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Effect of Rhizomes Group (Galangal and Ginger) in Treatment of Liver Cancer

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Abstract. Galangal (*Alpinia officinarum*), is the rhizome of *Zingiberaceae* family. It grows in south China and south east of Asia. Ginger (*Zingiber officinale*), belong to the rhizomes of *Zingiberaceae* family. It grows in Hawaii Island, Florida, south California and New Mexico. In order to study the therapeutic effects of these rhizomes on liver cancer, aflatoxin B_1 (AFB_1) 0.1 ml/100gm was administered intraperitoneal in male Wister Albino rats for a period of 10 days to induce liver cancer. Galangal and ginger, were given separate as water extract to rats for a period of 20 days. The animals were scarified and blood was tested for some key enzymes such as: Aseparate aminotransferase (AST), alanine aminotransferase (ALT), gamma- glutamyl transferase (GGT), and other non-enzymatic biochemical parameters including bilirubin, urea, uric acid, creatinine, cholesterol, triacylglycerols, glucose and hemoglobin. Part of the liver samples were taken to determine the content of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and total proteins, the other part was used for histological examination. The results of this study demonstrated that galangal extract prevented the proliferation of liver cancer at the initiative stage by inhibiting AFB_1 effects on DNA and regeneration of hepatocyte cells, ginger was also found to be active but less potent.

Introduction

The disease under study is liver cancer, which is considered the most risky disease threaten the health of people worldwide. There are two classes of liver cancer, primary cancer which constitutes small percent and contains hepatic cellular carcinoma and a rare type called angiosarcoma. The secondary cancer is formed in liver by metastases from other organs in the body, such as breast, lung, colon and others. Liver cirrhosis is the (primary) cause of liver cancer. In addition, some chemical substances considered as a cause of liver cancer, for example, chloredinic acid. There is a relation between liver cancers incidence and consumption of some types of food contaminated with *Aspergillus flavous* that secreted some secondary metabolite substance called aflatoxins, which is a toxin causes liver cancer in human and animal [1]. Several compounds have been discovered with inhibitory effects on the tumor promoting stage and interestingly many of them were derived from plants [2]. There are increasing interests in the antioxidant compounds of herbs that retard the oxidative degradation of lipids and play an important role in the prevention of diseases [3].

The *Zingiberaceae* is a well-known herbs family in Southeast Asia and numerous of its species are being used in traditional medicine, which is found to be quite effective in the treatment of several diseases [3].

Galangal (*Alpinia officinarum*), is a species of the *Zingiberaceae* that grows in South-east Asia from India to the Philippines. The rhizomes are used in traditional medicine in skin infection caused by a fungus, indigestion, colic and dysentery [4]. The effects of studies of flavonoids and polyphenoles in *vitro* and *vivo*, are found, which may be related to their antioxidation as water–soluble protectors against lipid peroxidation and other free radical – mediated cell injury [5]. The concept of chemoprevention, which is the use of natural or synthetic compounds to prevent the development of cancers, has get a lot of interest in scientific committee [6]. Galangal is capable of protecting

cells from toxic effects of a variety of hazardous chemicals [7].

Ginger (*Zingiber officinale*), is a species of the *Zingiberaceae* that grows in Hawaii Island, South California and New Mexico. Ginger has along history of medicinal use for condition such as headaches, rheumatism, gastrointestinal diseases, including dyspepsia, nausea and diarrhea, cytoprotective and anti-ulcergenic [8, 9]. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities were found [10, 11, 12]. More potent synthetic is gingerol, a major component of ginger, which is providing a basis for the design analogues with similar potencies to aspirin, as platelet activation inhibitors and antioxidant [13, 14]. Its inhibitory effects on the viability and DNA synthesis of human promyelocyctic leukemia were discovered [15-17].

The aim of this study is to find out the biochemical changes of liver cancer caused by Aflatoxin B_1, on the blood and liver tissues of male Wister Albino Rats. Also, to study effects of two herbs extracts used for treatment of liver cancer as well. Also, to study the histological changes of liver tissue and cells caused by treatment and to record the degree of cancer trauma and prognosis of the treatments.

