BIOCHEMICAL EFFECT OF COSTUS SPECIOSUS ON EHRLICH ASCITES CARCINOMA (EAC) IN FEMALE ALBINO MICE

Abdel-Maksoud, H.[1]; Elsenosy, Y.A.[1]; El-Far, A. H.[2] and Randa, M. Khallaf [1]
[1]*Biochemistry Department, Faculty of Vet. Med. Moshtohor, Benha University, Egypt.*
[2]*Biochemistry Department, Faculty of Vet. Med. Damanhour University, Egypt.*

A B S T R A C T

Traditional medicine has a long history of serving peoples all over the world. In recent years, The medicinal plants received a considerable interest as it contain several phytochemicals such as vitamins, carotenoids, flavonoids, polyphenols, alkaloids, saponins, etc. These phytochemicals possess antioxidant activities, which, are being used traditionally for the prevention and treatment of many diseases, including cancer. The present study is discussing one of these plants (Costus Speciosus) and its biochemical effect on Serum glucose, serum cholesterol (Chol), carcinoembryonic antigen (CEA) and carbohydrate antigen (CA 19-9) On experimentally induced Ehrlich ascites carcinoma (EAC) in female mice. This study was carried out on 160 female mice which were allocated into four equal groups of 40 mice each. Group I: (Control group): received no drugs .Group II: (Tumor non-treated group): intraperitoneally (i.p) injected in the right thigh with 0.2 ml of Ehrlich ascites adenocarcinoma (2.5×10^6 tumor cells with single cell suspension) .Group III: (*C. speciosus* treated group): treated with *C.speciosus* rhizomes powder mixed with food (5gm / kg. after EAC injection) and Group IV: (*C.speciosus* group): received *C.speciosus* rhizomes powder mixed with food. Ehrlich ascites carcinoma induction induced an increase in CEA and CA19-9 levels which became a decrease after *C.speciosus* and treatment. Also, *C.speciosus* potentially decreased serum glucose and cholesterol level at different periods of inoculation. These results suggest that *C.speciosus* and its constituents may be help in cancer treatment by its antiapoptotic and anticarcinogenic effects, also the results showed hypoglycemic and hypolipidemic effects of *C.speciosus* rhizomes .

Key Words: Anticancer, Medicinal herbs, Costus Speciousus, Diosgenin, Ehrlich ascites carcinoma

(BVMJ-26(1):200-212, 2014)

1. INTRODUCTION

Cancer is a term describing conditions characterized by uncontrolled cellular proliferation and differentiation (Ponder, 2001). Several factors are known to increase the risk of cancer, including smoking, dietary factors, certain infections, exposure to radiation, lack of physical activity, obesity, and environmental pollutants (Anand et al., 2008). Experimental models of cancer have played an important role in cancer drug discovery, as they serve as tools determinants of therapeutic success or failure (Durrett, 2013). Ehrlich ascites carcinoma is one of these models, it is an undifferentiated carcinoma that has high transplantable capability, no regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor-specific transplantation antigen. Ehrlich ascites carcinoma has a resemblance with human tumors which are the most sensitive to chemotherapy due to the fact that it is undifferentiated and that it has a rapid growth rate (Kabel et al., 2013). *C.speciosus* is a tropical Zingiberaceae plant, which is wide spread throughout Southeast Asia. It is considered as an important component in many human and veterinary medicines; *C. speciosus* is

widely used in treating various diseases (Eliza et al., 2009a; Vijayalakshmi and Sarada, 2008). The *C. speciosus* extracts showed significant antioxidant activity, which is partially related to its high polyphenolic content (Vijayalakshmi and Sarada, 2008). Recent studies have indicated that costunolide and eremanthin isolated from *C. speciosus* possess normo-glycemic and hypolipidemic activities in streptozotocin-induced diabetic rats (Eliza *et al.,* 2009b). Dasgupta and Pandey, (1970) reported that, Diosgenin is a steroidal saponin considered the major constituent isolated from *C. speciosus*. Anticancer activity is one of its Pharmacological properties (Raju et al., 2004). Accordingly, the purpose of the present study was to investigate the effect of *C. speciosus* against EAC-induced tumor in female albino mice.

2. MATERIALS AND METHODS

2.1. Experimental animals:

A total number of 160 Australian female albino mice, 12-16 weeks old and average body weight 20 - 25 g were used in the experimental study, and obtained from Research Institute of Ophthalmology, Giza, Cairo. Animals were housed in separate metal cages (2-3 per cage). Fresh and clean drinking water was supplied ad-lib through specific nipple.

2.2. Plant material

C. speciosus rhizomes were washed, cutted, grinded and refined. The ground powder was mixed with the ration by the concentration of (5gm /kg ration).

2.3. EAC Induction:

The experimental induction of tumor in female mice was carried out at the National Cancer Institute Egypt. Every 1 ml of Ehrlich ascites adenocarcinoma was diluted with 4 ml of normal saline. Each mouse was intraperitoneal (i.p) injected in the medial aspect of the right thigh with 0.2 ml of Ehrlich ascites adenocarcinoma (2.5×10^6 tumor cells with single cell suspension (Zeinab, 2009). The tumor developed and become palpable in all injected animals 5-7 days post tumor inoculation (Omayma et al., 2011).

