

BIOLOGICAL EVALUATION OF GINGER RHIZOMES, AND GARLIC BULBS EXTRACTS

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ABSTRACT

The study aimed to use two extracts from garlic and ginger at different four doses (50, 100 and 200, 400 mg/kg) for ginger and garlic, respectively, and the extracts were used to decrease the harmful effect of over toasted bread. The extracts were added to the basal diet containing 10% over toasted bread. Thirty –six male albino rats were used in the animal experiment and the rats received tested diets and water in groups (1-6) for 30 days. The rats' weights and the eaten and uneaten food were recorded then blood samples were collected in tubes containing EDTA for blood analysis. The obtained results throughout chemical analysis for plant samples and the blood can be summarized as follows:

Our results showed that in ginger total carbohydrates, lipid, protein, ash and fiber were higher than garlic sample, but the content of moisture and vitamin C was higher in garlic sample than ginger sample and the contents of iron, phosphorus, calcium, potassium, zinc and total content of polyphenols and flavonoids in ginger sample were higher than garlic sample. As well as, fourteen polyphenols were detected in the two samples except catechin and salicylic acid. Pyrogallol was the highest polyphenol in garlic and ginger samples, respectively. In addition, most flavonoids were found in garlic and ginger samples, garlic has higher content of rutin, luteolin, quercetin, hyperoside and their values were 5.31, 32.53, 20.48, 13.99 ppm respectively than ginger. Also, our results revealed that the content of acrylamide in over toasted bread was 315.7 microgram/ g of sample and over toasted bread did not appear any effect on the body weight gain, daily food intake, and food conversion ratio. In addition, over toasted bread did not change the levels of total protein, albumin, globulin, antioxidant capacity and glucose, the change was not significant $P < 0.05$. So, the plant extracts (ginger and garlic) have got therapeutic effect for increasing both hemoglobin and hematocrit to normal level, while over toasted bread in positive group led to increase the levels of liver enzymes, total cholesterol, LDL, VLDL, triglycerides and decrease SOD level, but the sixth group which contains (400 mg/kg b.w) could successfully decrease previous blood parameters and increase SOD level.

Keywords: Ginger, garlic extracts, over toasted bread, polyphenols.

INTRODUCTION

Consumption of plant –derived foods, especially fruits and vegetables has been linked to decrease risk of cancer. Laboratory studies with animals and cells in culture have shown cancer preventive activity of chemicals isolated from green, yellow, orange fruits and vegetables. (Dong 2000). Since ancient times, spices and herbs have been used for preventing food spoilage and deterioration, and for extending shelf life of food. Many of the spices and herbs used today have been valued for their antimicrobial effects and their medicinal powers in addition to their flavor and fragrance qualities (Ceylan and Fung, 2004)

Ginger (*Zingiber Officinale Roscoe, Zingiberaceae*) is one of the most commonly used spices around the world, especially in the South Eastern Asian countries. Ginger is also a medicinal plant has been widely used in Chinese, Ayurvedic (Indian complementary medicine) and Unani-Tibb medicines. Since antiquity, for a wide group of ailments(diseases) that include arthritis, rheumatism, sprains, muscular aches , pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia , fever, infectious diseases and helminthiasis (*Dedov et al., 2002*).

Ginger has been demonstrated to have various pharmacological activities such as antiemetic, antiulcer , anti-inflammatory, antioxidant, antiplatelet, glucose- and lipid – lowering, cardiovascular and anti-cancer activities (*Shukla and singh, 2007*).

Garlic (*Allium Sativum*) is used as a vegetable spice and medicinal herb. Recent research on garlic has used it in the form of tablets, fresh, dried raw, boiled and cooked preparation (*Gorinstein et al., 2006*). Commercially available garlic preparations in the form of garlic oil, powder and pills are also widely used for therapeutic purposes. Garlic exhibits a wide range of properties including immunomodulatory, hepatoprotective, antimutagenic, anticarcinogenic, and antioxidant effects (*Horie et al., 1989*) and (*Al-Numair, 2009*).

Over toasted bread contains acrylamide. Long-term exposure to acrylamide may cause damage to the nervous system both human and animals to a certain extent (*Tilson, 1981*), and acrylamide is also considered as a potential genetic and reproductive toxin (*costax et al., 1992*), with mutagenic and carcinogenic properties in experimental mammals both in vitro and in vivo study (*Dearfield et al., 1995*).

The aim of this study is to evaluate ginger rhizomes, and garlic bulbs extracts as anticarcinogenic effects.

MATERIALS AND METHODS

MATERIALS:

Precarcinogenic diets:-

Bread: bread was over toasted until brown color in an oven at 140-150 C°

Antioxidant plants: such as ginger rhizomes, and garlic bulbs.

Bread and antioxidant plants: were bought from local Mansoura market.

Solvents: were obtained from Gomhoreha Company for chemicals in Cairo.

Animals: thirty –six male albino rats weighting between (182-252gm) were obtained from the animal house of National Research Institute, Cairo, Egypt.

