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Phytochemical screening and toxicity studies for ginger extracts with evaluation of some biochemical parameters and anticoagulant bioactivity

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Abstract

In the present study, the chemical investigation of the bioactive fractions for the aqueous and ethanolic extracts for the rhizomes of *(Zingiberofficinale),* which resulted in the characterization of (32) compound for the aqueous extract and (65) compounds for the ethanolic extract which were identified using GC-MS analysis and (12) compound was mainly identified using LC-MS analysis for the aqueous and ethanolic compound. The final formulation of ginger extracts used for the toxicity study. The acute toxicity was evaluated as per OECD guidelines 423. Ginger extracts was fed at 5000 mg/kg body weight to prepared overnight fasted male rats (8-10 weeks old; 180-200g). In the acute toxicity study, no mortality or clinical signs of toxicity were observed at a maximum recommended dose level of 5000 mg/kg; therefore, the LD_{50} is >5000 mg/kg in rats. The animals were observed daily for clinical signs of appearance, behavior, toxic symptoms, abnormality and mortality with no symptoms of poisoning. Sub-acute toxicity of ginger extracts was studied by feeding the extracts at 1250 mg/kg equal 1/0 of the LD_{50} of ethanolic plant extract and 1770 mg/kg equal 1/0 of the LD_{50} of aquas plant extract daily to rats as determined and mentioned in our study and it is also supported by OECD guidelines 407. The repeated administration of ginger extract for 28 days in rats at the maximum dose level of 1250 mg/kg of the ethanolic extract and 1770 mg/kg of the aquas extract did not induce observable toxic effects when compared to its corresponding control animals, while hemophilia and the anti-clotting effect were clearly visible at rising doses administration up to 20 gm/kg from the ethanolic extract and 25 gm/kg from the aqueous extract. After 28 days, animals were terminal sacrificed, and gross pathological changes were recorded, the hematology and biochemistry profile of treated rats was similar to control animals, and the difference (P>0.05). The histopathology for (liver, kidneys and heart) of all the control and treated animals was normal. The screening and evaluation for bioactive constituent of the extracts exhibited potent anti-platelet aggregation bioactivity and vaso-relaxing activity.

Keywords: Hemostasis, Anticoagulant, Ginger, Zingiberofficinale, LC-MS, GC-MS, Medicinal plants.

1. Introduction

Ginger (*Zingiberofficinale* Roscoe), belonging to the family Zingiberaceae, is one of the most commonly consumed dietary condiments in the world. (1)

The oleoresin from the rhizome of ginger contains many bioactive components that is believed to exert a number of remarkable pharmacological and physiological activities. Gingerols & shogaols, are the major constituents of fresh ginger rhizomes, 6-gingerol and 6-shogaol present anti-platelet aggregation activity *in vitro* (2)

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About 60 related constituents have been identified from the alcoholic extracts of fresh ginger rhizomes [3][4]. The use of natural or alternative medicines has increased markedly over the last few years. Especially the older adults are using complementary and alternative medicine, herbal remedies, and dietary supplements without advice from a physician on the assumption that these substances will have a positive effect. [5]

Scientifically, this might not be a safe or advisable practice. A few toxicity studies with ginger are reported in animals. *In vitro* investigations have repeatedly shown that an aqueous ginger extract inhibited the formation of thromboxane B2 is an inactive metabolite/product of thromboxane A2 responsible for platelet aggregation induced by several aggregating agents, an inhibition that has been explained by an inhibitory effect of ginger on platelet cox enzyme (leading to a reduced amount of the pro-aggregatory TXB)[6]. The gingerols, primarily 8-gingerol and 8-paradol, may be the major active principles that inhibit platelet activation [7], the lack of a complete understanding of its mechanisms of action suggests caution in its therapeutic use [8].The aim of the present study, therefore, was to assess the toxicity of a purified ginger extract in rats as to support its safety for human use [9].

2- Experimental:

Chemicals used for Quantitative Phytochemical Analysis:

Chemicals used for tannins and total phenolics determination were sodium tungstate, phosphomolybdic acid, HCl, and orthophosphoric acid (85%), lithium sulfate, liquid bromine, sodium carbonate (7.5%) and Gallic acid. As well, aluminum chloride, sodium nitrite (5% W/V), NaOH (1M) and catechin (CE) were used for estimation of total flavonoids. While, chemicals used for total alkaloids evaluation were bromocresol green (BCG) dye, chloroform, atropine, solvents used for plant extraction like hexane, distilled water and ethanol and all the other chemicals were of analytical grade and were purchased locally from (El- Gomhoria Co. Egypt).

Determination of total alkaloids:

Bromocresol green (BCG) dye was used to estimate the total alkaloids according to[10].BCG solution was prepared by heating (69.8 mg) BCG with 3 ml NaOH (2N) and 5 ml of distilled water, and then completed to one liter with distilled water. After that, ten microliters of the sample were mixed with 3ml BCG solution. Thirty minutes later 0.5 ml of chloroform were added and shaken for 2 min. The lower layer was separated after 30 min. The extraction was continued for three times. A set of reference standard solution (0.1 %) atropine was prepared, and followed the steps described above. The absorbance of color was read at 417 nm. The total alkaloid content was expressed as atropine equivalent (AE)/gm.

Determination of total phenols&Extraction procedure:

Plant was washed with distilled water and placed in an oven to dry at 45˚C for 4 days. Then it was ground in an electric grinder into fine powder. Extraction was performed as described by [11].In addition,0.5 gm of the plant powder was extracted with (2x10 ml) of 80% aqueous methanol using electric homogenizer for 5 min., then centrifuged (10 min & 3000 r.p.m), and the extract was poured into pre-weighed small conical flasks. Methanol was removed under reduced pressure. The solid residue (crude extract) was weighed and dissolved in distilled water to a 5 ml volume.

Quantification of total phenols:

The amount of total phenols in extracts was determined by Folin-Ciocateu method as modified by [12].The Folin- Ciocalteu reagent was prepared by adding 100 gm sodium tungstate, 25 gmphosphomolybdic acid, 100 ml HCl, and 50 ml orthophosphoric acid (85%) to 700 ml deionized water in conical flask. The flask was refluxed for 10 hours, cooled and then 150 gm of lithium sulphate were added followed by few drops of liquid bromine to make the solution yellow in color, then the final volume was completed by deionized water to 1 liter. Two hundred microliters of the plant extract were introduced into test tubes; 1 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 760 nm was measured against blank containing all the components except the sample. Gallic acid standard (5 gm %) was used, and the total phenolic content was expressed as mg gallic acid per gm dry weight of the original sample (mg GA/g d.w.).

Determination of tannins & Extraction procedures:

Whole plant was washed with distilled water and placed in an oven to dry at 45˚C for 4 days. Then it was ground in an electric grinder into fine powder. 0.5 gm of the powder was transferred to a 250 ml conical flask contained 75 ml distilled water. The flask was heated gently and boils for 30 min., then centrifuged at 2000 r.p.m. for 20 min. and collect the supernatant for tannins determination as reported by [14].

Quantification of tannins:

The amount of tannins in extracts was determined by Folin-Ciocateu method as modified by. The Folin-Ciocalteu reagent was prepared by adding 100 gm sodium tungstate, 25 gmphosphomolybdic acid, 100 ml HCl, and 50 ml orthophosphoric acid (85%) to 700 ml deionized water in conical flask, refluxed for 10 hr. cooled, then 150 gm lithium sulphate were added. Few drops of liquid bromine were added to make the solution yellow in color; the final volume was completed by deionized water to 1 litre. Two hundred microliters of plant extracts were introduced into test tubes; 1 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 760 nm was measured against blank containing everything except the sample. tannic acid standard (5 gm %) was used, and the total phenolic content was expressed as mg tannic acid per gm dry weight of the original sample (mg tannic acid /gmd.w.).

