Metabolic effects of Lactuca sativa extract on experimentally induced Ehrlich ascites carcinoma

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A B S T R A C T

The present study aimed to determine the effect of Lactuca sativa (lettuce) extract on alleviating Ehrlich ascites carcinoma (EAC) in female mice. EAC was induced by injection of 0.2 ml of (2.5×10^6 tumor cells with single cells suspension) in the medial aspect of the right thigh of female albino mice, a mixture of L. sativa extract powder was supplemented at doses of (100 and 200 gm/10 kg normal ration), respectively, from the first day of experiment for 21 days. The obtained results indicated that feeding on Lettuce extract powder significant increased serum glucose, albumin and high density lipoprotein cholesterol (HDL-c) towards the normal levels. meanwhile, significant decreases triacylglycerols (Tg), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c) concentrations, as well as the activity of liver enzymes involving alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP); in addition to a significant decreasing of the tumor size were observed in EAC- L, sativa extract powder supplemented mice when compared to EAC-cells bearing mice. From the obtained results it could be concluded that lettuce extract is a natural, cheap, less toxic vegetable with great potential therapeutic effect against Ehrlich ascites carcinoma and other complications resulted from malignant tumors.

Keywords: *Ehrlich ascites carcinoma, lettuce, L. sativa, glucose, lipid profile, liver function tests.*

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1. INTRODUCTION

Cancers are a group of diseases, involving uncontrollable cell division and unregulated cell growth. There are over 200 different known cancers that affect humans (Cancer Research U.K, 2012). Cancers are non-communicable diseases and continue to represent the second largest causes of mortality in the world. The prevention and control of cancers in developed and developing countries deserve urgent attention (Islam et al., 2014). Where it was responsible for 8.8 million deaths in 2015 and it was expected to increase to 11.5 million in 2030 globally, nearly 1 in 6 deaths is due to cancer.

Cancers may be caused by combined genetic and non-genetic changes induced by environmental factors that cause inappropriate activation or inactivation of specific genes leading to neoplastic transformations, or

abnormal cell growth (Jemal et al., 2006 and Parsa, 2012).

Ehrlich ascites carcinoma (EAC) is undifferentiated carcinoma, having a specific tumour transplantation antigen, it has high transplantable capacity, no regression, rapid proliferation, shorter life span and 100% malignancy; in addition, EAC has resemblance with human tumours that are the most sensitive to chemotherapy (Kabel et al., 2013).

Experimental models of cancer have played an important role in the cancer drug discovery, in addition, they serve as tools for the elucidation of the molecular basis of neoplastic transformation (Durret, 2013).

Herbal medicines have been proven to be important sources of novel agents with a pharmaceutical potential, nowadays, many anticancer drugs used are either natural or were derived from natural fruits and vegetables which are rich in medical biological functional compounds (involving flavenoides, carotenoids and catechins) that acted as sources of different kinds of antioxidants, free radical scavenging agents and metal ion chelating components (Anilakumar et al., 2017).

Lactuca sativa plant belongs to Asteraceae family. The main components of its extract are 15-oxalyl and 8-sulfate conjugates of guaianolidesesquiterpene lactones, lactucin, deoxylactucin, and lactucopicrin; as well as, triterpenoids, saponins and simple phenols;with large amounts of phytochemicals such as flavonoids and polyphenols. So, using of lettuce as dietary supplement should be exploited (Harsha and Anilakumar, 2013 and Alireza et al., 2014).

This study aimed to determine the metabolic and hepatoprotective activities of Lettuce extract as one of the natural supplements against Ehrlich ascites carcinoma.

2. Materials and methods

Experimental Animals:

A total number of 40 Australian experimental female Albino mice obtained from Research Institute of Ophthalmology (where the experiment was held), Giza, Cairo, 12-14 weeks old and weighed 20-25 gm., mice were housed in separated metal cages, allowed free access of fresh and clean drinking water and were kept at constant environmental and nutritional condition for 15 days for acclimatization before the beginning of the experiment.