METHODS:

Chemical analysis:

Moisture, crude fibers, ash, crude protein, crude fat, ascorbic acid, Iron, and Phosphorus contents were determined according to A.O.A.C. (2009). Carbohydrates were calculated by differences. Minerals (Ca, K, Cu, and Zn) by using Atomic adsorption spectrometry Berkin Elemer (2380Ass) according

to A.O.A.C. (2000). Total phenolic compounds were determined according to A.O.A.C (2000). Total flavonoids was determined as mg catechin/100g sample according to *Ivanova et al. (2010)*, while identification of phenolic compounds were determined by HPLC according to the method of *Goupy et al. (1999)* and Flavonoid compounds were determined by also HPLC according to the method of *Mattila et al., (2000)*. Total acrylamide in over toasted bread was determined as described by *Wang et al., (2008)*.

Preparation of ginger extract:

The plant was dried in the shade. The dried rhizome was powdered mechanically and extracted by cold percolation with 95% ethanol for 24h. The extract was recovered and 95% ethanol was further added to the plant material and the extraction continued. The process was repeated three times. The three extracts were collected together and the combined extract was concentrated under reduced pressure (55 mmHg) at 45-60 C^o. Solvent free extract (30g) was equivalent to 1 kg of dried ginger powder (W/W). The concentrate was weighed and the dose equivalent to 200 mg of the crude plant /kg body weight (W/W) was calculated. The extract was prepared according to *Bhandari et al., (1998)*.

Preparation of garlic extract:

Garlic was peeled and dried in an oven (Galen Kamp) at 55C^o for 3h. The dried garlic was ground to pass through a 1 mm sieve and about 5.0 g of garlic was extracted by 150ml of each solvent methanol, ethanol, diethyl ether, acetone, hexane and ethyl acetate, extracts then subjected to shaking at room temperature over night at a speed of 1000 vib/min. The extracts were filtered and residue was again extracted with 100 ml of each solvent. This procedure was repeated three times to ensure the complete extraction of phenolic compounds. Then, the filtrate was subjected to rotary evaporation at 40 C^o under reduced pressure for drying and removal of solvent. The extracts were weighed to calculate the yield and were stored under -20 C^o before adding the extract to the diet according to *Iqbal and Bhanger, (2007)*.

Yield of extracts:

Ginger rhizomes were extracted by ethanol, while garlic was extracted by methanol, ethanol, diethyl ether, acetone, hexane and ethyl acetate. Yield of extracts was 9%, and 2.5% for garlic bulbs and ginger rhizomes, respectively. Plant extracts yield was calculated on dry weight basis.

Design of biological experiment:

Thirty – six male albino rats were housed in stainless steel wire cages under normal healthy conditions and fed one basal diet for 7 days as adaptation period. Diets were offered to the rats in a special non-scattering feeding cup to avoid loss of food and contamination. Tap water provided to rats by mean of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage. The composition of basal diet(g/kg) according to *Reeves et al, (1993)* (AIN – 93G diet) consist of cornstarch(397.486), casein(200), dextrinized cornstarch(132), sucrose(100), soybeanoil(70), fiber(50),mineralmix(35), vitamin mix(10), L-cystine(3), choline bitartrate(2.5), tert-butylhydroquinone (0.014) . After adaptation period (7 days) animals were fed on different tested diets for thirty (30) days

and the body weight was recorded on days 0, 7, 14, 21, 28 of the experiment. The diet and uneaten food were weighed every day. Rats were fasted overnight, anesthetized using diethyl ether and the blood was collected from the orbital venous plexus by capillary tube into tube containing EDTA for analysis. The blood samples were centrifuged at 5000 r.p.m for 15 min to separate serum, and then kept in plastic vials at -20C^o until analysis. Liver, kidney, testis, brain, spleen, and heart were separated, weighed then kept in saline solution and stored at -20C^o until histopathological examination. The rats were divided into eight groups (six rats in each group) as follows:

Group (1): control rats were fed basal diet (negative control).

Group (2): rats were fed basal diet contains 10% over toasted bread (positive control).

Group (3): rats were fed basal diet contains 10% over toasted bread and ginger extract corresponding to 50mg /k.g body weight / day.

Group (4): rats were fed basal diet contains 10% over toasted bread and ginger extract corresponding to 100mg /k.g body weight / day.

Group (5): rats were fed basal diet contains 10% over toasted bread and garlic extracts corresponding to 200 mg /k.g body weight / day.

Group (6): rats were fed basal diet contains 10% over toasted bread and garlic extract corresponding to 400 mg /k.g body weight / day.

Biochemical Assays:

Determination of Glutamic pyruvic transaminase (GPT) activity:

Glutamic pyruvic transaminase (GPT) was determined according to the method described by *Reitman and Frankel (1957)*.

Determination of Glutamic oxaloacetic transaminase (GOT) activity:

Glutamic oxaloacetic transaminase (GOT) was determined according to method of *IFCC (1977)*.

Determination of serum triglycerides:

Triglyceride measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g. diabetes mellitus, nephrosis and liver obstruction. Triglycerides are determined after enzymatic hydrolysis with lipases as described by *Trinder et al., (1969)*.

Determination of serum total cholesterol:

Total Cholesterol was determined by enzymatic colorimetric method using kits provided from Beta Lap. Company according to *Trinder , (1969) .* Determination of high density lipoprotein cholesterol (HDL – cholesterol) in serum:

Low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol fractions are precipitated from serum or plasma by means of a magnesium chloride dextran sulfate reagent, according to *(Finley et al., 1978)*. High density lipoprotein (HDL) cholesterol is then determined in the supernatant fluid, using a cholesterol reagent and the derived dilution factor in the calculation.