Determination of total flavonoids:

Total flavonoids content was measured by the aluminum chloride colorimetric methodaccording to [15].Ten microliters of plant ethanol extract were added to 1 ml of distilled water, followed by the addition of 100 µl of sodium nitrite (5% W/V) and allowed to stand for 5 min., then 100 µl $Alcl_3$ (10 %) were added and incubated for min., followed by the addition of NaOH (1 M) and volume was made up to 5 ml with distilled water. After 15 min., the solution was mixed completely and the absorbance was measured against blank at 510 nm. Total flavonoids were expressed as mg catechin (CE) as standard per gm sample (mg CE/gmd.w.).

Determination of total sapponins:

According to [16] measured weight (5g) of the powdered sample was mixed with 50 ml of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55ºC; it was then filtered through Whatman filter paper (No 42). The residue was extracted with 50 ml of 20% ethanol and both extracts were poured together and the combined extract was reduced to about 40 ml at 90ºC and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Re extraction by partitioning was done repeatedly until the aqueous layer become clear in color. The saponins were extracted, with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60ºC in the oven and reweighed after cooling in a desiccator. The process was repeated two more times to get an average. Saponin content was determined by difference and calculated as a percentage of the original sample thus:

(%) = − *h* 100 1 Where: -W1 = Weight of evaporating dish W2 = Weight of evaporating dish + sample

Chemicals used for estimation of biochemical parameters:

Chemicals used for the analysis of acid phosphates were potassium ferricyanide, carbonate buffer (pH 4.5& 10.4), disodium phenylphosphate, 0.5 N NaOH, 0.5 N NaHCO3 and 4-aminoantipyrine solution.In case of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase AST chemical used were phosphate buffer (pH 7.2), 0.2 mM α-ketoglutaric and 200 mM L-aspartate, 2,4-dinitrophenyl hydrazine and 0.4 N NaOH. Likewise,α, β-esteraseschemical used werediazo blue sodium lauryl sulphate solutions. Biuret reagent was used for estimation of total soluble protein. ELISA Kit., Rat Acetylcholinesterase) AChE) ELISA Kit., ALP and Albumin kits from Diamond Diagnostics were used from (El-Nasr Co. Egypt).

Chemicals used for histopathological studies:

Absolute alcohol, canada balsam and neutral formalin were purchased from (El-Nasr Co. Egypt). Xylene, haematoxylene and eosin stain were obtained from

(Cheme Co. Egypt). Parrafin wax was supplied from (El-Gomhoria Co. Egypt).

Extraction of the plant material and identification:

The ginger rhizomes were collected from the faculty of pharmacy – Heliopolis University for sustained development, Medicinal Plant Reserve. (Latin name: *Zingiberofficinale*; plant part: *Rhizome*) it was identified and authenticated by Prof. Mostafa Kamel, Agriculture branch at NRC, The National Research Center.

Preparation of plant extracts:

The entire whole plant samples comprised of leaves, roots, stems, and flowers were cleaned from the dust and debris, cut into small pieces and shade dried at room temperature (25 °C) for 7 days. The dry plant materials were ground in a grinder to a coarse powder. Extraction was carried out according to the method adopted by [17] with slight modifications where ground plant was soaked in the chosen solvents instead of using Soxhlet procedure. Serial exhaustive extraction was used in which we start with a non-polar solvent to a moderately polar and finally polar solvent (hexane, acetone, methylene chloride and ethanol 70%). Sample of 1500 gm powder was soaked in the first solvent hexane for 72 hrs. in brown colored bottles, used as containers and was provided with tight stoppers. After that, the bottles were intermittently shaken for 2 hrs. daily. The combined extract was filtered by using Whitman's No.1 filter paper on the Buchner funnel. The extract was concentrated by rotary evaporator under reduced pressure at temperature not exceeding (40-50 °C) in a water bath. The marc was subsequently subjected to extraction with another solvent (acetone, methylene chloride and ethanol 70%, respectively) and the same precedent procedure was used. The crude extracts were weighed and kept in a deep freezer until use.

Aqueous ginger extract was prepared from available ginger (Zingiberofficinale) roots. The ginger roots were peeled on crushed ice, and (100 gm) of ginger was cut into small pieces and homogenized in 75 ml of cold, sterile 0.9% NaCl in the presence of crushed ice. The homogenization was carried out in a blender at high-speed bursts for total 15 minutes. Ethanolic ginger extract was prepared by (100 gm) fresh ginger was peeled in fine pieces of variable sizes and extracted with 90% ethanol in a rotary evaporator for 72 h. The solvent was removed under reduced pressure to give the dry extract, 10% yield w/w (with respect to the crude material) and a dose equivalent to 200 mg of the crude drug per kg body weight was calculated, by support from Prof. SamyFarag, natural products branch at NRC, The National Research Center.

Analysis of the mineral content& elements composition of Zingiberofficinale rhizome:

This analysis has been done at the faculty of technology& development, soil and plant analysis unit. Zagazig University. Ginger rhizomes plant samples were disintegrated using hydrogen peroxide and nitric acid in a special microwave oven until they were reduced to their atomic elements for examination. Accurate volumetric measurements were made for the samples before being subjected to the nitric acid solution. The mineral analysis was carried out as described by [18]using Atomic Absorption Spectrophotometer (PyeUnican Sp9, Cambridge, UK.). The minerals determined were sodium (Na), calcium (Ca), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), cadmium (Cd) and lead (Pb) as mentioned at Table ()

Gas chromatography-mass spectrometry (GC-MS) conditions:

The identification of chemical components of the ethanolic & aqueous extract of *ginger* was carried out by gas chromatography mass spectrometry (GC-MS) at the Central Laboratory, Faculty of Science, Ain Shams University. Gas chromatography analysis (GC-MS) was performed by Agilent 6890N with using capillary column DB1, 30m; 0.53mmID; 1.5um Film, GC-MS analysis was performed using a Shimadzu QP 5050A mass spectrometer which coupled with a Shimadzu 17A gas chromatograph fitted with a split-split less injector and a DB-5 fused silica capillary column (30 m × 0.25 mm i. d., 0.25 µm film width). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection port was maintained at 250 °C, and the split ratio was 40:1. Oven temperature programming was done from 40 to 160 °C at 5 °C /min, and it was kept at 250 °C for 5 min. interference temperature was kept at 250 °C. Ionization mode was electron impact ionization and the range of the scanning from 40 amu to 400 amu. Mass spectra were obtained at 0.5 sec. interval. The spectra of the compounds were matched with NIST and Wiley library. The structures were defined by the

percentage similarity values and confirmed by genesis.

Liquid chromatography-mass spectrometry (LC-MS) conditions:

The identification of chemical components of the ethanolic extract of *Ginger* was carried out by liquid chromatography mass spectrometry (LC/MS) at Center for Drug Discovery Research and Development, Faculty of Pharmacy, Ain shams University. Liquid chromatography analysis (LC/MS) was performed by ESI-MS negative and positive ion acquisition mode which carried out on a XEVO TQD triple quadruple instrument.