2.4. Experimental design:

Mice were randomly allocated into four main equal groups, 40 animals each, placed in individual cages and classified as follow: Group 1 (control normal group): Comprised from 40 female mice that received no drugs served as control non-treated for all experimental groups and having normal diet daily. Group 2 (Tumor non-treated group): Included 40 female mice, each mouse were intraperitoneal (i.p) injected with EAC, for tumor-induction and received no drugs. Group 3 (*C. speciosus* treated group): Comprised 40 female mice, each mouse were intraperitoneal (i.p) injected with EAC, for tumor-induction. Mice were fed *C. speciosus* rhizomes powder among normal diet daily from the first day of experiment and along its duration. Group 4 (*C. speciosus* group):Included 40 female mice, received *C. speciosus* rhizomes powder among ration from onset of experiment and along its duration.

2.5. Sampling:

Blood samples were collected from all animals groups (control and experimental groups) four times along the duration of experiment after14, 24, 34 and 44 days from the onset of EAC Injection and treatment with *C. speciosus*. All samples were collected in the morning following over-night fasting.

2.6. Preparation of Blood samples and Biochemical analysis:

The animals were anesthetized with ethyl ether before blood sampling. Blood samples were collected by occular vein puncture and sacrification at the end of each experimental period in dry, clean, and screw capped tubes. Serum was separated by centrifugation at 3000 r.p.m for 5 minutes. The clear serum Samples were

proceed directly for glucose determination, and then kept in a deep freeze at -20° C until used for subsequent biochemical analysis. Serum glucose, total cholesterol, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) were analyzed according to the methods described by Tietz, (1995), NCEP expert panel, (1988), Begent and Rustin, (1989) and Minamide et al., (2000) respectively.

2.7. Statistical analysis:

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS, 13.0 software, 2009). Values of $P<0.05$ were considered to be significant.

3. RESULTS

Biochemical effect of *C. speciosus* administration on serum Glucose as a metabolic Biomarker, Serum total cholesterol resembling lipids profile , carcino-embryonic antigen and carbohydrate antigen 19-9 as a tumor marker in normal and EAC induced tumor in female mice were statistically analyzed and represented in the tables (1 & 2).

3.1. Serum glucose

Effect of treatment with *C.speciosus* on serum glucose concentration in normal and EAC induced tumor in female mice were presented in the table (1).

3.1.1. Effect of treatment factor:

A non-significant decrease in serum glucose concentration was observed in EAC induced tumor in female mice after 14 days, In addition , a significant decrease in serum glucose concentration after 34 days. Meanwhile, a non-significant increase in serum glucose concentration was observed after 24 days. This increase became significant after 44 days when compared with normal control group. Treatment with C.speciosus to EAC induced tumor in female mice resulted in a non-significant decrease in serum glucose concentrations all over the periods of the experiment when compared with EAC non-treated group. Administration of *C.speciosus* to normal mice resulted in a non-significantly decreased serum glucose concentration after 14 and 34 days of treatment. Meanwhile, a non-significant increase in serum glucose concentration was observed after 24 and 44 days of treatment when compared with normal control group.

3.1.2. *Time impact:*

G_2: first time $\equiv$ second time $\ll$ fourth time $\gg$ third time.

G_3: first time $\equiv$ second time $\equiv$ fourth time $\gg$ third time.

G_4: first time $\equiv$ second time $\equiv$ third time $\ll$ fourth time.

Note: $(\equiv)$ sign means that there was no significant difference ,where there may be a non-significant decrease or increase between different times , while $(\gg, \ll)$ signs mean there were significant differences according to the direction of the sign.

3.2. Serum total cholesterol:-

Effect of treatment with *C.speciosus* on serum total cholesterol concentration in normal and EAC induced tumor in female mice are presented in the table (1).

3.2.1. Effect of treatment factor:

A non-significant increase in serum total cholesterol concentration was observed in EAC induced tumor in female mice after 14, 24 and 44 days. Meanwhile, a non-significant decrease in serum total cholesterol was observed after 34 days when compared with normal control group. Treatment with *C.speciosus* to EAC induced tumor in female mice

The table (1): Effect of treatment with *C. speciosus* rhizomes on Serum Glucose and Total Cholesterol concentrations in normal and EAC-induced tumor in female mice.

Animal Groups	Glucose (mg/dL) (1St) 14 Days	(2nd) 24 Days	(3rd) 34 Days	(4th) 44 Days	Total Cholesterol (mg/dL) (1St) 14 Days	(2nd) 24 Days	(3rd) 34 Days	(4th) 44 Days
Group I: (Control group)	133.90 ±4.29^{aA}	101.23 ± 5.92^{aB}	113.2±17.56^{aB}	115.1 ±15.1^{aB}	89.73 ± 3.52^{aA}	84.83 ±15.70^{bA}	94.73 ± 8.10^{aA}	74.70 ± 12.59^{aA}
Group Π : (EAC induced group)	105.8±15.63^{abB}	112.17 ±10.45^{aB}	52.23 ± 3.81^{bC}	132.27 ±8.18^{aA}	94.37 ± 1.28^{aA}	90.20 ± 7.98^{bA}	84.33 ± 21.99^{aA}	95.20 ± 3.15^{aA}
Group III: (EAC induced and *C.speciosus* treated group)	95.23 ± 9.32^{bA}	108.83 ± 7.67^{aA}	43.17 ± 1.90^{bB}	112.6±15.87^{aA}	72.50 ± 6.22^{bB}	174.67±15.9^{aA}	62.17 ± 1.14^{aB}	64.23 ± 8.92^{bB}
Group IV: (Control *C.speciosus* treated group)	110.0±11.95^{abB}	105.07 ±10.58^{aB}	111.70±5.19^{a}B	132.00 ±3.00^{aA}	88.37 ± 4.28^{aA}	57.97 ± 4.62^{bB}	74.50 ± 6.86^{aA}	65.63 ± 11.29^{aAB}

The table(2): Effect of treatment with *C.speciosus* rhizomes on carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) concentrations in normal and EAC-induced tumor in female mice.