Calculation of low density lipoprotein cholesterol (LDL – cholesterol):

LDL can be calculated according to (Friedewald *et al.*, 1972) as follows:

$$\text{LDL (mg /dL)} = \text{Total cholesterol} - \text{HDL} - \frac{\text{TG}}{5}$$

Calculation of very low density lipoprotein cholesterol VLDL– cholesterol:

V-LDL was estimated by the following equation :

$$\text{V-LDL(mg /dL)} = \text{Total cholesterol} - (\text{HDL-C} + \text{LDL-C}).$$

Determination of serum glucose:

Glucose was determined using a colorimetric enzymatic method . The method is based on the hydrogen peroxide indicator reaction which couples 4 – aminoantipyrine to phenolic compound as first proposed by (Trinder *et al.* 1969). This method has been validated in an expensive study by Penock *et al* (1973)

Determination of serum total protein:

Serum total protein was determined according to (Tietz *et al.* 1970).

Determination of Albumin:

Colorimetric determination of albumin in serum or plasma samples was performed according to Dumas *et al.* (1971).

Determination of food conversion (FCR):

Food conversion ratio (FCR) was determined using the following equation according to Youssef *et al.*, (1995).

$$F.C.R = \frac{\text{Daily food consumed (g)}}{\text{Daily body weight gain (g)}}$$

Determination of total antioxidant capacity:

Total antioxidant capacity was determined by colorimetric method according to koracevic *et al.* (2001).

Determination of hematocrit:

Hematocrit was measured using heparinized tube according to Inory (1954).

Determination of blood superoxide dismutase:

It is based on the method of Beachamp & Fridovich, (1971) and depends on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium (NBT) by the reaction of photoreduced riboflavin and oxygen.

Determination of hemoglobin:

Hemoglobin was determined by a colorimetric method according to Franco *et al.* (1984).

Statistical analysis:

The results were analyzed by the analysis of variance (ANOVA) using the procedure by Statistical Analysis System (SAS) program according to SAS (1996). Significant differences were determined at the level $P \geq 0.05$.

RESULTS AND DISCUSSIONS

Chemical composition of study ginger and garlic samples:

Data in table (1) showed that moisture, ash, crude protein, crude fat and total carbohydrates contents were determined in ginger and garlic

samples. All results were calculated as (g/100g dry weight).It was clear that ginger has low percentage values for moisture(10%) and high values of carbohydrates, fat ,ash ,protein and fiber which were 67.6, 3.5 ,7.2 ,8.7 and 3% respectively compared with garlic bulbs which have a high moisture content (63%)

Table (1): Chemical composition of study ginger and garlic samples:

Raw materials \ constituents	Moisture %	Crude fiber %	Ash%	Crude protein %	Crude fat %	Total carbohydrates %
Ginger	10	3	7.2	8.7	3.5	67.6
Garlic	63	2.5	1.3	6	0.3	26.9

The obtained results by *Mustafa et al.(1993)* revealed that the powdered ginger rhizomes sample contains 9% protein, 60 – 80% carbohydrates, 3% crude fiber, 8% ash and 9 – 12 water. Our results agreed approximately with previous results. As well as *Blumenthal,(2000)* reported that the moisture content was found to be 65% , lipid content was 0.15%, protein was found to be 2% ,carbohydrates content was 28% and fiber was found to be 1.5% in garlic bulb.

Minerals and vitamin C contents of ginger and garlic samples:

Elements play an important role in human nutrition. For example, phosphorus is an essential element for human bones skeleton and iron represent an important physiological function in the hemoglobin, other elements are important for activity of some enzymes and vitamins. From Table (2), it could be seen that phosphorus values were 227 and 150 mg /100g while iron values were 10 and 1.5 mg /100g in ginger and garlic, respectively.

Our results showed that ginger has a high content of iron, phosphorus, calcium and potassium elements. Our results did not agree with *Gusev and Grishina (1963)* who mentioned that iron content of garlic varied from 20 mg to 100mg absolute dry weight. And also a low content of K, Ca, and Zn in garlic sample.

Vitamin C decreases risk of cardiovascular disease and it is the main water soluble antioxidant in human plasma.

The content of vitamin C was 30 and zero mg/ 100g in garlic and ginger rhizomes, respectively.

On the other hand, *Oboh et al., (2010)* mentioned that vitamin C content of the white ginger was (0.91 / mmol/ 100g).Our results did not agree with the previous result, it may be due to the difference in the chemical analysis methods, dryness method and other factors.

Table (2): Minerals and vitamin C contents of ginger and garlic samples:

Raw materials \ constituents	Fe mg/100 g	P mg/100 g	Ca ppm	K ppm	Cu ppm	Zn ppm	Vitamin C mg/100g
Ginger rhizomes	10	227	18.7	108.8	0.025	0.189	zero
Garlic bulbs	1.5	160	15.8	28	0.030	0.182	30

(Fe, P, Ca, K, Cu and Zn) are elements of iron, phosphorus, calcium, potassium, copper, and zinc, respectively.