Identification of the component

Interpretation and identification of the mass spectrum of GC-MS and LC-MS were compared with the records and database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components present in the tested materials were ascertained according to [17]. The confirmation of structure and mass characterization was carried out using mass bank free online database (https://massbank.eu/MassBank/).

Blood samples collection and preparation of plasma

These determinations were done at the Faculty of medicine University of Zagazig, Egypt.

The blood was collected by cardiac puncture and allowed to clot for 30 min. at room temperature. The clotted blood was then centrifuged at 3500 RCF for 30 min. The serum was separated and stored at -25°C until protein and enzyme analyses were performed. Blood was stored at room temperature and, within 30 minutes of collection, complete blood counts, including platelet number and indices of platelet size and granularity, were determined on whole blood in EDTA with an automated hematologyanalyzer, using the methods recommended by the manufacturer. Clotting times (PT and PTT) were measured on plasma within 30 minutes of collection with an automated coagulometricanalyzer, using the methods recommended by the manufacturer. Collection of blood samples were obtained from cardiac puncture using sterile syringes, and placed separately in containers containing trisodium citrate to prevent the clotting process. Centrifugation was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma for PT test. Each plasma sample was separately poured in plane containers using automatic pipette and stored at room temperature [19].

In vitro anticoagulant test of Zingiberofficinale extract for determination of the prothrombin time, the plasma sample of each individual was divided into five groups each of 50 µL. Group 1 (n=30) was tested first to determine the normal PT (positive control group) using the stable, liquid, combined calcium/ thromboplastin rabbit brain (DiaMed LTD, UK) as a gold standard. Four volumes of ginger aqueous extract (25, 50, 75 and 100µL) were added separately to the remaining four groups of plasma samples in a water bath with gentle shaking. Then thromboplastin reagent (100 µL) was added separately to the mixture of each plasma sample using pipettor volume adjustment. Stop watch was used for measuring the time of the clot formation[20][21]. Thromboplastin reagent was added to the plasma in order to counteract the sodium citrate and allow clotting to proceed 14 [22].After 28 days of administration with different treatments separately from Zingiberofficinale ethanolic extract & aquas extract, the animals were sacrificed at the end of the experimental period. Blood samples were collected into clean, dry sterile and labeled centrifuge tubes, the blood was allowed to flow smoothly into labeled heparinized& centrifuge tubes, the obtained plasma left to clot and the serum was obtained by centrifugation at 4000 r.p.m. for 15 min. and the clear supernatant serum was aspirated gently using sterile pipettes into clean, dry sterile and labeled stoppered vials and stored at -20 ^{0}C till used it in examination of biochemical parameter.

Histopathological Assessment and Vital Organs Measurement

These determinations were done at the faculty of medicine University of Zagazig, Egypt.

The liver, kidneys and heart were immediately excised from the euthanized animals, freed from adventitia, blotted dry, weighed, sectioned and fixed in 10% formalin for histological studies. Fixed sections were passed through xylene, alcohol and water to ensure that the tissue was totally free of wax and alcohol. Each section was then stained with hematoxylin and eosin for photo microscopic assessment using light microscope at a magnification of 400. In order to minimize bias, the pathologist had no knowledge of the doses and treatments given to the different groups of experimental rats [23]. At the

end of the study, qualitative data on the weights of vital organs (heart, liver and kidneys) were assessed by a careful dissection of each organ from the euthanized animals. Isolated organs were blotted dry and weighed fresh on a sensitive balance. Then the organs were then fixed in 10% formalin for histopathological examination. Each weighed organ was standardized for 100g.

Body weight of each rat using the formula below:

$$\text{Standardized organ weight} = \frac{\text{Weight of each organ}}{\text{Body Weight of rat}}$$

Hematological and Biochemical Parameters

Blood samples were collected through cardiac puncture from each diethyl-ether anaesthetized rat into different EDTA-coated sample bottles. The blood samples were analyzed for red blood cells count (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cells count (WBC) and differential WBC (neutrophil, eosinophil, basophil, lymphocyte and monocytes). These parameters were analysed within 24 h post-collection using automated haematology analyser according to manufacturer's protocols (Sysmex Haematology-Coagulation Systems®), at the Department of Haematology, Faculty of medicine University of Zagazig, Egypt.

Whole blood of each rat was centrifuged at 2500 rpm for 20 min at 10°C to separate the serum. The activities of serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined at 340 nm using the method described by [24]. Serum creatinine and urea level was estimated by endpoint colorimetric test using diagnostic kits. These determinations were done spectrophotometrically at the faculty of medicine University of Zagazig, Egypt, using analytical kits according to standard procedures of manufacturer's protocols.

Statistical analysis: The data were analyzed by one – way analysis of variance (ANOVA) using Graph pad prism software. Following ANOVA, Dunnett' pair – wise comparison of means of treated groups with control group mean was carried out individually.

The data is presented as the mean ± SEM (standard error of the mean), and p- value< 0.05 was considered as significant.

Toxicological studies:

Acute Toxicity Study in Rats: No clinical signs of toxicity were observed in the animals at administration dose for ginger extracts at 5000 mg/kg body weight of the ginger extract. No mortality was observed in the animals administered with the ginger extract at 5000 mg/kg body weight. Individual body weight was recorded prior to oral administration (day 1) and on days 7 and 14 following oral administration.

The gain in body weights was normal in all rats **Table (1)** all animals were sacrificed at the end of the study and they did not reveal any abnormality of gross pathological significance. Based on these findings of the acute oral toxicity of the ginger extracts in rats, the LD50 of the extract may be classified as GHS (Globally Harmonized System) category 5 ranges (2000-5000 mg/kg) as per OECD Guideline No. 423, December 2001.

Table (1): Body weight, body weight changes and mortality in acute toxicity study

Dose mg/kg	Rat Number	Sex	Bodyweight (g)					Number of dead rats /Number of tested rats
			Day 1	Day 7	Day 1 Day 14	Day 14	Day 14 Day 1	
5000	1	Male	161	170	9	171	10	0/5
	2	Male	164	172	8	179	15	
	3	Male	162	174	12	174	12	
	4	Male	165	180	15	180	16	
	5	Male	172	182	10	186	14	

Table (2): Determination LD_{50} of extract ethanolic ginger extract

Groups	Dose Mg/kg	Dose Difference Mg/kg(a)	No of rats Per group	No of Dead	Mean Dead (b)	a*b
1	Control	----	4	0	0	0
2	500	500	4	0	0	0
3	700	200	4	0	0	0
4	900	200	4	0	0	0
5	1500	600	4	0	0	0
6	2000	500	4	0	0	0
7	3000	1000	4	0	0	0
8	5000	2000	4	0	0	0
9	20.000	15000	4	4	2	30.000

$LD_{50}= LD_{100}–\Sigma\ (a \times b) / n$, LD_{50} (oral) = 12.500 gm/kg

Table (3): Determination LD_{50} of aqueous ginger extract

Groups	Dose Mg/kg	Dose Difference Mg/kg(a)	No of rats Per group	No of dead	Mean Dead (b)	a*b
1	Control	----	4	0	0	0
2	500	500	4	0	0	0
3	700	200	4	0	0	0
4	900	200	4	0	0	0
5	1500	600	4	0	0	0
6	2000	500	4	0	0	0
7	3000	1000	4	0	0	0
8	5000	2000	4	0	0	0
9	10000	5000	4	0	0	0
10	20000	10000	4	0	0	0
11	25000	5000	4	4	2	10000

$LD_{50}= LD_{100}–\Sigma\ (a \times b) / n$, LD_{50} (oral) = 17.700 gm/kg

Table (4): LD_{50} of Ginger extracts against albino rats

Rodenticide used	No of group	Dose mg/kg b. wt.	No of animals per group	No. of dead animals	LD_{50}
Ginger	Control	0.0	3	0	
	1/10 LD_{50} Ethanolic	1.25	3	0	12.5 gm/kg b. wt.
	1/10 LD_{50} Aqueous	1.77	3	0	17.7 gm/kg b. wt.