Materials and Methods

Subject:

In this study, eighty male Wister Albino rats, weighing 70-100 gm were maintained in clean cages. The rats were fed with commercial pelleted diet obtained from King Fahad Medical Research Center in Jeddah. The rats were divided into 4 groups, each group contains 20 rats. All groups except the first group were injected with concentrate 20 µl (0.1ml/ 100 gm) B.W aflatoxin B_1 [18], time of the experiment is 30 days. The first group acted as non tumor bearing control (NTB-C). The second group consisted of rats, which were injected i/p with 20 µl (0.1 ml/100gm) B.W aflatoxin B_1 one time from the first day, and left for 10 days and served as tumor bearing rat control (TBR-C). After 10 days of aflatoxin B_1 injection (i.p), all the following groups from 11^{th} to 30^{th} day were treated by allowing the animals to drink the plant extracts as the following: Third group consisted of tumor bearing rat treated with galangal (TBRga). The fourth group consisted of tumor bearing rat treated with ginger (TBRgi).

Methods

After 10 and 20 days of treatment, the rats were anesthetized with ether and blood was collected from the heart and some enzymatic biochemical parameters were determined including some enzymes such as aspartate aminotransferase (AST) [19], alanine aminotransferase (ALT) [20] and gama glutamyl aminotransferase (GGT) [21] and non-enzymatic biochemical parameters include bilirubin [22], urea [23], uric acid [24], creatinine [25], cholesterol [26], triacylglycerols [27], glucose [28] which were measured by Diminsion (DAD BEHRING Company, Germany). Also, blood samples were used for the determination of hemoglobin [29] measured by Reflotron (Boehringer Company, Germany). The rats were killed by cervical decapitation and livers from each group were removed and divided into 2 parts, first part used for the determination of RNA, DNA [30] and total protein [31]. The second part were put in formalin solution (10 %) and stained by Hematoxyline and Eosine (H & E) to be used for histological examination [32].

Rates in day 10 were treated (for third and fourth groups) by the 2 herbs each one alone. Herbs extract were prepared by heating distilled water (400 ml) to 80°C and soaking 20 gm of herbs for about 60 min, after cooling to room temperature the dose was given orally through special drinking bottles daily [33].

Statistical analysis

Collected data were calculated by T-test and ANOVA using SPSS program version 11. Sigma plot program version 9 were also used.

Results

Table 1 shows some key enzymes from the group of tumor bearing rats (TBR-C) which are compared with the non tumor bearing control (NTB-C). The results show a slight change in the activity of AST, a decrease in the activity of ALT and a significant increase in the activity of GGT.

Table 2 shows non-enzymatic biochemical parameters. There is a highly significant increase in bilirubin levels slight decrease in urea level, increase in uric acid and creatinine levels. There is also a highly significant decrease of cholesterol and triacylglycerol levels. Additionally, an increase in glucose level and a decrease in hemoglobin level are also noticeable.

Table 3 shows a significant decrease in RNA levels, a decrease in DNA and total protein obtained from liver tissue.

Figure 1-a, 1-b and figure 2-a, 2-b, show histological examination of the liver tissue. They

Table 1. Mean value ± SE of some key enzymes (AST, ALT, GGT) in tumor bearing rat treated with rhizomes group (galangal and ginger)

Groups	*Non tumor bearing control*	*Tumor bearing rat control*	*Tumor bearing treated with galangal*		*Tumor bearing treated with ginger*	
Parametes	(NTB-C)	(TBR-C)	($TBRga_{10}$)	($TBRga_{20}$)	($TBRgi_{10}$)	($TBRgi_{20}$)
AST (U / L)	259.9±41.5	242.3±43.7	210.3±100.4	144.7±12	198.7±51.7	205.6±23.4
ALT U / L)(	88.8±20.2	66.5±11.1	75.1±97.8	68.8±6.2	52.3±11.6	117.8±28.9
GGT (U / L)	10±0.76	13.6±0.34*	10.3±1.37*	14±0.26	12.3±0.75*	13.8±0.48

Data are presented as mean ± S. E.; S. E. =Standard error; * Significant P < 0.05; highly significant P < 0.001** ;
*** very highly significant P < 0.000