Total polyphenols and flavonoids content of ginger and garlic samples:

Phenolic compounds can play an important role in preventing body cells and organs from injuries by hydrogen peroxide, damage by lipid peroxides and scavenging or neutralizing free radicals. Table (3) has shown the polyphenols determined as mg pyrogallol equivalent / 100g for investigated plant samples.

It was noticed that ginger rhizomes had high content of total polyphenols (328.9 mg pyrogallol Eq. / 100g sample) while, garlic had low value of polyphenols (180.526mg pyrogallol equivalent / 100g).

On the other hand, *Bozin et al., 2008* reported that total phenol content in dried garlic bulb extract was (0.18 mg gallic acid (G.A) equivalent /100 g).

As well as, *Oboh et al., 2010* showed that the phenol content was (61.89 mg G.A E.q / 100g) in white ginger.

Table (3): Total polyphenols and flavonoids content of investigated ginger and garlic samples:

Extracts	constituents	Total polyphenols (mg pyrogallol Eq / 100g)	Total flavonoids (mg catechin Eq /100g)
Ginger rhizomes		328.9	155.94
Garlic bulbs		180.526	16.90

E.q: equivalent

Our results showed that total flavonoids content was 155.94 and 16.90 mg catechin E.q /100g in samples of ginger and garlic, respectively.

Polyphenolic compounds in ginger rhizomes and garlic bulbs:

High performance liquid chromatography (HPLC) procedure was used for qualitative and quantitative analysis of polyphenolic compounds in garlic and ginger extracts. Fourteen polyphenolic compounds were found in garlic and ginger extracts. They were available at authentic samples namely: pyrogallic, chlorogenic acid, catechein, synergic, caffein, P.coumaric acid, ferulic acid, coumarin, nariginin, cinnamic, vanillic, chrisin, P. Benzoic and salicylic acid.

From Table (4), it was clear that both investigated samples contained pyrogallic, caffein, P. coumaric and ferulic acid with different concentrations. It could be noticed that pyrogallic acid was the predominant identified component in garlic and ginger at concentrations of 17258.8 and 4703.8 ppm respectively, followed by Synergic (128.30ppm) , and Ferulic acid (94.70ppm) in garlic.. While vanillic, chrisin and P.hydroxy benzoic were only found in ginger rhizomes. But in another study of *Ghoneim ,(2012)* reported that the content of phenolic compounds in ginger ranged from 0.021 to 119.26mg/100g and values of gallic acid ,catechol ,chlorogenic, catechien , P.Benzoic, coumaric ,salicylic , ,caffeine ,cinnamic,and chrisin were 119.26, 8.17 ,6.11 ,36.66 ,1.99 ,1.78 ,11.11 , 10.39 ,0.95 , and 0.021 mg/100g respectively ,but vanillic ,syringic and caffeic were not detected.

In a previous study by *Rong et al. (2009)* reported that the contents of 6-gingerol, 6-shogaol and galanolactone in ginger extract were quantified to be 9065.87, 159.87 and 95.38 ppm, respectively.

Table (4) : Polyphenols of ginger and garlic extracts :

Polyphenols(ppm)	Extracts	
	Ginger extract	Garlic extract
Pyrogalllic	4703.80	17258.8
Chlorogenic acid	-	296.60
Catechein	-	-
Synergic	-	128.30
Caffein	76.70	89.40
P. coumaric	9.80	14.49
Ferulic	77.1	94.70
Coumarin	23.80	18.46
Nariginin	-	31.70
Cinnamic	-	9.20
Vanillic	38.30	-
Chrisin	22.20	-
P.hydroxy benzoic	80.80	-
Salicylic acid	-	-

ppm: part per million

Flavonoids of ginger and garlic extracts:

High performance liquid chromatography (HPLC) procedure was used for qualitative and quantitative analysis of flavonoids compounds in garlic and ginger extracts. Six flavonoids compounds were available as authentic samples namely: Rutin, Luteolin, quercetrin, hypersoid apigenin and kampferol.

From Table (5), it was noticed that investigated samples contained rutin, luteolin, querectrin and hypersoid, while apigenin exhibited only in ginger extract (78.01ppm), and kampferol appeared only in garlic extract (26.39ppm).

Garlic extract had high concentrations from luteolin and quercetrin and their values were 32.53 and 20.48 ppm, respectively.

On the other hand, *Miean and Mohamed (2001)* and *Leighton et al. (1992)* claimed that contents of apigenin , quercetrin and myricitin in garlic extract were 217, 47 and 693ppm, respectively , While Isorhamnetin , kaempferol and luteolin were not detected . Those results differed from our results , because our results proved that kampferol and luteolin were detected in garlic extract and their values were 26.39 and 32.53 ppm , respectively . The difference due to some factors such as, irrigation, fertilization, soil, climate and chemical analysis methods.

Table (5): Flavonoids of ginger and garlic extracts:

Extracts	Flavonoids (ppm)					
	Rutin	Luteolin	Quercetrin	Hypersoid	Apigenin	kampferol
Ginger	0.55	0.80	1.02	5.14	78.01	-
Garlic	5.31	32.53	20.48	13.99	-	26.39

Content of acrylamide in over toasted bread sample:

Acrylamide was determined in over toasted bread and our results have shown that the concentration of acrylamide was 315.7 microgram (µg) / gram of the sample. This value equals 0.3gram / kilogram of the sample and also be equal to 300mg / kilogram of over toasted bread sample. While *Wang et al.,(2008)* reported that the amounts of acrylamide in eight food samples such

as sweet potato crisps , fried sweet dumpling and sesame ball were 134, 136, and 68 µg / k.g of the sample, respectively, and our results have shown that the amount of acrylamide in over toasted bread was too higher than the amounts obtained by them.