LD_{50} Estimation of Zingiberofficinale aqueous and ethanolic extracts:

The Karber arithmetic method for toxicity determination& calculation of LD_{50} to of ethanol and water extracts of *Zingiberofficinale* plant. As shown in tables (2, 3&4) for ethanolic extract of *Zingiberofficinale*, eight groups containing four rats in each group, were treated with a single dose of a different conc. prepared in tween 80 (500, 700, 900, 1500, 2000, 3000, 5000 and 20.000 mg/kg), as well as control group. In the case of *Zingiberofficinale*aquas extract there are ten groups containing four rats in each group. All animals were treated with a single dose of a different conc. prepared in Tween 80 (500, 700, 900, 1500, 2000, 3000, 5000, 10.000, 20.000 and 25.000 mg/kg) in addition to control group. Animals were kept under continuous observation to record any change in behavior or physical activities and the final LD_{50} value was determined from Karber's formula.

$$LD_{50} = LD_{100} - \Sigma (a \times b) / n$$

n= total number of animals in a group.

a= the difference between two successive doses of administered extract /substance.

b= the average number of dead animals in two successive doses.

LD_{100} = Lethal dose causing the 100% death of all test animals.

The chromatogram of reversed-phased columns of the ethanolic extract of ginger (Fig.4) identifies peaks of major components, 6-shogaol, and 10-shogaol with retention times at 10.17, 20.28, 22.93, 30.79. 33.05 and 40.97min, respectively. Identification of the peaks were done by comparing them with the HPLC of reference standards for each of the compounds.

The results correspond to previous reports that these compounds were the main constituents of ginger [30]. Negative ion electrospray LC-MS-MS chromatograms of ginger extracts showing the detection of gingerols; and 136 u showing the detection of shogaols, gingerdiones, hydroxyl-shogaols, dehydrogingerols, and paradols.

As shown in (Fig.4), the peaks eluting at 25.2, 33.2 and 40.8 min were identified as 6-gingerol, 8-gingerol and 10-gingerol based on the comparison of retention times and tandem mass spectra with those of authentic standards.

The peak eluting at 47.7 min was tentatively identified as 12-gingerol based on its *m/z* value at 377 and similar fragmentation pattern as those of other gingerols. The peak eluting at 16.4 min (Fig.4) showed loss of 194 u from the deprotonated molecule of *m/z* 264, which corresponded to 4-gingerol. It should be noted that there is an abundant peak with 4-gingerol, and its tandem mass spectrum could not be interpreted.

The peak eluting at 37.0 min showed a deprotonated molecule of *m/z* 347, which is less than the deprotonated molecule of 10-gingerol, this was assumed by [32]. By comparing the product ion tandem mass spectrum of this peak with that of 10-gingerol, the peak eluting at 37.0 min was tentatively identified as the dehydrogenation product of 10gingerol.

The abundant product ions of *m/z* 192 and *m/z* 153 suggested that dehydrogenation might have occurred at C6 on the alkyl side chain, this dehydrogenation product of 10-gingerol has been named 6-dehydro-10-gingerol.The peaks eluting at 35.3, 42.9 and 50.1 min in Fig. (4) were identified as 6-, 8- and 10-shogaol based on the comparison of the retention times and tandem mass spectra with those of authentic standards.[33]reported that the peaks eluting at 38.2, 45.8 and 52.5 min were assigned as 6-, 8- and 10-paradol based ontheir *m/z* values of 277, 305 and 333, respectively, and their product ion.

It was observed that all paradols have longer retention times than shogaols during reverse phase-HPLC. In addition, the retention times of these compounds during reverse phase-HPLC increased with increasing hydrophobicity as the alkyl chain length increases.No peak corresponding to the deprotonated molecule of 12-shogaol or 12-paradol (*m/z* 359 and 360) was detected during constant neutral loss scanning of 136 u, possibly due to the low concentration of these compounds in or absence from the ethanolic ginger extract.

There are two classes of homologues, namely 6-dehydro-gingerols and 6-hydroxy-shogaols, with the same molecular masses as gingerdiones in commercial extracts of ginger rhizomes.

The peak eluting at 37.0 min was detected during the neutral loss scanning of 194 u instead of 136 u, and its tandem mass spectrum was different from those of the peaks eluting at 43.9 min and 52.5 min. Therefore, the peak eluting at 37.0 was tentatively identified as 6-dehydro-10-gingerol.

The peaks eluting at 43.9 min and 52.5 min (Fig. 4) corresponded to isomeric compounds that formed deprotonated molecules of *m/z* 347 and abundant product ions of *m/z* 211 (base peak) corresponding to a loss of 136) and *m/z* 169.

The deprotonated molecule of *m/z* 347 is 16 u higher than 10-shogaol.In addition, the base peaks of *m/z* 211 are 16 mass units higher than the base peak of 10-shogaol and correspond to a neutral loss of 136 u, which is class characteristic of shogaols, paradols and gingerdiones.

Since the masses of these compounds preclude them from being gingerols or paradols, they are probably 6-hydroxy-10-shogaol and 10-gingerdione. The peak eluting at 43.9 min and prior to the 10-shogaol peak at 50.2 min (Fig. 10) was probably 6-hydroxy-10-shogaol, since hydroxylation of 10-shogaol should increase the polarity of the molecule and reduce its retention time during reverse phase HPLC.

6-dehydro-gingerol series were detected in both LC-MS-MS chromatograms of the neutral loss scanning of 194 u and 136.

However, 6-dehydro-6-gingerol and 6-dehydro-8-gingerol were only detected in the LC-MS-MS chromatogram of neutral loss scanning of 136 u (Fig.5) and 6-dehydro-10-gingerol was only observed in LC-MS-MS with neutral loss scanning of 194 u (Fig.5a).Peaks from gingerdiols were not detected in neutral loss scanning of either 194 u or 136 u, which indicated that gingerdiols did not fragment via C4-C5 cleavage or benzylic cleavage. This observation also suggested that the ketone group on C3 is critical for the negative ion electrospray CID fragmentation patterns discussed above for gingerol-related compounds.

In addition, derivatives from gingerol-related compounds such as methyl gingerols or 1-dehydro-gingerdiones (Fig.5a) were not detected using the current method. For methyl gingerols, cleavage at C4-C5 bond could be their characteristic fragmentation pattern. However, this homolog lacks the 2-methoxy-4-methylphenol moiety and thus cannot be detected during the neutral loss scan of 194 u. For 1-dehydro-gingerdiones, the double bond at C1-C2 would possibly prevent the benzyl cleavage, and therefore, this homolog cannot be detected during the neutral loss scan of 136 u.

Despite that complexity of the composition of ginger extract, neutral loss scanning of 194 u or 136 u can filter out the isobaric compounds without the 4-(4-hydroxy-3-methoxyphenyl) butan-2-one moiety or 2-methoxy-4-methylphenol moiety to selectively detect most gingerol-related species (except for gingerdiols).