Animal Groups	CEA (ng/ml)				CA 19-9 (U/ml)			
	(1^{St}) 14 Days	(1^{St}) 14 Days	(1^{St}) 14 Days	(1^{St}) 14 Days	(1^{St}) 14 Days	(2^{nd}) 24 Days	(3^{rd}) 34 Days	(4^{th}) 44 Days
Group I: (Control group)	1.67 ± 0.24 ^{aA}	1.19 ± 0.11 ^{aA}	1.03 ± 0.20 ^{abB}	0.67 ± 0.20 ^{bB}	3.49 ±0.45^{bA}	2.80±0.37^{bA}	4.27±0.48^{bcA}	3.50±0.48^{bA}
Group Π : (EAC induced group)	1.59 ± 0.21^{aA}	1.90 ± 0.60^{aA}	1.20 ±0.27^{aAB}	0.87±0.07^{bB}	9.00±0.79^{aB}	9.1±0.87^{aB}	12.42±2.30^{aA}	10.88±3.30^{aAB}
Group III: (EAC induced and *C.speciosus* treated group)	1.93 ± 0.24^{aA}	0.96 ± 0.23^{aB}	0.86 ±0.27^{abB}	1.64 ±0.43^{aA}	4.73±0.73^{bB}	3.68±0.71^{bB}	8.78±1.15a^{bA}	6.80±1.11a^{bA}
Group IV: (Control *C.speciosus* treated group)	1.19 ± 0.24^{aA}	0.77 ± 0.31^{aAB}	0.39 ± 0.06^{bB}	0.38 ± 0.05^{bB}	2.63±0.19^{cA}	2.88±0.17^{bA}	3.26±0.80^{cA}	3.35±0.46^{bA}

Data presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same row are significantly

significantly decreased serum total cholesterol concentration after 14 days and 44 days of treatment, in addition to non-significant decrease in serum total cholesterol concentration after 34 days was observed. However, after 24 days a significant increase of serum total cholesterol was observed when compared with EAC non-treated group. Administration of *C.speciosus* to normal mice resulted in a non-significant decrease in serum total cholesterol concentrations all over the periods of the experiment when compared with normal control group.

3.2.2. Time impact:

G_2: second time ≡ first time ≡ third time ≡ Fourth time

G_3: second time ≫ first time ≡ third time ≡ Fourth time

G_4: second time ≪ first time ≡ third time ≡ Fourth time

Note: (≡) sign means that there was no significant difference, where there may be a non-significant decrease or increase between different times, while (≫,≪) signs mean there were significant differences according to the direction of the sign.

3.3. Serum carcinoembryonic antigen (CEA):

Effect of treatment with *C.speciosus* on serum CEA concentration in normal and EAC induced tumor in female mice are presented in the table.

3.3.1. Effect of treatment factor:

A non-significant decrease in serum CEA concentration was observed in EAC induced tumor in female mice after 14 days. Meanwhile, a non-significant increase in serum CEA concentration was observed 24, 34 and 44 days when compared with normal control group.

Treatment with *C.speciosus* to EAC induced tumor in female mice non-significantly increased serum CEA concentration after 14 days, in addition to, a significant increase in serum CEA concentration was observed after 44 days. Meanwhile, a non-significant decrease in serum CEA concentration after 24 and 34 days was observed when compared with EAC non-treated group. Administration of *C.speciosus* to normal mice showed a non-significant decrease in serum CEA concentrations all over the periods of the experiment when compared with normal control group.

3.3.2. Time impact:

- G_2: first time ≡ second time ≫ forth time ≡ third time
- G_3: first time ≡ fourth time ≫ second time ≡ third time.
- G_4: first time ≡ second time ≫ *or* ≡ third time ≡ fourth time.
- Note: (≡) sign means that there was no significant difference, where there may be a non-significant decrease or increase between different times, while (≫,≪) signs mean there were significant differences according to the direction of the sign.

3.4. Serum carbohydrate antigen 19-9 (CA 19-9):

Effect of treatment with *C.speciosus* on serum CA 19-9 concentration in normal and EAC induced tumor in female mice are presented in the Table.

3.4.1. Effect of treatment factor:

A significant increase in serum CA 19-9 concentration was observed in EAC induced tumor in female mice all over the periods of the experiment compared with normal control group. Treatment with *C.speciosus* to EAC induced tumor in female mice significantly decreased serum CA 19-9 concentration after 14 and 24 days of treatment, in addition to, a non-significant decrease in serum CA 19-9 concentration was observed after 34 and 44 days when compared with EAC non-treated group. Administration of *C.speciosus* to normal mice resulted in no change in serum CA 19-9 concentration after 24 days of treatment. Meanwhile, a significant decrease in serum CA 19-9 concentration was observed after 14, this decrease became non significant after 34

and 44 days of treatment when compared with normal control group.

3.4.2. Time impact:

G_2: first time ≡ second time ≪ third time ≡ fourth time.

G_3: first time ≡ second time ≪ third time ≡ fourth time.

G_4: first time ≡ second time ≡ third time ≡ fourth time.