Table 2. Mean value ± SE of som non- enzymatic biochemical parameters (bilirubin, urea, uric acid, creatinine, cholesterol, triacylglycerol, glucose and hemoglobin in tumor bearing rat treated with rhizomes group (galangal and ginger)

Groups	*Non tumor bearing control*	*Tumor bearing rat control*	*Tumor bearing treated with galangal*		*Tumor bearing treated with ginger*	
Parametes	(NTB-C)	(TBR-C)	($TBRga_{10}$)	($TBRga_{20}$)	($TBRgi_{10}$)	($TBRgi_{20}$)
Bilirubin (mg / dl)	0.17±0.2	1.26±0.15***	0.29±0.22***	0.1±0.22***	0.55±0.28***	0.72±0.37***
Urea (mg /dl)	26.3±1.03	24.9±1.05	26±1.04	20±1.4*	22.8±1.3	21.2±2.3
Uric acid (mg / dl)	2.2±0.3	3.11±0.5	2.7±0.6	2.4±0.99	1.8±0.2*	3.4±0.6
Creatinine (mg / dl)	0.3±0.27	0.4±0.24	0.16±0.33**	0.33±0.33	0.23±0.13*	0.30±0.45
Cholesterol (mg /dl)	115.5±2.9	65.3±2.5***	62±4.5	58.2±3.1	70.7±6.8	73.3±1.7
Triacylglycerol (mg / dl)	149.9±6.2	66.4±2.1***	43.7±4.5	72.6±9.7	36.3±6.9*	101.5±26.8
Glucose (mg / dl)	134.5±4.6	168.5±5.3***	160±5.7	181.3±18.3	174.5±11.4	200.5±23.7*
Hemoglobin (mmol / l)	10.5±0.7	9.4±.41	7.9±0.4	8.6±0.53	8.2±0.62	7.7±0.68*

Data are presented as mean ± S. E.; S. E. =Standard error; * Significant P < 0.05; highly significant P < 0.001 **
*** very highly significant P < 0.000

Table 3. Mean value ± SE for liver content of DNA, RNA and total proteins (gm/100gm) in tumor bearing rat treated with rhizomes group (galangal and ginger)

Groups	*Non tumor bearing control*	*Tumor bearing positive control*	*Tumor bearing rats treated with galangal*	*Tumor bearing rats treated with ginger*
Parameters	(NTB-C)	(TBR-C)	(TBRga)	(TBRgi)
RNA (gm/100gm)	2.06±0.40	1.29±0.52*	2.19±0.91*	2.47±0.22***
DNA (gm/100gm)	0.75±0.34	0.47±0.69	0.63±0.52	0.67±0.95
Totalprotein (gm/100gm)	6.84±0.39	5.54±0.85	9.64±0.62*	8.69±1.77*

Data are presented as mean ± S. E.; S. E. =Standard error; * Significant P < 0.05; *** very highly significant P < 0.000

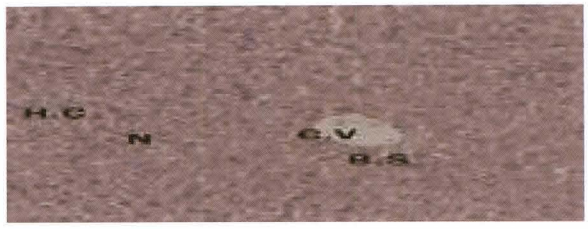

Fig (1-a). Part of liver from control group (NTB-C) showing hepatic cells (H.C) around the central vein (C.V),nucleus (N), and blood sinusoid (B.S). Hematoxyline & Eosine (H&E) (X 400)

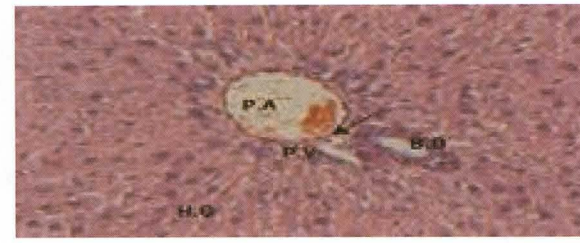

Fig. (1-b). Part of liver from control group (NTB-C) showing portal area (P.A), which is contain portal vein (P.V), bile duct (B.D), inside the endothelial tissue (arrow) and laminal of hepatic cells (H.C). Hematoxyline & Eosine (H&E) (X 400)