When compared with the LC-MS in scan mode, the current LC-MS constant neutral loss scanning method provided a rapid and simple procedure for peak identification.

Since standards of 6-, 8- and 10-gingerol and 6-, 8- and 10-shogaol were available, standard curves were prepared, and the LC-MS-MS based method for the quantitative analysis of these compounds in ginger dietary supplements was validated. Although shogaols fragmented to form only one abundant product ion for SRM analysis, $[M-H-136]^-$ (Fig.5& Fig.5a), gingerols formed abundant $[M-H-194]^-$ ions as well as product ions of *m/z* 193.

The formation of either gingerol product ion was suitable for quantitative analysis (Fig. 5& Fig. 5a). However, monitoring both species is useful for gingerol identification and for the detection of possible interference, since the ratio of both of these abundant gingerol product ions should be constant.

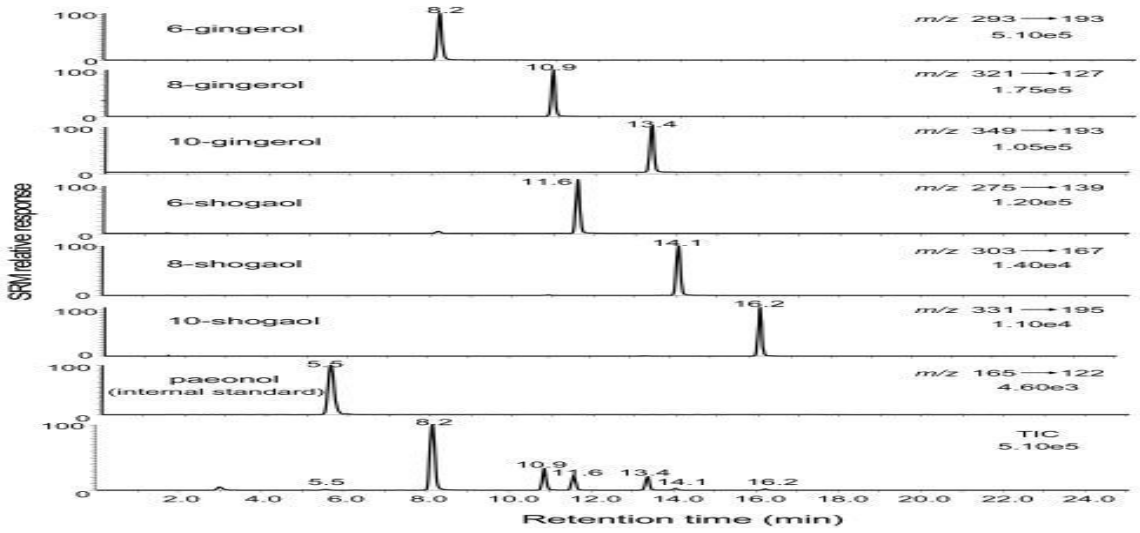


Fig. (5): Qualitative LC-MS chromatograms of the extracts of ginger

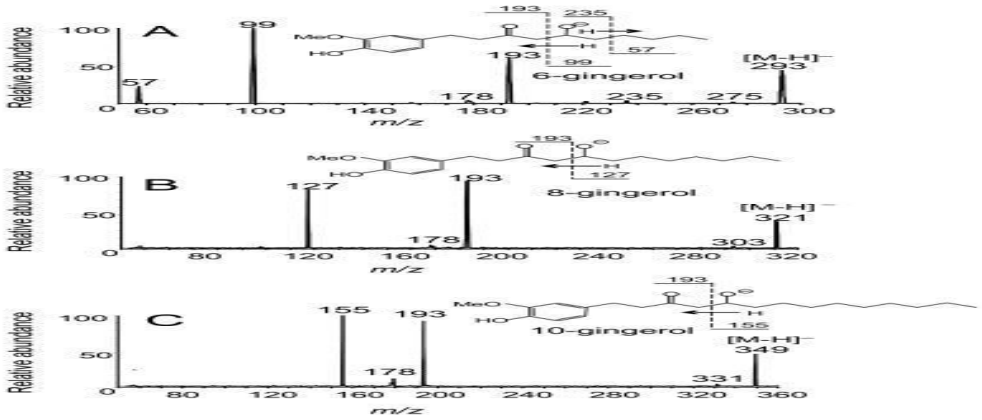


Fig. (5a):Qualitative LC-MS chromatograms of the extracts of ginger

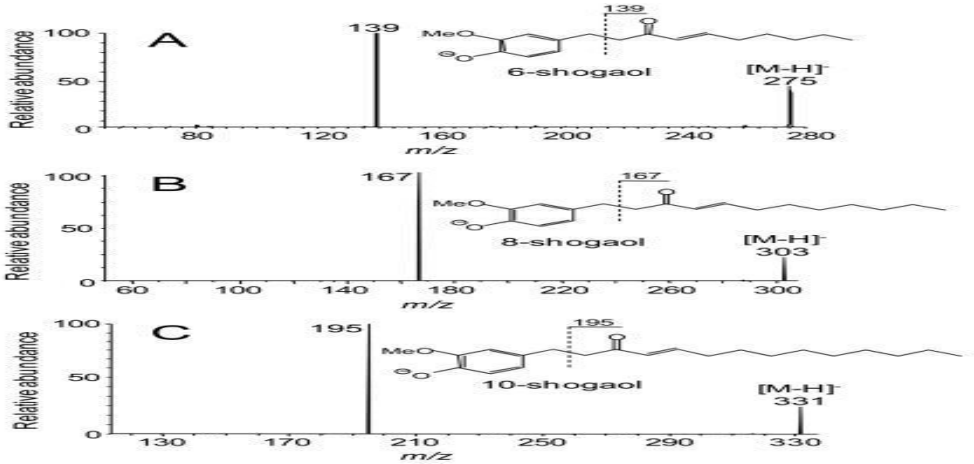


Fig. (5b): Qualitative LC-MS chromatograms of ethanolic extract of ginger

Table (5): LC-MS/MS data of phenolic compounds and quantity of phenolic compounds in aqueous extract of ginger and ethanol extract of ginger.

No.	Compounds	Retention times	Collision Energy (V)	Aqueous extractof ginger (mg/kg)	Ethanolic extract of ginger (mg/kg)
1	Pyrogailol	3.26	20	142.4	264.3
2	Hydroxybenzoic acid	4.40	10	321.1	29.4
3	Ferulic acid	5.85	10	88.8	224.7
4	Vanillin	5.50	10	101.2	89.4
5	p-Coumaric acid	5.96	10	291.4	170.2
6	Gallic acid	2.01	10	29.8b	39.6
7	Ascorbic acid	4.95	12	_b	31.3
8	Caffeic acid	4.45	11	9.8	91.2
9	Syringic acid	4.47	10	_b	_b
10	Ellagic acid	8.03	10	_b	_b
11	Quercetin	7.98	10	_b	_b
12	α-Tocopherol		20	_b	_b

[a]It was used for internal standard

[b]These values are below the limits of the quantification

Negative ion electrospray product ion tandem mass spectra of the deprotonated molecules of 6-gingerol; 8-gingerol; and 10-gingerol.

