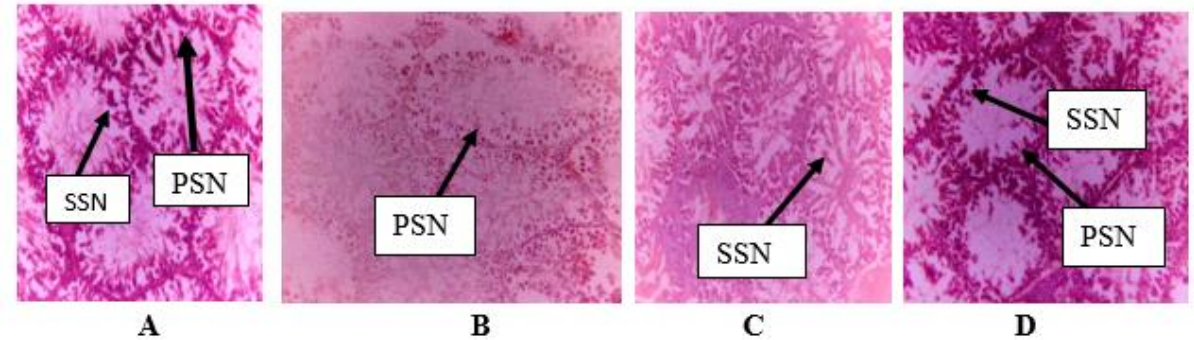

Figure 16: Photomicrographs of testes sections of rats after 14 days' recovery period (stained with hematoxylin and eosin at ×250 magnification). A= Control (distilled water); B = GE 500 mg/kg; C= GE 1,000 mg/kg; D= GE 2,000 mg/kg; PSN= primary spermatogenic cell necrosis, SSN= secondary spermatogenic cell necrosis

Discussion

Ginger and its components have been widely utilized as flavours in foods, beverages and confectionery, and are also used for their medicinal values in pharmaceutical and nutraceutical products despite limited toxicological reports on its long-term safety profile. In this study, we assessed the safety profile of aqueous ginger extract in a 90-day sub-chronic toxicity study in rats.

Changes in relative organ weight (ROW) of an animal reveals its physiological status and can be used as a sensitive index of organ toxicity (Zakaria et al. 2016). It is one of the toxicity tests usually employed by regulatory authorities for safety assessment of new compound, drug, food additives or chemicals (Lazic et al. 2020). In this study, repeated daily oral administration of aqueous ginger extract did not affect the relative organ weights of the heart, liver, kidney, lungs, brain, thyroid glands, ovaries and testes of the rats, signifying that the extract was nontoxic. This result is in contrast to that of a previous toxicological study that reported an increase in the relative organ weight of the liver after a 60-day sub-chronic toxicity study (Idang et al. 2019).

Liver is usually the organ of primary target for chemical or drug-induced systemic toxicity, and liver function testing in animals can indicate the safety or otherwise of such chemical (Tabernilla et al. 2021). Serum levels of liver transaminases (ALT, AST, ALP) are important clinical biomarker in detecting hepatocellular damage, liver disease or injury (Meunier and Larrey 2019). Additionally, reduction in serum albumin and total proteins indicate liver injury (Rasekh et al. 2008). Furthermore, liver parenchymal cells or hepatocytes that make up 60% of the cells in the liver are responsible for metabolic activities, bile acid and albumin secretion (Tabernilla et al. 2021). Any injury or damage to the liver cells is usually accompanied by inflammatory infiltration, increase in the kupffer cells, hyperplasia, necrosis and other abnormalities (Abbasnezhad et al., 2022; Tabernilla et al. 2021). In this study, our findings revealed that there were no significant differences in the serum levels of liver transaminases when compared with the control, implying that the aqueous ginger extract was not toxic to the liver tissues. However, the results from the histopathological analysis of the liver revealed necrosis at the highest dose tested. It is possible that the necrosis observed was due to mild and reversible tissue damage, not significant enough to alter serum transaminases, especially ALT. This was evident from the normal liver architecture shown in all the treatment groups two weeks after discontinuation of the ginger extract. This finding was further supported by the serum albumin and protein levels, which showed no significant differences between the treatment groups and the control group.