Effect of (over toasted bread) and antioxidant ginger and garlic extracts on body weight gain, daily food intake and food conversion ratio in rats:

Gain in body weight, daily food intake and food conversion ratio of rats fed on over toasted bread and plant extracts for a month in negative control group, positive control group and other groups fed the experimental diets are shown in Table (6). Data showed non significant changes among groups in body weight gain, daily food intake and food conversion ratio of rats compared with a positive group (P > 0.05).

From Table (6) It was noticed that acrylamide in over toasted bread did not appear any adverse effect on rats during the experiments.

Our results agree with *Weidner and Sigwart (2000)* illustrated that effect of ginger extract at doses of 100, 333 and 1000 mg / kg on body weight gain and food consumption were similar in all groups compared with control group.

In addition, the data obtained are not consistent with *Trnqvist, 1997* who found a decrease in body weight gain per day in rats exposed to 5–10mg acrylamide/kg body weight. While the dose of acrylamide in our experiment was less than the dose used by him, and our findings have cleared that the concentration of acrylamide was 0.3g/ kg of the sample and over toasted bread in the diet was ten percent (10%).

Table (6): Effect of (over toasted bread) and ginger and garlic extracts on body weight gain, daily food intake and food conversion ratio in rats:

parameters \ Treatments	Final body weight gain (g)	Daily body weight gain (g)	Daily food intake (g)	Food conversion ratio (FCR)
Group (1) :Basal diet (-control)	70.33 ^{ba}	2.34 ^{ba}	16.47 ^a	7.69 ^b
Group (2): Basal diet + 10% over toasted bread(+control)	73.67 ^a	2.46 ^a	18.42 ^a	8.01 ^b
Group (3) :+ control diet + ginger extract 50mg/K.g b.w	55.83 ^{bac}	1.86 ^{bac}	18.46 ^a	10.72 ^{ba}
Group (4): + control diet + ginger extract 100mg/K.g b.w	49.83 ^{bac}	1.66 ^{bac}	18.66 ^a	15.28 ^{ba}
Group (5): + control diet + garlic extract 200 mg / K.g b.w	65.16 ^{ba}	2.17 ^{ba}	17.08 ^a	8.63 ^b
Group (6): + control diet + garlic extract 400 mg / K.g b.w	49 ^{ba} ^c	1.63 ^{bac}	16.61 ^a	11.62 ^{ba}
P. Value:	0.09	0.09	0.20	0.14
L.S.D:	26.78	0.89	2.38	8.86
	N.S	N.S	N.S	N.S

L.S.D: Least significant difference.

N.S: Non significant at P > 0.05

b.w: Body weight.

a, b and c: Means within the same column with different superscripts are significant

Effect of (over toasted bread) and ginger and garlic extracts on hemoglobin, hematocrit and total antioxidant capacity:

Data in Table (7) illustrated that hemoglobin and hematocrit were decreased in the positive group compared with the negative group, and the values were 12.23 g/dl and 37.67% for hemoglobin and hematocrit, respectively. The highest value was 14.21g/dl for hemoglobin in negative group but the lowest value was 12.23 g/dl in positive group, while the fourth group which contains ginger extract at dose of 100mg / k.g body weight and sixth group which contains garlic extract at dose of 400 mg/kg b.w had values of (14.05and 14.03g/dl), respectively.

For hematocrit, our results have shown that the highest value was 43.62% in the negative group, but the lowest value was 37.67% in the positive group. The groups which followed the highest value were the fourth group which contains ginger extract at a dose of 100mg / k.g body weight. Our results showed high significant difference among the groups compared with the positive group $P < 0.01$. In the present study ginger extract and garlic extract proved that they have got therapeutic effect for increasing both hemoglobin and hematocrit to normal level.

Ballat and Smith (1992) reported that garlic extract protect the erythrocytes membrane against oxidative stress inhibiting the formation of abnormally dense erythrocytes which are believed to play an important role in the clinical manifestations of sickle cell anaemia patients, the previous result supports our results for influential effect of garlic extract.

With respect to total antioxidant capacity for serum, our results have shown non significant difference among groups compared with a positive group $P > 0.05$.

Our results did not agree with *Afshari et al., (2007)* who illustrated that plasma antioxidant capacity in diabetic treated with ginger group was higher when compared with positive and negative groups. The contradiction may be due to the difference in concentration of ginger and chemical analysis, on the other hand our results agreed with *Shati et al., (2009)* who found that water extract of ginger with alcohol showed no significant changes in total antioxidant capacity for serum and significant changes in total antioxidant capacities in liver and kidney tissues when compared with the control group.

On the other hand *Benkeblia, 2004* claimed that organic and aqueous extracts of garlic had significant antioxidant potential. But our results have shown the treatment of garlic extract at 400mg / k.g body weight per day increased the antioxidant capacity from 1.64 mmol/L in a positive group to 1.94 mmol /L but no significant increase $P > 0.05$.