The product ion of *m/z* 193 and neutral loss of 194 u were observed as abundant fragmentation patterns in the tandem mass spectra of all gingerols due to characteristic fragmentation at C4-C5 revealed that the fragmentation patterns of the deprotonated molecules of 8-gingerol and 10-gingerol (*m/z* 321 and *m/z* 349, respectively) were similar to those of 6-gingerol and also appeared to be dominated by cleavage at the C4-C5 bond (Fig.5 and Fig. 5a). In particular, the product ions of *m/z* 127 and 155 were formed by the neutral loss of 194 u from the deprotonated molecules of 8-gingerol and 10-gingerol, respectively, and the other abundant product ion of *m/z* 193 in each tandem mass spectrum (Fig. 5 and Fig. 5b) most likely resulted from cleavage at the C4-C5 bond with the loss of an aldehyde. It should be noted that these gingerol fragmentation patterns are similar to those reported by [(34)(35)]. Therefore, CID of deprotonated gingerols appears to produce abundant cleavage at C4-C5 with either abundant neutral loss of 194 u or formation of the corresponding $[M-H-192]^-$ ion as class characteristic product ions.The deprotonated molecules of 6-, 8- and 10-shogaol were observed at *m/z* 275, *m/z* 303 and *m/z* 331 during negative ion electrospray. Unlike the fragmentation at C4-C5 occurring in gingerols, benzylic cleavage of the C1-C2 bond probably produced base peaks of *m/z* 139, 167 and 195 for 6-, 8- and 10-shogaol, respectively, and a class-characteristic neutral loss of 136 u (2-methoxy-4-methylenecyclohexa-2,5-dienone). Negative ion electrospray product ion tandem mass spectra of the deprotonated molecules of 6-shogaol; 8-shogaol; and 10-shogaol. Neutral loss of 136 u due to benzylic cleavage of 6-, 8- and 10-shogaol resulted in the formation of the base peaks at *m/z* 139, 167 and 195, respectively.

Gingerols, shogaols, gingerdiones, gingerdiols, and paradols share the same 1, 2, 4-trisubstituted benzene ring consisting of a 3-methoxy group, a 4-hydroxyl group and an unbranched 1-alkyl chain. Within each of these classes of ginger compounds, the unbranched alkyl chain varies in length from 4, 6, 8, 10, or 12 carbons. Based on the product ion tandem mass spectra of the gingerols and shogaols shown in Fig. (5), Fig.(5a) and Fig. (5b).

Although a reference standard of 12-gingerol was not available, its fragmentation pattern is predicted to be same as other gingerols. Similarly, the fragmentation of 12-shogaol, whose reference standard was also not available, should be same as the other shogaols. In

consideration of the structures of paradols and gingerdiones, their fragmentation patterns could be expected to be similar to those of shogaols rather than gingerols. In particular, benzylic cleavage might be characteristic of paradols and gingerdiones. The product ions of *m/z* 193 and $[M-H-194]^-$ appear to be class characteristic of gingerols, and the formation of $[M-H-136]^-$, corresponding to benzyl cleavage, is predicted to be class characteristic of ofshogaols, paradols, and gingerdiones.. The total amount of phenolic and flavonoid content of the ethanolic extract and the aqueous extract of ginger were shown in Tables (5-10).

Total phenolic contents of the ethanolic extract and the aqueous extract of ginger were 52.8 and 137.5µg/mg gallic acid equivalents, respectively. On the other hand, flavonoids are widely distributed in different parts of plants, fulfilling many functions.

They make up one of the most widespread, groups of plant phenolics. Due to their importance in human health, it would be useful to have a better understanding of flavonoid concentration and biological activities that could indicate their potentials as therapeutic agents, and also for predicting and control ling the quality of food and medicinal plants.

Total flavonoids contents of the ethanolic extract and the aqueous extract of ginger were 3.9 and 25.1µg/mg quercetin equivalents, respectively. They are the most important plant pigments for flower coloration, producing yellow, red or blue pigmentation in petals designed to attract pollinator animals. Previously total phenolic content of ginger was found to be highest with 50 % aqueous ethanoi extraction. Authors suggested that this is because 50% queousethanoi has greater dielectric constant, thus yields the greater release of total phenolic content. The amount of total phenols also appears to be locations dependent. It has been reported that reported that total phenolic content is higher in leaf than in rhizomes of different ginger species. Looking at overall data, it was clear that the ethanolic extract of ginger showed superior antioxidant capacity compared to and the aqueous extract, which is consistent with the higher levels of total phenols and flavonoid contents in the ethanolic extract.

Thus, these results suggest that a better antioxidant activity of the ethanolic extract could be due to considerable amount of polyphenols found in the ethanolic extract. After determination of total phenolic and flavonoid contents, phenolic acids of the aqueous extract and the ethanolic extract was determined by LC-MS experiment.

Flavonoids influence platelet aggregation in concentrations that can be achieved in vivo. Some flavonoids can influence platelet aggregation [(36)]. Ginger extracts rich in polyphenols present interesting area of research as some extracts show antiaggregatory effect[(37)].

Table (6): Biochemical parameters of male albino rats in 28 days repeated dose:
The doses used for biochemical hematological or histopathological studies were1/10 LD_{50} from the aquas extract (1770 mg/kg b. wt.) and 1/10 LD_{50} from the ethanol extract (1250 mg/kg b. wt.)

Tested parameters		**ALT (U/L)**		**AST (U/L)**		**ALP (U/L)**		**AChE (U/L)**	
Control		134.16	±1.211	35.45	±0.684	375.16	±0.921	471.20	±0.787
Ginger plant extract doses (mg/kg b.wt.)	1250 mg/kg b. wt.	124.12	±5.21	40.56*	±5.96	395.22	±11.54*	354.35	±0.735*
	1770 mg/kg b. wt.	122.33	± 6.32	30.44	±6.35**	450.38	±12.45*	456.33	±0.975*

Table (7): Biochemical parameters of male albino rats in 28 days repeated dose:

Tested parameters		**Urea (mg/dl)**	**Creatinine (mg/dl)**	**Glucose (mg/dl)**	**T.P. (mg/dl)**	**Alb (mg/dl)**
Control		26.29 ±0.333	0.73 ±0.033	128.20 ± 0.787	5.16 ±0.450	3.26 ±0.142
Ginger plant extract doses (mg/kg b.wt.)	1250 mg/kg b. wt.	20.77 ±1.33*	0.60 ±0.08	150.45 ±2.55*	5.60 ±0.45	3.21 ±0.18*
	1770 mg/kg b. wt.	23.33 ±1.33	0.55 ±0.05	120.25 ±3.66	4.71 ±0.65*	3.20 ±0.14*

ALT = Alanine aninotransferase, AST = Aspartate amino transferase, ALP = Alraline phosphatase; T.P = Total protection; Alb. = AlbuminAChE = Acetyl Choline Sterase. Analysis of variance (ANOVA) was used for statistical analysis data or presented as the Mean ± SEM (Standard Error of Mean); n=s, Significant difference levels: *P≤ 0.05; **P ≤compared with the respective control group. ALT = Alanine aminotransferase, AST = Aspartate amino transferase, ALP = Alkaline phosphatase; T.P = Total protection; ALP = Albumin, ACh E = Acetyl Cholinesterase.