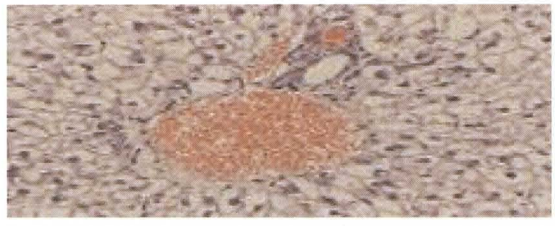

Fig (2-a). Part of liver from tumor group (TBR-C) showing degenerative, necrotic hepatic cells, hemorrhage in the portal area. Hematoxyline & Eosine (H&E) (X 400).

Fig (2-b). Part of liver from tumor group (TBR-C) showing hepatoma focci (arrow). Hematoxyline & Eosine (H&E) (X 400).

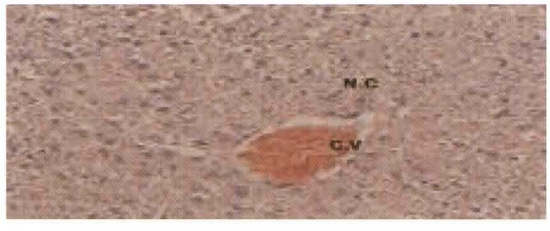

Fig (3-a). Part of liver from tumor group (TBRga) showing hepatomic focci, hypostasis and congestion in the central vein (C.V), necrosis in some liver cells (N.C). Hematoxyline & Eosine (H&E) (X 100).

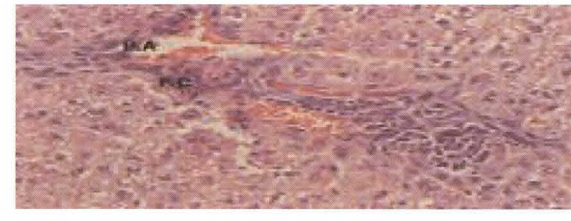

Fig (3-b). Part of liver from tumor group (TBRga) showing dilatation in portal area (P.A), congestion and fibrous cells (F.C). Hematoxyline & Eosine (H&E) (X 400).

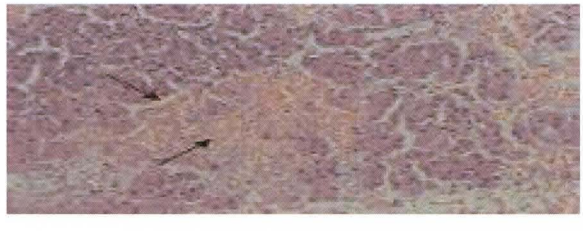

Fig (4-a). Part of liver from tumor group (TBRgi) showing hepatoma focci (arrow) and hemorrhage (head arrow). Hematoxyline & Eosine (H&E) (X 200).

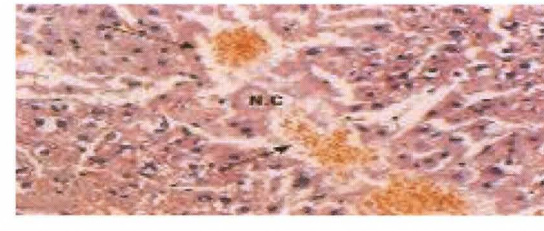

Fig (4-b). Part of liver from tumor group (TBRgi) showing necrosis in some liver cells (N.C) and hemorrhage (arrow). Hematoxyline & Eosine (H&E) (X 200).

indicate that the liver cells were seen without nucleus, degenerative, hepatoma focci as well as a decrease in the number of kupffer cells.