. Note: (≡) sign means that there was no significant difference ,where there may be a non-significant decrease or increase between different times , while (≫,≪) signs mean there were significant differences according to the direction of the sign.

4. DISCUSSION

Cancer is an unnatural cell growth, where they can loss their natural function and spread through of the blood, at all the body(Maxmen, 2012). Ehrlich ascites carcinoma (EAC) is one of the experimental breast tumor derived from spontaneous mouse adenocarcinoma. Similar to other tumors developing in body cavities, (Ulakoglu and Altun, 2004). The obtained data in table (1) revealed that, a non-significant decrease in serum glucose concentration in EAC induced tumor in female mice after 14 days, in addition , a significant decrease in serum glucose concentration after 34 days. Meanwhile, a non-significant increase in serum glucose concentration was observed after 24 and 44 days when compared with normal control group. These results are nearly similar to those recorded by Omayma *et al.,* (2011) who reported that, serum glucose concentration significantly decreased in EAC- induced tumor in female mice. Also, Hussein and Boshra, (2013) and Ali et al., (2014) demonstrated that, subcutaneous implantation of Ehrlich tumor cells resulted in a significant decrease in plasma glucose compared to the normal group mice. Carbohydrate metabolism plays a central task in cancerous condition and it is one of the most common and profound in malignant tissues. The high glycolysis rate is important for rapid proliferating cancers (Eigenbrodt and Glossmann, 1980). Tumors related to the occurrence of hypoglycemia can, as a general rule, be divided into three groups. First, tumors can produce excess insulin such as pancreatic insulinomas or ectopic insulin-producing tumors. Second, hypoglycemia can be caused by tumor-related factors such as destruction of the liver and adrenal glands by massive tumor infiltration. Finally, hypoglycemia rarely can be induced by the production of substances interfering with glucose metabolism including insulin receptor antibodies (Marks and Teale, 1998). Glucose utilization is also inversely correlated with treatment response in a number of tumors, while changes in tumor glucose utilization during the first weeks of chemotherapy are significantly correlated with patient outcome (Padma et al., (2003). Therefore, glucose utilization appears to be a useful metabolic biomarker for diagnosis, prognosis and prediction of tumor response to a variety of therapies (Weber, 2006). In present study decrease in serum glucose in EAC-induced tumor may be due to malignant cells transport glucose at a much faster rate than normal cells. It has been suggested that increased glucose transport in malignant cells is associated with increased and deregulated expression of glucose transporter proteins, with the overexpression of GLUT1 and/or GLUT3 (Macheda et al., 2005). The phenomenon of carcinogen-induced increased glucose uptake might be similar to insulin response, which stimulates glucose uptake by inducing the translocation of the glucose transporter from intracellular storage sites to the plasma membrane, where the transporter facilitates the diffusion of glucose (Ray, 2012). Also, decrease of serum glucose may be due to general changes in energy metabolism associated with tumor growth (Hussein and Azab, 1997). On the other hand, Burt et al., (1981) reported that, the glucose turnover

rate was significantly greater in tumor-bearing rats compared to non-tumor-bearing controls rats, as was the rate of glucose recycling and the rate of gluconeogenesis both energy demanding process. The obtained data in table (1) revealed that, treatment with *C.speciosus* to EAC induced tumor in female mice resulted in a non-significant decrease in serum glucose concentrations all over the periods of the experiment when compared with EAC non-treated group. These results are nearly similar to those reported by Bavarva and Narasimhacharya, (2008) and Rajesh *et al.,* (2009) who reported that, aqueous extract and methanolic extracts of *C. speciosus* were highly effective in bringing down the blood glucose level. Also, El-Far and Abou-Ghanema, (2013) suggested that, a significant decrease of serum glucose concentration after 30 days supplementation of *C. speciosus* in the buffaloes ration. In present study, serum glucose concentration decrease after supplementation of *C. speciosus*, this finding might be attributed to both the increase in insulin units released by the beta cells of islet of Langerhans and the increase in sensitivity of cell receptors to insulin consequently increased glucose utilization or increases one of them. The hypoglycemic action of eremanthin, a component of *C. speciosus* was caused by potentiation of insulin release from the existing beta cells of islets of Langerhans and increased the sensitivity of insulin to uptake glucose (Li et al., 2004). Generally, blood glucose levels were decreased by *C. speciosus* supplementation due to the increase in glycogenesis and glycolysis and the reduction in gluconeogenesis (Bavarva and Narasimhacharya, 2008). Costunolide isolated from *C. speciosus* was found to possess normo-glycemic and hypolipidemic effect in streptozotocin-induced diabetic rats (Eliza et al., 2009a). Diosgenin significantly decreased plasma glucose in streptozotocin-induced diabetic rats by comparison to the diabetic controls suggesting its anti-diabetic properties. These results Proved that the diabetic state were normalized by treatment with diosgenin (McAnuff et al., 2005). In present study a non significant increase in serum total cholesterol concentration was observed in EAC-induced tumor in female mice after 14,24 and 44 days. Meanwhile, a non-significant decrease in serum total cholesterol was observed after 34 days of the experiment. Ehrlich carcinoma had been characterized by increase cellular content of triglycerides and cholesterol esters (Ozaslan et al., 2011). The chemopreventive activity of statins against cancer is suggested to depend on inhibition of cholesterol synthesis and, thereby, cell growth (Takahashi and Nishibori, 2007). The pronounced increase in serum cholesterol levels in tumor mice is in agreement with results reported previously by Segura et al., (2001) who demonstrated that, Following implantation of Ehrlich tumor cells, morphological and metabolic changes occur such as structural deterioration, decreased number of mitochondria, decreased DNA and RNA synthesis, loss of intracellular purine and pyrimidine nucleotides, nucleosides and bases, a decline of ATP concentration and turnover, decreased protein synthesis, decreased glutathione concentration and increased triglycerides, cholesterol esters and free fatty acids. Also, Habib et al., (2010) reported that, the serum cholesterol concentration significantly increased in EAC- induced tumor in female mice when compared to normal group. Abnormal lipid metabolism, leading to increased lipid synthesis, is found to play an important role in the pathogenesis of malignancies (Tania et al., 2010). The increased lipogenesis in cancer is reflected in over expression and hyperactivity of lipogenic enzymes such as ATP citrate lyase (ACL), acetyl-CoA carboxylase (ACC), or the fatty acid synthase (FAS) (Kuhajda, 2000). The obtained data showed that, after 34 days of the experiment there is a non-significant decrease of cholesterol value, This alteration is due to metabolic