Table (8): Bleeding parameters of male albino rats in 28 days repeated dose:

Tested parameters		**BT (Bleeding time)**		**PTT**		**Platelets (K/µl)**	
Control		18.6	±0.9695	30.4	±0.4540	954.5	±11.437
Gingerplant extract doses (mg/kg b.wt.)	1250 mg/kg b. wt.	20.5	±0.1697**	32.2	±0.2540*	777.2	±0.3624
	1770 mg/kg b. wt.	19.8	±0.2540*	34.3	±0.4432**	856.4	±0.3842

Table (9): Hematological parameters of male albino rats in 28 days repeated dose:

Tested parameters		**Hb (g/dl)**	**HCT %**	**MCV**	**MCH**
Control		15.1 ±0.1924	41.07 ±0.3842	59.00 ±0.6584	22.8 ±0.2633
Ginger plant extract doses (mg/kg b.wt.)	1250 mg/kg b. wt	15.9 ±0.9568**	44.10 ±2.36	59.8 ±3.42	23.9 ±1.14
	1770 mg/kg b. wt	14.7 ±0.9744*	42.3 ±2.17	61.1 ±3.66	25.4 ±1.06

Table (10): Hematological parameters of male albino rats in 28 days repeated dose:

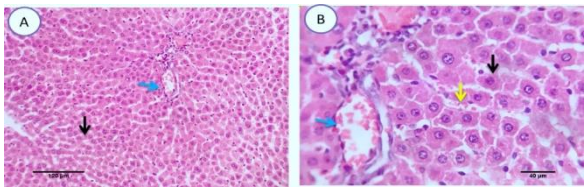
Tested parameters		**RBCs (K/µl)**	**WBCs (K/µl)**	**Neutrophils Band (%)**	**Lymphocytes %**
Control		7.808 ±0.1755	11.4 ±0.3795	6.998 ±0.4465	84.65 ±0.9665
Ginger plant extract doses (mg/kg b.wt.)	1250 mg/kg b. wt	8.11 ±0.6522*	15.9 ±0.6344	7.569 ±0.3262	81.46 ±4.2877*
	1770 mg/kg b. wt	8.17 ±0.5907	16.8 ±1.2677**	8.377 ±0.8001**	87.99 ±3.2177*

Analysis of variance (ANOVA) was used for statistical analysis data or presented as the Mean ± SEM (Standard Error of Mean); n=s, Significant difference levels: *P≤ 0.05; **P ≤compared with the respective control group. BT = Bleeding Time; PTT = Prothrombine Time, RBCs = Red Blood Cells; Hb = Henmoglobin; HCT = HenatoCrit; MCV = Mean Corpuscular; MCH = Mean Corpuscular Hemoglobin; WBCs = White Blood Cells.

Histopathological section of liver, kidney and heart in 28 days repeated dose toxicity study.
The histopathologic finding:

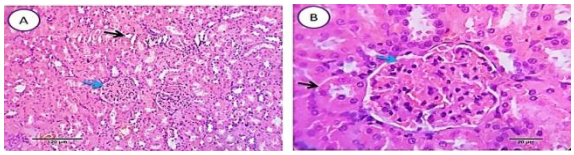
Group 1. (Control free)

Liver:



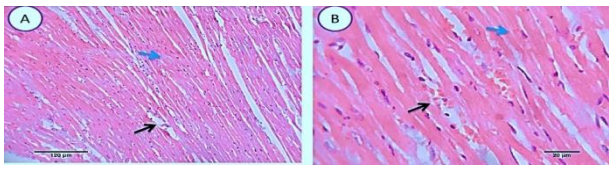
(Fig. 6a): photo-micrograph from liver, control group, showing normal hepatic parenchyma with preserved lobular pattern, central veins (blue arrow), Sinusoids and hepatic cords (black arrow). Scale bars 120, 40 um

Kidney:



(Fig.6b): photo-micrograph from kidney, control group, showing normal normalhistomorphology of nephron unites, with preserved glomerular tuft endothelium(blue arrow) , interstitial blood vessels and epithelial lining of proximal and distal tubules(black arrows) Scale bars 120, 20 um

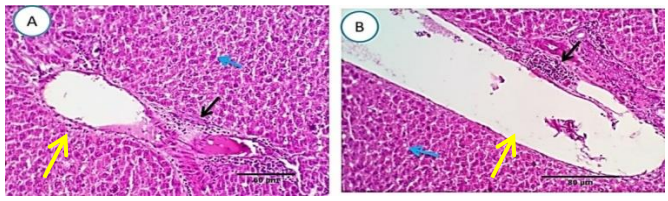
Heart:



(Fig.6c): photo-micrograph from heart, control group, showing normal cardiomyocytes (blue arrow) and intermuscular capillaries (black arrow). Scale bars 120, 20 um.

Group 2: 1/10 LD_{50} (Ethanolic ginger extract: 1250 mg/kg b. wt.)
Liver

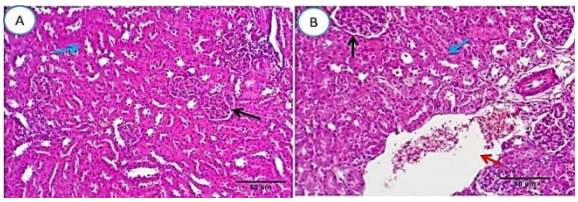
Examined serial sections denoted apparently normal hepatic parenchyma, lobular arrangement, portal triads structures, vascular tributaries and biliary tree, however some sections showed vascular dilatation and mild portal round cells aggregation.



(Fig. 7a): Photomicrograph of liver showing normal hepatic parenchyma(blue arrows), vascular dilatation(yellow arrows)and mild portal round cells aggregation(black arrows). Scale bars 60,80 um.

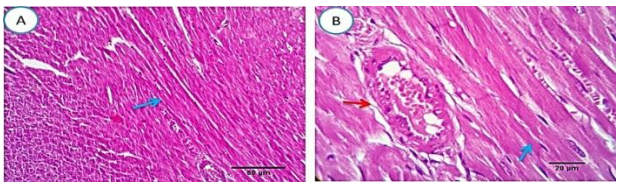
Kidney

Sections from kidney and heart revealed apparently normal histo-morphology of the corresponding tissues respectively , a part of mild to moderate vascular dilatation and blood engorgement in both tissues.



(Fig. 7b): Photomicrograph of kidney showing apparently normal glomerular (black arrows) and tubular (blue arrows) structures . Renal vascular dilatation with blood engorgement is seen (red arrow). Scale bars 60,60 um.

Heart

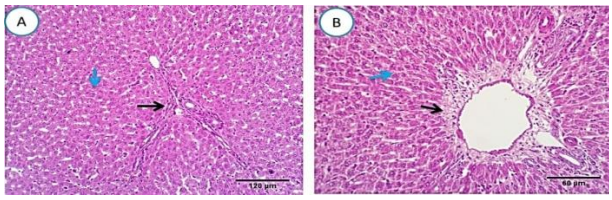


(Fig. 7c): Photomicrograph of heart showing apparently normal cardiac muscle fibers (blue arrows)and vascular structures Some intermuscular blood vessels appears mildly dilated(red arrow).Scale bars 60,20 um.

Group 3: 1/10 LD_{50} (Aquas ginger extract: 1770 mg/kg b. wt.)

Liver

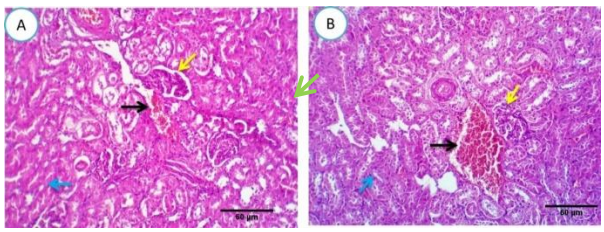
Examined sections from liver of this group revealed apparently normal hepatic parenchyma , stromal and vascular structures . The portal triad of some sections showed mild edematous fibroblastic and biliary proliferative reactions.