Table (7) Effect of (over toasted bread) and ginger and garlic extracts on hemoglobin, hematocrit and total antioxidant capacity.

parameters \ Treatments	Hemoglobin g/dl	Hematocrit %	Total antioxidant capacity mmol/L
Group (1): Basal diet (control)	14.21 ^a	43.62 ^a	1.94 ^a
Group (2): Basal diet +10% over toasted bread (+control)	12.23 ^b	37.67 ^b	1.64 ^a
Group (3): (+control) diet + ginger EX (50mg/K.g b.w)	14.00 ^a	43.00 ^a	1.94 ^a
Group (4): (+control) diet + ginger EX (100mg/K.g b.w)	14.05 ^a	43.13 ^a	1.90 ^a
Group (5): (+control) diet +garlic EX (200mg/K.g b.w)	13.90 ^a	42.69 ^a	1.98 ^a
Group (6): (+control) diet+garlic EX (400mg/K.g b.w)	14.03 ^a	43.07 ^a	1.92 ^a
P. value	0.003	0.003	0.96
L.S.D	0.95	2.86	0.56
	**	**	N.S

a, b and c: Means within the same column with different superscripts are significant.

L.S.D: Least significant difference

N.S: Non significant (P > 0.05)

EX.: extract

b.w: Body weight

** : high significant (p<0.01)

Effect of (over toasted bread) and antioxidant ginger and garlic extracts on liver functions:

Table (8) showed the effect of over toasted bread and antioxidant plant extracts on liver functions in rats, the data showed that the positive group had high significant increase in GPT and GOT. Ginger and garlic extracts could decrease liver serum enzymes in all groups from group (3) to group (6). There was a very high significant change among all groups compared with positive group P < 0.0001.

It could be seen from Table (8) that the highest value was observed in the positive group (96.85 and 144.48 U/L) for GOT and GPT, respectively.

But the lowest value for GPT was 44.47 U/L in the sixth group treated with garlic extract at dose of 400mg/k.g b.w followed by group (4) treated with ginger extract at dose of 100 mg /kg b.w and its value was 45.65U/L. So, the garlic extract was the best for reducing GPT enzyme. For GOT enzyme, our obtained results have shown the lowest value in negative group followed by the sixth group with value of 41.75 U/L treated with garlic extract at dose of 400mg/k.g/ day, and fifth group treated with garlic extract at dose of 200mg/k.g body weight / day. So, It could be summarized that garlic extract was the best extract to reduce high levels of GOT and GPT in blood serum.

From the same table there was not significant difference between the groups and positive group (P > 0.05) for total protein. It was noticed that over toasted bread containing acrylamide reduced significantly protein, albumin and globulin and the ratio between albumin and globulin in the positive

control, and the extracts led to increase total protein, albumin and globulin , and albumin /globulin ratio ,but the increase was not significant compared with the negative control group (p>0.05.)

Our results are in accordance with *Gedik et al.,2005* who reported that garlic extract could decrease the high levels of serum AST (GOT) and ALT (GPT) in the BDL (Bile duct ligation and scission) rats group and the results were significant compared with the positive (BDL) group P < 0.01.

And also our results agreed with *Shati et al., (2009)* who showed that ginger extract alleviated of the high levels of liver serum enzymes (GOT ,GPT)and their values were in alcohol group (121.9, 133) respectively, after using ginger extract liver enzymes were significantly reduced to 58 and 75.2 for GOT ,GPT ,respectively and also bilirubin was elevated in alcohol group ,but the ginger extract could significantly reduce bilirubin from 11.9 to 6.6.

Table (8) Effect of (over toasted bread) and ginger and garlic extracts on liver functions:

parameters	GOT U/L	GPT U/L	Total protein g/dl	Albumin g/dl	Globulin g/dl	Ratio between albumin and globulin
Treatments						
Group (1): Basal diet (-control)	37.68 ^c	47.29 ^c	6.25 ^a	4.36 ^a	1.88 ^a	2.32 ^a
Group (2): Basal diet +10% over toasted bread (+control)	96.85 ^a	144.48 ^a	5.30 ^b	3.70 ^b	1.60 ^b	2.32 ^a
Group (3): (+control) diet +ginger EX (50mg/k.g b.w)	45.10 ^{cb}	68.19 ^b	5.92 ^{ba}	4.09 ^{ba}	1.83 ^{ba}	2.25 ^a
Group (4): (+control) diet+ ginger EX (100mg/k.g b.w)	44.72 ^{cb}	45.65 ^c	6.14 ^a	4.29 ^a	1.85 ^{ba}	2.32 ^a
Group (5): (+control) diet+ garlic EX (200mg/K.g b.w)	42.60 ^{cb}	65.53 ^b	5.91 ^{ba}	4.03 ^{ba}	1.89 ^a	2.18 ^a
Group (6): (+control) diet + garlic EX (400mg/k.g b.w)	41.75 ^{cb}	44.47 ^c	5.94 ^{ba}	4.05 ^{ba}	1.89 ^a	2.31 ^a
P- value	<0.0001	<0.0001	0.16	0.23	0.42	0.99
L.S.D	7.82	11.54	0.69	0.55	0.27	0.45
	***	***	N.S	N.S	N.S	N.S

GPT: Glutamic pyruvic Transaminase.