disturbance of tumor cells as confirmed by the finding of Lanza-Jacoby et al., (1984) and Obeid and Emary, (1993) who reported that, the level of total cholesterol concentration tended to decrease during the later stages of tumor growth. Also, Ali et al., (2014) observed that, a significant decrease in plasma cholesterol (TC), after subcutaneous implantation of Ehrlich tumor cells into female mice. In present study results revealed that, treatment with *C. speciosus* rhizomes to EAC induced tumor in female mice significantly decreased serum total cholesterol concentration after 14 and 44 days of treatment, in addition to non-significant decrease in serum total cholesterol concentration after 34 days was observed. However, after 24 days a significant increase of serum total cholesterol was observed when compared with EAC non-treated group. Also, administration of *C. speciosus* to normal mice resulted in a non-significant decrease in serum total cholesterol concentrations all over the periods of the experiment when compared with normal control group, these results are nearly similar to those reported by El-Far and Abou-Ghanema, (2013) who observed that, a significant decrease in serum total cholesterol concentration after supplementation of *C. speciosus* in the buffaloes ration. *C. speciosus* affects the lipid metabolism by a significant decrease in serum total cholesterol. This finding came in accordance with that stated and the hexane extract of the rhizome possesses a hypolipidemic activity (Daisy et al., 2008). Moreover, costunolide isolated from the plant significantly decreases serum total cholesterol (Eliza et al., 2009b). In addition, the ethanolic extract of *C. speciosus* of administration reduced plasma and hepatic total cholesterol concentration in diabetic rats (Bavarva and Narasimhacharya, 2008). Moreover, some studies reported that diosgenin, a component of *C. speciosus*, suppressed cholesterol absorption and increased cholesterol secretion through biliary excretion (Accatino et al., 1998; Kamisako and Ogawa, 2003). Diosgenin possesses hypolipidemic effects on the model of high-cholesterol fed rats (Son et al., 2007). It has stronger preventive and therapeutic activities than the total saponin of dioscorea panthaica in the hypercholesterolemia induced by cholesterol in mice or rats (Ma et al., 2002). In the present study, a non significant decrease in serum CEA concentration was observed in EAC induced tumor in female mice after 14 days. Meanwhile, a non-significant increase in serum CEA concentration was observed after 24, 34 and 44 days. Moreover, a significant increase in serum CA 19-9 concentration was observed in EAC induced tumor in female mice all over the periods of the experiment compared with normal control group.

These results are nearly similar to those reported by (Perkins et al., 2003) who reported that, an increase in carcinoembryonic antigen (CEA) level is associated with adenocarcinoma, especially colorectal cancer. In present study, treatment with *C. speciosus* to EAC induced tumor in female mice, non-significantly increased serum CEA concentration after 14 days. In addition , a significant increase in serum CEA concentration was observed after 44 days. Meanwhile, a non-significant decrease in serum CEA concentration after 24 and 34 days was observed when compared with EAC non-treated group. At the same level treatment with *C. speciosus* to EAC induced tumor in female mice, significantly decreased serum CA 19-9 concentration after 14 and 24 days of treatment, in addition to, a non-significant decrease in serum CA 19-9 concentration was observed after 34 and 44 days when compared with EAC non-treated group. In present study, administration of *C. speciosus* to normal mice showed a decrease in serum CEA and CA 19-9 concentrations all over the periods of the experiment when compared with normal

control group. These decreases might be due to the chemo protective effect of *C. speciosus* and its main constituent (diosgenin). Diosgenin has played a significant role as chemo preventive and therapeutic agent against some cancers by over-expressing HER2 gene (Raju and Mehta, 2009). By growth inhibition and induction of apoptosis, diosgenin is an inhibitor of human colon carcinoma cells (Raju and Bird, 2007).

Diosgenin has anticancer activity, where diosgenin increase activation of p53 that leads to activation of its target genes as (BAX and NOXA), release of apoptosis-inducing factor, suppresses proliferation and induce apoptosis in cells of human colon carcinoma (Wang et al., 2004), osteosarcoma (Corbiere et al., 2003), leukemia (Liu et al., 2005) and human erythroleukemia (Legar et al., 2004). The anti-proliferative effects of diosgenin are mediated through cell cycle arrest, disruption of Ca^{+2} homeostasis (Liu et al., 2005), the activation of p53, release of apoptosis-inducing factor, generation of reactive oxygen species (ROS), and modulation of caspase-3 activity (Corbiere et al., 2004).