Table 1 shows some key enzymes after treatment with galangal and ginger. For 10 and 20 days ($TBRga_{10}$), ($TBRga_{20}$), ($TBRgi_{10}$) and ($TBRgi_{20}$) respectively. In ($TBRga_{10, 20}$), there are decreases in the activity of AST was seen. In ($TBRga_{10, 20}$), there is an increase in the activity of ALT, but this activity decreases when compared with the activity of (NTB-C). In ($TBRgi_{10}$), there is a decrease in the activity of ALT, but this activity increases in ($TBRgi_{20}$). In ($TBRga_{10}$), we see significant decreases in the activity of GGT, however, this activity increases in ($TBRga_{20}$). In ($TBRgi_{10}$), there is a decrease in the activity of GGT but this activity increases in ($TBRgi_{20}$) when compared to (TBR-C), (NTB-C).

Table 2 shows non-enzymatic biochemical parameters. In ($TBRga_{10, 20}$), there is a very highly significant decrease in the level of bilirubin. In ($TBRgi_{10, 20}$), there are a very highly significant decrease in the level of bilirubin when compared with (TBR-C), but this level increases when compared with (NTB-C). In ($TBRga_{10}$), we detect slight increases in the level of urea in ($TBRga_{20}$), there is a decrease in this level compared to (TBR-C) and (NTB-C). In ($TBRgi_{10, 20}$), a decrease in the level of urea was seen. In ($TBRga_{10, 20}$), there is a decrease in the level of uric acid. In ($TBRgi_{10}$), there is a decrease in the level of uric acid levels, but this level increases in ($TBRgi_{20}$) are detected. In ($TBRga_{10}$), very highly significant decreases in the creatinine level. Additionally this level decreases in ($TBRga_{20}$) when compared with (TBR-C), but slightly increases when weighed against (NTB-C). In ($TBRgi_{10}$), we see very highly significant decreases in the level of creatinine, which slightly changes in ($TBRgi_{20}$) compared with (TBR-C), and this level become similar with (NTB-C). In ($TBRga_{10,20}$), there is a slight decrease in the level of cholesterol. In ($TBRgi_{10, 20}$), an increase in the level of cholesterol is seen when compared with (TBR-C), but decreases when this level is compared with (NTB-C). In ($TBRga_{10}$), there is a decrease in the level of triacylglycerol, but this level increases in ($TBRga_{20}$) when compared with (TBR-C), but decreases when compared with (NTB-C). In both ($TBRgi_{10, 20}$), there are significant decreases in the triacylglycerol level. In ($TBRga_{10}$), there is a decrease in the level of glucose, increases in ($TBRga_{20}$) when compared with (TBR-C) and (NTB-C). In ($TBRgi_{10}$), there is an increase in the level of glucose, and a significantly increase in ($TBRgi_{20}$). In ($TBRga_{10}$), we see decreases in the level of hemoglobin and significant decreases in ($TBRga_{20}$). In ($TBRgi_{10}$), there are decreases in the level of hemoglobin and additional significantly decreases in ($TBRgi_{20}$).

Table 3 show results after treatment with galangal (TBRga). There are significant increases in the level of RNA when evaluated against both (TBR-C) and (NTB-C). After treatment with ginger (TBRgi), very highly significant increases are shown in the level of RNA when compared with (TBR-C), and an increase when compared with (NTB-C). After treatment with galangal (TBRga), there are increases in the level of DNA contrasted with (TBR-C), but decreases were also noticed when compared to (NTB-C). After treatment with ginger (TBRgi), there is a very highly significant increase in the level of DNA when compared with (TBR-C), but decreases when compared to (NTB-C). After treatment with galangal (TBRga), significant increases in the level of total protein are seen when compared with (TBR-C). Aditionally, this level increases contrasted with (NTB-C). After treatment with ginger (TBRgi) significant increases in the level of total protein are shown compared to (TBR-C) as well as an increase compared to (NTB-C) obtained from liver tissue.

Figure 3-a, 3-b, 4-a, 4-b show the histological examination of liver tissue from the group of tumor bearing rats receiving treatment with galangal (TBRga), indicates hepatomic focci, necrosis in some liver cells around the portal area, dilatation in portal area (P.A), congestion and fibrous cells (F.C). In the tumor bearing rats receiving treatment of ginger (TBRgi) show hepatomic focci, hemorrhage and necrotic in some liver cells.