The measurement of serum urea and creatinine, electrolyte concentration of sodium and potassium are usually used to evaluate renal function and test for any damage to the kidney (Loha et al. 2019; Awotunde et al., 2019; Ekeanyanwu and Njoku 2014; Ramaiah 2011). An increase in serum level of urea and creatinine might be an indication of renal dysfunction or impaired glomerular filtration (Hor et al. 2012; Gad et al. 2013; Zakaria et al. 2016). Urea is a by-product of protein metabolism and an increase in serum urea might affect kidney function (Awotunde et al., 2019). In this study, there was a significant increase in the serum concentration of urea of the animals treated with 2,000 mg/kg ginger extract compared to the control group. This is an indication of a possible damage to renal tubules or glomerular dysfunction due to toxic effect of the ginger extract at the highest dose tested. This was further supported by the histology of the kidney, revealing moderate tubular and slight glomerular necrosis at doses of 1,000 and 2,000 mg/kg respectively. However, the necrosis observed in the kidney tissues was reversed after a 14-day recovery period. The results of this study contradicted those of a previous study which reported no changes in the serum urea level or histopathology of kidney after sub-chronic administration of ginger in Wistar rats (Jeena et al. 2011).

Maintenance of electrolytes balance is very important kidney function and disturbances in serum electrolytes can be attributed to tubular or glomerular dysfunction (Molla et al., 2020; Dhondup and Qian, 2017). In this study, there were significant increases in serum levels of sodium, potassium, and bicarbonate. The elevation of sodium level is an additional evidence indicating a possible renal dysfunction induced by the ginger extract at doses of 1,000 and 2,000 mg/kg. However, after a 14-day recovery period, the serum electrolyte levels became normal with the exception of sodium which decreased significantly. Oral administration of ginger extract at high doses of 1,000 and 2,000 mg/kg is likely to induce adverse effects or toxicity on vital organs such as the liver and kidney. The liver and kidney are major organs involve in the metabolism, detoxification and excretion of food, drugs, herbs and other agents (Vaidya et al. 2010). These organs were exposed to the toxic compounds present in the extract that exerted the pathological lesions observed in this study.

Histopathological examination of vital organs is an important aspect of toxicological assessment of bioactive compounds (Traesel et al. 2016). Histological analysis after toxicity studies can provide clear evidence of organ damage or injury (Olayode et al. 2020). In this study, the histological analysis showed slight myocardial necrosis, hyperplasia of inflammatory cells and slight alveoli congestion in the lungs and slight necrosis of neurons in the brain at doses of 1,000 and 2,000 mg/kg. However, these effects were reversed after a 14-day recovery period. The hyperplasia of inflammatory cells and slight alveolar congestion observed in this study suggest that at high doses, ginger extract could impair the pulmonary circulation of oxygen and other gases. In a previous study, ginger oil at the dose of 500 mg/kg did not cause any necrosis to the brain and lungs (Jeena et al. 2011). Our study showed normal architecture of thyroid glands, suggesting that repeated daily administration of aqueous ginger extract for 90 days at all doses tested did not cause any histological changes in the gland architecture or toxicity to the glands.

Histological assessment of the ovaries and testes showed some form of pathological lesions. The extract induced glandular hyperplasia and endometrial necrosis in the ovaries of the rats administered with 1,000 and 2,000 mg/kg of ginger extract. Similarly, spermatogenic and Leydig cell necrosis were observed in the testes of all the rats, including the control and extract treated groups. However, the observed lesions in the ovaries were reversed after a 14-day recovery period, but those of the testes were not reversed. Furthermore, the pathological changes observed in testes in the current study cannot be attributed to the effect of the extract administration since the lesions were also seen in the control group. In addition, even after the recovery period, the lesions were not reversed. However, previous study reported androgenic activity of ginger (Kamtchouing et al. 2002). Also, oral administration of ginger powder at a dose of 2,000 mg/kg for 35 days was reported to cause a significant reduction in the weight and ROW of testes in rats (Rong et al. 2009). The pathological lesions observed in the ovaries and testes of rats in this study were not supported by the results of their ROW, thus require further investigation.

Interestingly, the histopathological changes observed in organs in this study occurred mostly at a dose of the ginger extract higher than 500 mg/kg (≥ 1,000 mg/kg). At 500 mg/kg, no form of toxicity was observed. Thus, it can be inferred that repeated daily administration of aqueous ginger extract at a dose higher than 500 mg/kg might expose the organs to damage or toxicity. This is in agreement with the findings of a previous study (Jeena et al. 2011). The findings of this study show that despite the many nutritional and medicinal benefits of ginger (*Zingiber officinale*), indiscriminate consumption could expose users to potential harmful or adverse effects due to overdose or chronic use.

CONCLUSION

The findings of this study show that oral use of aqueous ginger extract could be nontoxic and relatively safe after 90 days of subchronic administration at a dose ≤ 500 mg/kg per day. However, at higher doses (1,000 and 2, 000 mg/kg per day), the extract could be slightly toxic to the organs (heart, liver, kidney, lungs, brain, ovaries), and induce histopathological changes, but the pathological lesions were reversed after two weeks. Therefore, caution should be exercised regarding the chronic use of ginger, especially at a dose higher than 500 mg/kg per day.

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