Conclusion

The present study demonstrated that, *C. speciosus* rhizomes as a dietary supplementation provided an effective treatment against cancer and has a potent chemo-preventative activity against a wide variety of tumors, since costus speciosus rhizomes was able to ameliorate serum biochemical parameters and prevent cell apoptosis.

5. REFERENCES

Accatino, L., Pizarro, M., Solis, N., Koenig, C. S. 1998. Effects of diosgenin, a plant-derived steroid, on bile secretion and hepatocellular cholestasis induced by estrogens in the rat. Hepatology 28: 129–140.

Ali, H.A.; Hussein, M.A. Walaa, E. M, 2014. Comparative Effects of Purslane seed oil (PSO) and 5-Flourourasil on Ehrlich ascites carcinoma (EAC) in female albino mice. International Journal of Pharma Sciences . 4 (1): 424-430.

Anand, P., Kunnumakkara, A.B., Kunnumakara, A.B., Sundaram, C.,Harikumar, K.B.

, Tharakan , S.T., Lai, O.S., Sung, B., Aggarwal, B.B. 2008. "Cancer is a preventable disease that requires major lifestyle changes". Pharm. Res. 25 (9): 2097-116. PMC 2515569. PMID 18626751.

Bavarva, J.H., Narasimhacharya, A.V. 2008. Antihyperglycemic and Hypolipidemic Effects of Costus speciosus in Alloxan induced Diabetic Rats. Phytother. Res. 22: 620–626.

Begent, R., Rustin, G. J. S. 1989. Tumor markers: from carcinoembryonic antigen to products of hybridoma technology, Cancer Surv; 8(1): 107-121.

Burt, M.E., Lowry, S.F., Gorschboth, C., Brennan, M.F. 1981. Metabolic alterations in a noncachectic animal tumor system. Cancer 47: 2138-2146.

Corbiere, C., Liagre, B., Bianchi, A., Bordji, K., Dauca, M., Netter, P., Beneytout, J. L. 2003. Different contribution of apoptosis to the antiproliferative effects of diosgenin and other plant steroids, hecogenin and tigogenin, on human 1547 osteosarcoma cells. Int J Oncol 22 (4): 899-905.

Corbiere, C., Liagre, B., Terro, F., Beneytout, J.L. 2004. Induction of antiproliferative effect by diosgenin through activation of p53, release of apoptosis-inducing factor (AIF) and modulation of caspase-3 activity in different human cancer cells. Cell Res 14: 188-196.

Daisy, P., Eliza, J., Ignacimuthu, S. 2008. Influence of Costus speciosus (Koen.) Sm. Rhizome extracts on biochemical parameters in Streptozotocin induced diabetic rats. J. Health Sci. 54: 675-681.

Dasgupta, B., Pandey, V.B. 1970. A new Indian source of diosgenin (Costus speciosus). Experientia, 26(5): 475-6.

Durrett, R. 2013. Cancer Modeling: A Personal Perspective. Notices of the AMS; 60 (3): 304-309.

Eigenbrodt, E., Glossmann, H. 1980. Glycolysis one of the keys to cancer. Trends Pharmacol Sci.1: 240-245.

El-Far, A. H., Abou-Ghanema, I. I. 2013. Biochemical and hematological evaluation of Costus speciosus as a dietary supplement to Egyptian buffaloes. African Journal of Pharmacy and Pharmacology. 7(42), pp. 2774-2779.

Eliza, J., Daisy, P., Ignacimuthu, S., Duraipandiyan, V. 2009a. Normo-glycemic and hypolipidemic effect of costunolide isolated from Costus speciosus (Koen.) Sm. in streptozotocin induced diabetic rats. Chem. Biol. Interact. 179: 329–334.

Eliza, J., Daisy, P., Ignacimuthu, S., Duraipandiyan, V. 2009b. Antidiabetic and antilipidemic effect of eremanthin from Costus speciosus (Koen.) Sm., in STZ-induced diabetic rats. Chemico Biol. Interact. 182(1): 67-72.

Habib, M.R., Abdul-Aziz, M., Karim, M.R. 2010. Inhibition of Ehrlich's ascites carcinoma by ethyl acetate extract from the flower of Calotropis gigantea L. in mice. J Appl Biomed 8: 47–54.

Hussein, M.A., Boshra, S.A. 2013. Atitumor and structure antioxidant activity relationship of colchicine on Ehrlich ascites carcinoma (EAC) in female mice. International Journal of Drug Delivery 5: 430-437.

Hussein, S.A., Azab, M.E. 1997. Effect of insulin treatment on some metabolic changes on experimentally induced tumor in female mice. Egyptian J. Biochemistry 15: 61-80.

Kabel, A.M., Abdel-Rahman, M.N., El-Sisi, A, Haleem, M.S., Ezzat, N.M., El Rashidy, M.A. 2013. Effect of atorvastatin and methotrexate on solid Ehrlich tumor. Eur J Pharmacol; 713(13): 47-53.

Kamisako, T., Ogawa, H. 2003. Regulation of Biliary cholesterol secretion is associated with abcg5 and abcg8 expressions in the rats: effects of diosgenin and ethinyl estradiol. Hepatol. Res. 26: 348–352.

Kuhajda, F.P. 2000. Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. Nutrition.16: 202 – 208.