(Fig. 8a): Photomicrograph of liver showing apparently normal hepatic parenchyma(blue arrow) , stromal and vascular structures(A ,black arrow) . The portal triad of some sections showed mild edematous fibroblastic and biliary proliferative reactions. (B, black arrow). Scale bars 120,60 um .

Kidney

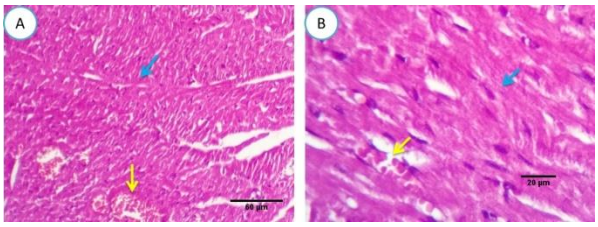
Serial sections from kidney of this group denoted normal histo-morphology of the nephron counterparts (glomeruli, proximal and distal convoluting tubules beside ascending and descending loops of Henle), the collecting tubules, renal pelvis, stromal and vascular structures were apparently normal, however a few sections showed mild vascular dilatation and blood engorgement beside degenerative and necrotic changes in a few distal convoluted tubules.



(Fig. 8b): Photomicrograph of kidney showing normal histo-morphology of the nephron counterparts(glomeruli, proximal and distal convoluting tubules beside ascending and descending loops of Henle)(blue and yellow arrows). mild vascular dilatation and blood engorgement(black arrow) beside degenerative and necrotic changes in a few distal convoluted tubules(green arrow) are seen. Scale bars 60,60 um.

Heart

Examined sections showed normal histo-morphological parenchymal (cardiomyocytessarcolemma membrane and Purkinjefibers), stromal(interstitial fibrous stroma) and vascular structures (coronary and intermuscular blood vessels).



(Fig. 8c): Photomicrograph of heart showing normal histo-morphological parenchymal , stromaland vascular structures (blue and yellow arrows).

Discussion

Ginger is an herb that has been used for thousands of years as a medicinal herb to treat a variety of ailments. Chemical and metabolic analysis have revealed that ginger comprises hundreds of compounds [(38)].

The major studied bioactive compounds include flavonoids and alkaloids, especially represented as gingerols, shogaols and paradols. Research interested in determination the role natural compounds in preventing disease has increased markedly over the last few years [(39)]. In spite of the abundance of research studies, many of the results are phenomenon based and provide data that are descriptive and observational rather than mechanistic. More studies in animals and humans on the pharmacokinetics of ginger and the constituents and on the effects of consumption over a long duration of time are needs there was still a lock of systemic safety evolution for ginger. In the assessment and evaluation of the toxic characteristics a test substance, determination of acute oral toxicity study in rats is usually an initial step.

Results of acute toxicity obtained in this study clearly indicate that the ginger extract is acutely safe and the LD_{50} for rats was found to be >5000 mg/kg. The repeated administration of the ginger extract to rats for 14 days demonstrated that oral administration of ginger extracts up to 5000 mg/kg to male rats was not associated with any abnormalities or mortalities in general conditions, behavior, growth, and food and water consumption of animals. Various parameters of

hematology and blood biochemistry were similar in both control and treated animals. The results of necropsy suggest that all of the examined organs treated by 1000 mg/kg of the ginger extract are normal. Converting rat dose to human equivalent dose, the 1000 mg/kg body weight in rats corresponds to about 11 g per day for a human weighing 70 kg [40].

While illustration the mechanism, it was noted that ginger acts on liver to reduce cholesterol biosynthesis and may also stimulate the conversion of cholesterol into bile acids and ginger elevate the liver enzymes[41][42]. It has also been demonstrated dial ginger enhanced the activity of pancreatic lipase and amylase when they were directly in contact with the enzyme [43]. Apart from effects on lipid profile, ginger dose-dependently inhibited the arachidonic acid-induced platelet aggregation, cyclooxygenase-derived thromboxane and prostaglandins, and prostacyclin synthesis, with a significant increase in fibrinolytic activity, in *in-vitro* and *in-vivo* studies [44][45]. these results indicate the anti-platelet effect of ginger.

In the present study, treatment of rats with ginger extracts up to (20gm/kg) demonstrated hemorrhage and mortality, where in the sub-acute study, the repeated administration of ginger extract for 28days, by oral route, to rats at the maximum dosage level of 12.5gm/kg it causes decline at the platelets count, also decrease the levels of urea creatinine and total protein compared to its corresponding control group of animals[46].

It was evident that there were proportional correlations between the different concentrations of *Zingiber officinale* aqueous extract and ethanolic required to inhibit clot formation and prolongation of prothrombin time and bleeding time. Accordingly, ginger extracts significantly (P = 0.01) inhibited the blood coagulation process and increased the prothrombin time. These findings demonstrated that, the extracts of *Zingiber officinale* possesses anticoagulant properties through prevention of coagulation process and clot formation.

The obtained results may indicate that, the use ginger may intensify the properties of prescribed anticoagulants [47]. Thus, the concomitant use of excessive amounts of ginger and anticoagulants and/or antiplatelet compounds could increase the risk of bleeding, due to an interaction between ginger and oral anticoagulant [48].

It is therefore essential to investigate the physiological role of active constituents of ginger and their potential effects on blood coagulation.

In this study the effects of the extracts of ginger as an anticoagulant agent had been investigated, using the principles of prothrombin time test in rats. The prothrombin time for all of them was found to be within the range (30.4±456 seconds) and blooding time was (18.6±0.969). When extract of zingiber officinal was significantly (P≤0.01) showed prolongation in the (32.2±0.254) <, prothrombin time (34.3±0.443) and prolongation in becoming time (20.5±0.169), (19.8±0.254).

Platelets are a natural source of growth factors. They circulate in the blood of mammals and are involved in hemostasis, leading to the formation of blood clots. If the number of platelets is too high, blood clots would form which may obstruct blood vessels and result in some events such as stroke, myocardial infarction, and pulmonary embolism or the blockage of blood vessels to other parts of the body, as the extremities of the arms or legs [48].

The traditional medicinal use of ginger is to promote the blood circulation for removing blood stasis

The above findings show that gingerol compounds and their derivatives are more potent anti-platelet agents than aspirin under the conditions described in this study. [8]-Paradol, a natural constituent of ginger, was found to be the most potent COX-1 inhibitor and anti platelet aggregation agent. The mechanism induced platelet aggregation inhibition may be related to attenuation of COX-l/Tx synthase enzymatic activity phenolic compounds for inhibition of AA-induced platelet aggregation and COX-1 activity [50].

Conclusion

Our results for estimation and identification the active components for ginger aqueous and ethanolic extracts using GC-MS and LC-MS analysis. Also., in the acute toxicity study for the mentioned extracts there is no toxic signs and symptoms or mortality was observed in any of the animals at the maximum 12.5 gm/kg body wight (LD_{50} > 12.5 gm/kg). In the sub-acute study, the repeated administration of ginger extracts for 28 days, oral at level dosage of (1250 and 1770 mg/kg) ethanolic and aqueous extract respectively did not induce observable toxic effects when compared to its corresponding control group of animals. The present study demonstrated that

Zingiber officinale extracts cause significant changes by increasing the bleeding and prothrombin time, while it causes decline for platelets count with normal impacts for the liver, kidney and heart histopathological examinations. So ginger extracts exhibit obvious anticoagulant effect.

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