Ration and additives:

The animals were fed on a constant ration through the course of the experiment in the form of concentrated diet (Allen et al., 1995).

Carbohydrate 59.52%	Minerals 1.49%
Protein 18%	Calcium 0.9%
Lipid 4.4%	Phosphorus 0.59%
Cellulose 3.1 %	Moisture 12 %

Preparation of the extract *powder of L. sativa as:*

1) L. sativa Extract (Leaves, stems and rhizomes) was obtained from a lettuce farm, kafr –elsheikh governorate, Biala city, then it was washed, cut, grinded, and refined then hit in the blender adding one cup of water.

2) Evaporating the liquid in a water bath for gaining the powder (a mixture of lettuce and its milky juice).

Tumor Induction:

The experimental induction of tumor in female mice was carried out at the National Cancer Institute, Egypt. Each 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 cells suspension) **(**Bhattacharyya et al., 2003).

Experimental design:

Ten Australian female albino mice were injected with normal saline only served as normal non tumor mice. While, the other 30 mice were injected with 0.2 ml of adenocarcinoma (2.5×10^6 tumor cells with single cell suspension) and randomly allocated into individual cages, 10 mice for each as follow:

Group 1(control healthy group): mice received ordinary ration with no drugs and served as control non-treated for all.

Group 2 (EAC cells bearing group): non treated group: They received ordinary ration without additives and served as EAC cells bearing group.

Group 3 (EAC-Lettuce extract powder treated group): EAC cells bearing mice were fed on L. sativa extract powder at a dose of (100 gm / 10 kg normal ration) from the first day of experiment for 21 days.

Group 4 (EAC- Lettuce extract powder treated group): EAC cells bearing mice were fed on L. sativa extract powder at a dose of (200 gm./10 kg normal ration) from the first day of experiment for 21 days.

At the end of the experimental period (21 days from feeding on the ordinary ration mixed with L. sativa extract powder), Mice were fasted overnight, and then tumor size was measured by the formula:

$V = \pi/6 \times L \times W \times H =$ mm **(**Richtig et al., 2004).

Blood sampling and Biochemical analysis:

Random blood samples were collected from the retro-orbital venous plexus (located at the medial canthus of the eye) of the all animal groups by means of heparinized capillary tubes in dry clean test tubes and incubated for half an hour at room temperature to allow clotting for serum separation. Clear sera were separated by centrifugation at 3500 r.p.m. for 15 minutes and then collected in Eppendorf's tubes using automatic micropipettes. Part of serum samples were processed freshly and immediately for determination of glucose (Caraway and Watts, 1987), albumin (Doumas et al., 1971), ALT (Murray, 1984a), AST(Murray, 1984b), ALP (Rosalki et al., 1993), LDH (Buhl and Jackson, 1978),Tg (Fossati, and principe, 1982),TC (Allain et al., 1974), HDL-c (Burnstein et al., 1970) and LDL-c (Friedewald et al., 1972).

Statistical analysis:

All data were expressed as means± Standard Errors (SE). The statistical significance was evaluated by the one-way analysis of variance (ANOVA) using SPSS (18.0) software and the individual comparisons were obtained by Duncan′s multiple rang test (DMRT). Values were considered statistically significant at $p<0.05$.

3. RESULTS

Data demonstrated in table (1) showed appearance of tumor with specific size in EAC cells bearing mice when compared to the control normal one. Meanwhile, feeding of EAC-cells bearing mice on ration mixed with L.sativa extract powder in the two doses (group 3 and 4) showed significant decreases in tumor size as compared to EAC- cells bearing mice (group 2).

Also, results in table (1) revealed significant increases in serum values of Tg,TC, LDL-c, ALT, AST, ALP and LDH accompanied with marked decreases in serum values of glucose, albumin and HDL-c in EAC-cells bearing mice when compared to the control normal one. Meanwhile, feeding on ordinary ration mixed with lettuce extract in the two EAC- treated groups resulted in biochemical alterations in serum values when compared to EAC-cells inoculated mice involving significant reduction in serum values of triacylglycerol, TC, LDL-c, ALT,AST, ALP and LDH, accompanied with marked increase in serum values of glucose albumin and HDL-c towards the normal values in EAC- Lettuce treated mice when compared with EAC- untreated

mice, with pronouncing to the effect of the ration mixed with 200 gm of L.sativa extract powder.