Lanza-Jacoby, S., Lansey, S.C., Miller, E.E., Cleary, M.P. 1984. Sequential changes in the activities of lipoprotein lipase and lipogenic enzymes during tumor growth in rats. Cancer Res. 44: 5062-5067.

Legar, D.Y., Liagre, B., Corbiere, C., Cook-Moreau, J., Beneytout, J.L. 2004. Diosgenin induces cell cycle arrest and apoptosis in HEL cells with increase in intracellular calcium level, activation of cPLA2 and COX-2 overexpression. Int J Oncol 25: 555-562.

Li, W.L., Zheng, H.C., Bukuru, J., De Kimpe, N. 2004. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J. Ethnopharmacol. 92:1–21.

Liu, G.J., Luo, N., Chen, J.Y., Cao, W., Luo, T. 2005. Study on several enzyme methods of extracting diosgenin.Zhengzhou J, Univ (Eng Sci). 26: 48-50.

Ma, H.Y., Zhao, Z.T., Wang, L.J., Wang, Y., Zhou, Q.L., Wang, B.X. 2002. (Comparative study on antihyper-cholesterolemia activity of diosgenin and total saponin of dioscorea panthaica. Zhongguo Zhong Yao Za Zhi 27: 528-531.

Macheda, M.L., Suzanne, R., Best, J.D. 2005. Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. J Cell Physiol 202: 654–662.

Marks, V., Teale, J.D. 1998. Tumours producing hypoglycaemia. Endocrine-Related Cancer 5 111–129.

Maxmen, A. 2012. The Hard Facts. Nature,485, S50-S51.

McAnuff, M.A., Omoruyi, F.O. Morrison, E.Y., Asemota, H.N. 2005. Changes in some liver enzymes in streptozotocin-induced diabetic rats fed sapogenin extract from bitter yam (Dioscorea polygonoides) or commercial diosgenin. West Indian Med J. 54(2): 97-101.

Minamide, M., Hosoi, I., Yanagi, S. 2000. CA 19-9-producing testicular tumor: a case report, Hinyokika Kiyo, 46(1): 45-47.

NCEP expert panel, 1988. (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults .circulation, 148:36-69.

Obeid, O.A., Emery, P.W. 1993. Lipid metabolism in cachectie tumoer-bearing rats at different stages of tumor growth. Nutr Cancer. 19: 87-98.

Omayma, A.R., Abou Zaid, Hassanein, M.R.R., EL-Senosi, Y.A., EL-Shiekha, M.F. 2011. Biochemical effect of some antioxidant on metabolic changes in experimentally induced tumor in female mice. Benha Veterinary Medical Journal, *Special ISSUE* [I]: 52—60.

Ozaslan, M., Karagoz, I.D., Kilic, I.H., Guldur, M.E. 2011. Ehrlich ascites carcinoma. Afr J Biotechnol. 10(13): 2375-2378.

Padma, M.V., Said, S., Jacobs, M., Hwang, D.R., Dunigan, K., Satter, M., Christian, B., Ruppert, J., Bernstein, T., Kraus, G., Mantil, J.C. 2003. Prediction of pathology and survival by FDG PET in gliomas. J.Neurooncol. 64(3): 227-237.

Perkins, G.L., Slater, E.D., Sanders, G.K., Prichard, J.G. 2003. Serum tumor markers. Am Fam Physician. 68(6):1075-1082.

Ponder, B.A.J. 2001. Cancer genetics. Nature, 411: 336-341.

Rajesh, M.S., Harish, M.S., Sathyaprakash, R. J., Shetty, A. R., Shivananda, T. N. 2009. Antihyperglycemic activity of the various extracts of Costus speciosus rhizomes. Journal of Natural Remedies, 9(2): 235-241.

Raju, J., Bird, R.P. 2007. Diosgenin, a naturally occurring steroid [corrected] saponin suppresses 3-hydroxy-3-methylglutaryl coa reductase expression and induces apoptosis in Hct-116 human colon carcinoma cells. Cancer Lett. 255: 194–204.

Raju, J., Mehta, R. 2009. Cancer chemopreventive and therapeutic effects of diosgenin, a food saponin. Nutr. Cancer 61: 27–35.

Raju, J., Patlolla, J.M., Swamy, M.V., Rao, C.V. 2004. Diosgenin, a Steroid Saponin of Trigonella foenum graecum (Fenugreek), Inhibits Azoxymethane-Induced Aberrant Crypt Foci Formation in F344 Rats and Induces Apoptosis in HT-29 Human Colon Cancer Cells. Cancer Epidemiol Biomarkers. Prev 13: 1392-1398.

Ray, S. 2012. Cancer Hypothesis. Carcinogen-induced enhanced glucose and nutrient uptake resulting increased glycolysis and oxidative phosphorylation leads to malignant transformation. Cancer Therapy (8), 159-170.

Segura, J.A., Ruiz-Bellido, M.A., Arenas, M., Lobo, C., Marquez, J., Alonso, F.J. 2001. Ehrlich Ascites Tumor Cells Expressing Anti-Sense Glutaminase RNA Lose Their Capacity to Evade the Mouse Immune System. Int J Cancer 2001. 91: 379-384.

Son, I.S., Kim, J.H., Sohn, H.Y., Son, K.H., Kim, J.S., Kwon, C.S. 2007. Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (dioscorea spp.), on high cholesterol fed rats. Biosci. Biotechnol. Biochem.71: 3063- 3071.

Takahashi, H.K., Nishibori, M. 2007. The antitumor activities of statins. Curr.Oncol. 14 (6): 246-247.