GOT: Glutamic oxalacetic transaminase.

a, b and c: Means with in the same column with different superscripts are significant

N.S: Non significant (P > 0.05)

***: very high significant at (P < 0.001).

EX: Extract

Effect of (over toasted bread) and antioxidant ginger and garlic extracts on lipid profile, glucose level and Superoxide dismutase (SOD):

From Table (9) our results have shown non significant change in glucose for all groups compared with the positive group control in glucose level thus, it could be noticed that acrylamide found in over toasted bread did not affect the glucose level in blood serum. So, effect of ginger and garlic extracts did not appear through the trial. The best extract was ginger rhizomes extract at dose of 100 mg / k.g body weight and it could decrease the glucose level from 91.65 mg / dl in positive group to 85.58 mg / dl but the reduction was not significant $P > 0.05$. Also our results did not agree with *Ahmed, (1994)* who reported that acrylamide caused an increase in glucose level and the disagreement due to the difference in amount of acrylamide.

Our results showed that the highest value of triglycerides was 105.23 mg /dl in the positive group, whereas the lowest value was observed for triglycerides was 76.55 mg / dl in group (6) treated with garlic extract at dose of 400 mg / k. g body weight per day, followed by group (4) which contains ginger extract at a dose of 100 mg / k. g body weight per day. With respect to total cholesterol, the obtained results have shown that the highest value was 157.81 mg cholesterol / dl in the positive group then the value decreased to 91.18 mg cholesterol / dl in the sixth group treated with garlic extract at a dose of 400 mg / k. g body weight daily. It was noticed that garlic extract decreased the high levels of total cholesterol and triglycerides in blood serum. In general, there was a very high significance among all groups compared with positive group $P < 0.0001$. *Al – Amin et al., 2006* reported that raw ginger at dose of 500 mg / k. g was significantly effective in lowering cholesterol and triglycerides levels in the ginger -treated diabetic rats.

From Table (9) our obtained results showed that the highest value of LDL was 90.72 mg / dl in the positive control, while the lowest value was 31.96 mg / dl in the negative control followed by group (6) treated with garlic extract at a dose of 400 mg / k. g body weight daily. So, the best treatment was garlic extract that could decrease LDL – C from 90.71 mg / dl to 35.38 mg / dl in group (6). With regard to HDL – C our results showed that extracts could decrease HDL – C except group (5) which contains garlic extract at dose of 200 mg/ kg b.w compared with the positive control ($P < 0.0002$).

The highest value was 46.23 mg / dl in group (5) followed by the positive control and its value was 46.04 mg/dl. It was concluded that acrylamide caused significant increase for HDL – C compared with negative control. Our result did not agree with many authors such as *Durak et al., 2004* who reported that garlic extract increased HDL-C level.

Also , our results showed that the highest value of VLDL was 21.04 mg / dl in positive control (2) , while the lowest value was 15.31 in group (6) which was treated with garlic extract at a dose of 400 mg / k. g body weight daily , followed by group (4) treated with ginger extract at a dose of 100mg /k.g b.w daily.

There was a very high significant change among all groups compared with the positive control ($P < 0.0001$).

It was noticed that the best extract was garlic extract in sixth group and acrylamide caused a significant increase for VLDL compared with negative group. Our results are in agreement with *Durak et al., (2004)* who claimed that serum total cholesterol, LDL-C, V – LDL –C, triglycerides levels and total / high – density lipoprotein cholesterol ratios were found to be significantly lowered after use of garlic extract at a dose of 1 ml per k. g of body weight daily for four months. In addition *Al – Amin et al ., (2006)* reported that raw ginger at a dose of 500 mg / k.g was significantly effective in lowering cholesterol and triglycerides levels in the ginger -treated diabetic rats .

Also from our results in Table (9) it could be seen that the highest value of SOD was 81.93% in the negative control, while the lowest value was 44.28% in positive control which contains only over toasted bread. So the present study has suggested that acrylamide led to decrease SOD. After the treatment with the extracts from group (3) to group (6), SOD increased compared with the positive control $P < 0.0001$. The best extract in increasing SOD enzyme was garlic extract at dose of 400 mg / k.g body weight daily in group (6) and its values was 65.88%. Our results agreed with *EL sharaky et al., (2009)* who reported that the administration of ginger extract at a dose of 100 mg / k g showed (SOD)enzyme activity was increased in liver tissues to 7U / mg protein compared with the level of SOD enzyme in bromobenzene control group (4U / mg protein) . SOD is an important cellular antioxidant enzyme, which converts superoxide radical into H_2O and O_2 .

And also *Dat and Tapat (2009)* illustrated that SOD activity was decreased by 25 – 30 % when compared with normal control group , but water garlic infusion increased SOD by (25 % , $P < 0.001$) compared with carcinogen DMBA group and it is known that 7.12 - Dimethylbenz (a) anthracene (DMBA) causes skin carcinogenesis when it is injected into rats.

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التقييم الحيوي لمستخلص الزنجبيل و الثوم.