Discussion

When comparing the tumor bearing rat control (TBR-C) with the non tumor bearing control (NTB-C) for some key enzymes and non-enzymatic biochemical parameters, the results showed a significant increase in the activity of GGT, very highly significant increase in bilirubin and glucose, and a very highly significant decrease in cholesterol and triacylglycerol serum level. Observed through liver tissue, a significant decrease in RNA level was seen. Histological examination showed that liver cells were seen without nucleus, degenerative, hepatoma focci and decreased in the number of kupffer cells. The results may be due to the crack up of liver cells in (TBR-C) and degeneration of GGT from the wall of hepatic cells to the blood, which correlate with the histological results.

There were significant increases in the level of

serum bilirubin following the administration of aflatoxin B_1 due to the degeneration of the heme of hemoglobin in red blood cells, which contradicted the results obtained by [34, 35]. The lipid content in the liver and plasma showed a decrease in triacyglycerols, free fatty acids and cholesterol levels. These results agree with [36, 37], which conclude that an increase of lipolysis in fat tissue and the metabolic alterations in the liver proceed catabolic reactions in peripheral tissues.

From the results, a decrease in the lipid content in tumor bearing rat was seen, which is considered one of the indicators of cancer. These results agree with [38] which reported that the turnover rate of glucose was significantly greater in tumor bearing rats compared with non tumor bearing rats since the rate of glucose recycling and the rate of gluconeogenesis which both energy demanding process were clear. Additionally, the decrease in DNA level due to AFB_1 DNA adduct shown a short time after the administration might be due to a decrease in protein synthesis, which agrees with [39] where they declared that significant changed in hepatic protein metabolism but not significantly changed in skeletal muscles were seen.

The tumor bearing rats had altered hepatic protein in a way similar to the results reported in [40] where they found a decrease in the level of total protein in the liver due to AFB_1 DNA adduct which may interrupt the transcription process of RNA causing a decrease in the synthesis of protein. Histology findings showed hepatoma focci, degeneration and necrosis of hepatic cells, cells without nucleus and a decrease in the number of kupffer cells which all agree with [41].

The biochemical parameters in the group of tumor bearing rat after treatment with galangal (TBRga) compared with the tumor bearing rat control (TBR-C) showed significant decreases in the level of urea since galangal suppressed genotoxicity of chemicals and helped kidney function to return to normal which showed a promising candidate for cancer chemoprevention [7]. A very highly significant decrease in the bilirubin level was recorded which might be due to the galangin from galangal, an antioxidant and scavenging free radical that also protect cells from tumor [6]. Significant increases in the level of RNA, DNA and total protein were shown from analysis liver tissues, which could be due to the flavonoid compounds present in galangal that inhibits AFB_1-DNA adduct [6]. Histological examination showed, hepatomic focci, necrosis in some liver cells around the portal area, which may be the effect of AFB_1 that is greater than the effect of galangal or could be due to the low dose of galangal in the treatment. This result opposed with [7] where they elucidated that galangin might protect cells from toxic effects.

Key enzymes and non-enzymatic biochemical parameters studied in the group of tumor bearing rat treated with ginger (TBRgi) and compared with the tumor bearing rat control (TBR-C), showed significant increases in triacylglycerol and glucose levels which might not be due to the effect of ginger on glucose because the tumor cells are insulin resistance [42]. Additionally, significant decreases in the level of hemoglobin, very highly significantly decrease in bilirubin were seen, which could be due to the resistance of phenolic compounds in ginger that have antioxidants that act to inhibit superoxide free radicals that are produced by the tumor necrosis factor secreted by the tumor cells [43,44]. Significant increases in total protein levels and a very highly significant increase in RNA levels were shown in liver tissues samples, which might be due to treatment by ginger, which contains eleven membered cyclic sesquiterpenes, zerumbone, 6-gingerol, 6- paradol that were found to exert inhibitory effects on the viability and DNA synthesis of human promyelocyctic leukemia. In addition, it markedly suppresses free radical generation, pro-inflammatory protein production accompanied by apoptosis [13, 14]. Histological examination showed, hepatomic focci, hemorrhage and necrosis in some liver cells, which connected with the biochemical parameters as a result from treatment. Ginger also contains diterpens which cause necrotic cancer cells [44]. In conclusion, this study demonstrated that galangal gave the best biochemical and histological results compared to ginger.

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