Tania, M., Khan, M.A., Song, Y. 2010. Association of lipid metabolism with ovarian cancer. Curr.Oncol.17: 6–11.

Tietz, N.W. ed. 1995. Clinical guide to laboratory tests. 3rd ed. Philadeiphia.WB Saunders; 268-273.

Ulakoglu, G., Altun, S. 2004. The effects of epirubicin on proliferation and DNA

synthesis of Ehrlich ascites carcinoma cells in vitro and in vivo. Biologia, Bratislava, 59/6: 727-734.

Vijayalakshmi, M.A., Sarada, N.C. 2008. Screening of Costus speciosus extracts for antioxidant activities. Fitoterapia. 79: 197-198.

Wang, Z., Fukuda, S. Pelus, L.M. 2004. Survivin regulates the p53 tumor suppressor gene family. Oncogene 23: 8146-8153.

Weber, W.A. 2006. Positron emission tomography as an imaging 123. biomarker. J.Clin.Oncol. 20: 3282-92.

Zeinab E. Hanafy. 2009. Ginger extract antimutagens as cancer chemopreventive agent against Ehrlich Ascites Carcinoma. Academic Journal of Cancer research 2: 61-67.

التأثير الكيميائي الحيوي للقسط البحرى على السرطان المحدث تجريبيا في إناث الجرذان

حسين عبد المقصود على[1]، ياقوت عبد الفتاح السنوسى[1]، علي حافظ على الفار[2]، رنده محمد سعيدخلاف[1]

[1]قسم الكيمياء الحيوية-كلية الطب البيطري بمشتهر-جامعة بنها. [2]قسم الكيمياء الحيوية-كلية الطب البيطري - جامعة دمنهور

الملخص العربي

في هذه الدراسة تم تقييم فاعلية القسط البحرى كنبات طبيعى فى تأثيره على الخلايا السرطانية وبعض المعدلات الأيضية المرتبطة بوجود السرطان. هذا وقد أستخدم لأجراء هذه الدراسة عدد160 من اناث الجرذان الاسترالية أعمارهم تتراوح من 12-14 أسبوع و أوزانها من 20- 25 جرام وقد قسمت إلي أربع مجموعات متساوية اشتملت كل مجموعة على عدد 40 من اناث الفئران وتم توزيعها كالآتي: المجموعة الأولى: (المجموعة الضابطة): اشتملت على 40 من اناث الفئران لم تعطى أي أدوية واستخدمت كمجموعة ضابطة للمجموعات الأخرى. المجموعة الثانية: (المجموعة المحدث بها السرطان تجريبيا (ايرليش استسقاء السرطان): تكونت من 40 من اناث الفئران تم حقنهم فى الغشاء البريتونى ب 2.5 × 10^6خلية سرطانية ومنذ يوم الحقن تم تغذيتها بالعليقة الاعتيادية . المجموعة الثالثة: اشتملت على 40 من اناث الفئران أيضا تمت معالجتها ببودرة القسط البحرى من خلال التغذية 5 جم /1 كجم. بعد احداث السرطان بها عن طريق الحقن في الغشاء البريتونى ب 2.5 × 10^6 خلية سرطانية ومنذ يوم الحقن تم تغذيتها بالعليقة المصحوبة بالعلاج . المجموعة الرابعة: اشتملت على 40 من اناث الفئران (الضابطة الايجابية) تتكون من 40 من اناث الفئران تم اعطائها القسط البحرى من خلال التغذية بخلط بودرة القسط البحرى بالعليقة 5 جم / 1 كجم. وقد تم تجميع عينات الدم (عينات صباحية بعد صيام طوال الليل) بعد تخدير الحيوان بالايثير الايثيلى في اليوم الرابع عشر من بداية التجربة تلاها السحبة الثانية اليوم الرابع والعشرين ثم الرابع والثلاثين وأخيرا الرابع والأربعين. وقد أسفرت نتائج التحاليل البيوكيميائية عن وجود انخفاض معنوى فى المستضد السرطاني الجنيني وكذلك المستضد الكربوهيدراتى 9-19 فى معظم فترات التجربة بعد المعالجة بالقسط البحرى بعد ان كانت هناك زيادة معنوية لمعدلات هذه البروتينات بعد الاصابة بالسرطان بالاضافة إلى انخفاض غير معنوى فى معدلات سكر الدم على مدار التجربة وذلك عند مقارنتها بالمجموعة المسرطنة فقط وأيضا كان هناك نقص معنوى فى تركيز الكوليستيرول فى الدم بعد اليوم ال 14 وآخر غير معنوى بعد اليوم 34 واليوم ال44 مع حدوث زيادة فى معدلات الكولستيرول ايضا بعد اليوم ال 24 للتجربة وهذا فى المجموعة الثالثة بعد العلاج والاصابى التجريبية بالسرطان وهذا بمقارنتها بالمجموعة المسرطنة فقط . وقد أوضحت الدراسة أن العلاج بالقسط البحري من خلال التغذية قد يكون له تأثيره كمادة مضادة للسرطان وكذلك مادة مضادة لموت الخلايا المبرمج وقد يكون لها دور فعال في التأثير على بعد المعدلات الأيضية المصاحبة للسرطان حيث كان للعلاج تأثيره كنقص الجلوكوز وكذلك الكوليستيرول الكلى مقارنة بمجاميع الكنترول والتى ورد ذكرها فى ابحاث اخرى سابقة.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(2):200- 212, يونيو 2014)