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هدفت الدراسة إلى استخدام مستخلصات الثوم والزنجبيل عند أربعة تركيزات مختلفة (١٠٠,٥٠ ملجم/كجم /يوم) للزنجبيل و(٤٠٠,٢٠٠ ملجم/كجم /يوم) للثوم على التوالي و استخدمت المستخلصات لتقليل التأثير الضار للخبز شديد التحميص. و تم إضافة المستخلصات إلى الوجبة الأساسية المحتوية على ١٠% خبز شديد التحميص في تجربة فئران التجارب. و تم استخدام ٣٦ (ستة و ثلاثين) فأرا و تناولت الفئران الوجبات المختبرة والماء في المجاميع (١-٦) لمدة ثلاثين يوما وأوزان الفئران والطعام المأكل وغير المأكل تم تسجيله وعينات الدم تم تجميعها في أنابيب مانعة للتجلط لتحليلها. النتائج المتحصل عليها من خلال التحليل الكميائي للعينات النابتية والدم يمكن تلخيصها كالآتي :

أوضحت النتائج أن عينة الزنجبيل أحتوت على أعلى نسبة من الكربوهيدرات الكليه والدهن والبروتين والرماد والألياف وهذه النسب أعلى مما في عينة الثوم, لكن الرطوبة وفيتامين C كان أعلى في عينة الثوم من عينة الزنجبيل, كما أن محتويات الحديد والفسفور والكالسيوم والبوتاسيوم والزنك والمحتوى الكلى من الفينولات والفلافونيدات في عينة الزنجبيل كانت أعلى من عينة الثوم , بالإضافة إلى أنه تم تحديد أربع عشرة مادة فينولية في العينتين ماعدا كاتيشين وحمض سلسليك. البروجالك كان البولى فينول الأعلى في الثوم و الزنجبيل على التوالي.بالإضافة إلى أن معظم الفلافونيدات وجدت في عينات الثوم و الزنجبيل , والثوم لديه محتوى أعلى من الزنجبيل من حيث روتين و لتولين و كورسترين والهيبيرسويدو كانت قيمهم ٣١.٥٣, ٣٢.٥٨, ٢٠.٩٩, ١٣.٩٩ جزء في المليون على التوالي. أيضا نتائجننا أوضحت أن محتوى الأكريلاميد فى الخبز شديد التحميص ٣١٥.٧ ملجرام/جرام عينة والخبز شديد التحميص لم يظهر أى تأثير على وزن الجسم المكتسب والمأكل اليومي ومعدل التحول الغذائى بالإضافة إلى أن الخبز شديد التحميص لم يغير مستويات البروتين الكلى والألبومين والجلوبيولين والقدرة المضادة للأكسدة والجلوكوز والتغير لم يكن معنويا $p>0.05$. لذا فالمستخلصات النباتية الزنجبيل و الثوم لديها تأثير علاجي على زيادة الهيموجلوبين والهيموتكريت كليهما إلى المستوى الطبيعى بينما الخبز شديد التحميص فى المجموعه الموجبة أدى الى زيادة مستويات إنزيمات الكبد والكوليسترول الكلى و LDL و VLDL والجليسريدات الثلاثية و خفض SOD لكن المجموعه السادسة المحتوية على مستخلص الثوم بتركيز ٤٠٠ ملجم /كجم استطاعت بنجاح خفض قياسات الدم السابقة ورفع مستوى SOD .

Table (9): Effect of (over toasted bread) and antioxidant ginger and garlic extracts on lipid profile, glucose level and Superoxide dismutase (SOD):

parameters	TG mg/dl	Total Cholesterol mg/dl	HDL – C mg/dl	L-D-L-C mg/dl	VLDL-C mg/dl	Glucose mg/dl	SOD%
Group(1) : Basal diet (-control)	80.63 ^{cb}	89.35 ^c	41.26 ^b	31.96 ^d	16.13 ^{cb}	83.53 ^a	81.93 ^a
Group(2) : Basal diet + 10% over toasted bread (+control)	105.23 ^a	157.81 ^a	46.04 ^a	90.72 ^a	21.04 ^a	91.65 ^a	44.28 ^d
Group(3) : (+ control) diet + ginger EX (50mg/k. g b . w)	84.80 ^b	99.95 ^c	38.54 ^b	44.46 ^{cb}	16.95 ^b	85.87 ^a	53.36 ^c
Group (4) : (+control) diet+ ginger EX (100mg/k.g b.w)	78.17 ^{cb}	94.19 ^c	38.65 ^b	39.91 ^{cd}	15.63 ^{cb}	85.58 ^a	62.15 ^b
Group (5) : (+control)diet+ garlic EX (200mg/K.g b.w)	79.13 ^{cb}	118.24 ^b	46.23 ^a	55.53 ^b	15.82 ^{cb}	86.58 ^a	51.39 ^c
Group (6) (+control) diet + garlic EX (400mg/k.g b.w)	76.55 ^c	91.18 ^c	38.32 ^b	37.56 ^{cd}	15.31 ^c	89.59 ^a	65.88 ^b
P- value	<0.0001	<0.0001	<0.0002	<.0001	<0.0001	0.80	<0.0001
L.S.D	8.15	13.31	3.92	11.71	1.63	9.8	5.8
	***	***	***	***	***	N.S	***

TG: Total glycerides

HDL-C: High density lipoprotein cholesterol

LDL-C: Low density lipoprotein Cholesterol

VLDL-C: Very low density lipoprotein cholesterol.

SOD: Superoxide dismutase

EX: Extract.

a, b, c and d : Means within the same column with different superscripts are significant. N.S: Non significant.

L.S.D.: Least significant difference.

***: Very high significant at (P<0