Table 1: The effect of L. Sativa extract powder supplement on the tumor size and serum levels of (glucose**;** triacylglycerols, total cholesterol, high density lipoprotein- cholesterol (HDL-c), low density lipoprotein- cholesterol (LDL-c), albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) in EAC-induced tumor groups, in comparison with control normal group.

Parameter	Group (1)	Group(2)	Group(3)	Group(4)
Tumor size mm.	-	1.27 ± 0.11^{a}	1.04 ± 0.06^{b}	0.62 ± 0.04^{c}
Glucose (mg/dl)	95.90 ± 2.14^{a}	63.30 ± 2.97^{d}	79.70 ± 1.12^{c}	91.70 ± 1.79^{bc}
Triacylglycerols (mg/dl)	108.70 ± 3.40^{d}	267.50 ± 5.32^{a}	170.80 ± 3.70^{b}	138.90 ± 2.91^{c}
Total cholesterol (mg/dl)	163.80 ± 3.83^{d}	265.30 ± 3.68^{a}	211.70 ± 1.59^{b}	186.10 ± 2.29^{c}
HDL-c (mg/dl)	37.90 ± 1.22^{a}	19.90 ± 0.82^{d}	26.80 ± 1.11^{c}	32.30 ± 1.09^{b}
LDL-c (mg/dl)	105.00 ± 3.52^{d}	169.80 ± 2.47^{a}	133.10 ± 1.99^{b}	116.30 ± 2.44^{c}
Albumin (g/dl)	4.74 ± 0.11^{a}	2.44 ± 0.15^{d}	3.16 ± 0.08^{c}	4.07 ± 0.10^{b}
ALT (U/L)	29.10 ± 1.23^{d}	83.78 ± 2.66^{a}	55.87 ± 1.63^{b}	35.83 ± 1.71^{c}
AST (U/L)	36.57 ± 1.61^{d}	91.79 ± 2.52^{a}	59.04 ± 1.89^{b}	42.31 ± 1.43^{c}
ALP (U/L)	95.10 ± 3.16^{d}	262.80 ± 5.44^{a}	161.30 ± 4.58^{b}	128.80 ± 4.72^{c}
LDH (U/L)	124.50 ± 2.25^{d}	382.70 ± 3.01^{a}	264.30 ± 5.81^{b}	228.00 ± 5.68^{c}

Groups1, 2, 3 and 4: Control normal group, EAC-cells bearing group, EAC- L-sativa extract powder supplemented group (100 gm) and EAC- L-sativa extract powder supplemented group (200 gm.), respectively. Data are represented as (Means ±S.E).Values with different letters within the same row significantly differed at ($p< 0.05$).

4. DISCUSSION

Ehrlich Ascites carcinoma is a breast tumor developed in body cavities, it was carried out in mice experimentally by spontaneous adenocarcinoma EAC cells fill the peritoneal cavity by rapid division leading to growth of tumours and ascites ended with severe pressure in the surrounding organs and damage of the organism (Sazzad et al., 2017). Lettuce should be the main ingredient in salads due to its known medicinal therapeutic properties such as anti-oxidant, anti-inflammatory, anti-microbial, analgesic, neuroprotective, sedative and its importance for immunological system (Nallamilli et al., 2018).

Data recorded in table (1) revealed that the inoculation of Ehrlich ascites tumor into the female mice lead to appearance of tumors with specific sizes in EAC cells bearing mice when compared to the control normal one.

Meanwhile, EAC-cells bearing mice were fed on rations mixed with L.sativa extract powder in the two doses (group 3 and 4) showed significant decreases in tumor sizes as compared to EAC- cells bearing mice(group 2).

Our results came in agreement with Fernandes et al., (2015); Hertweck and Dasgupta, (2017) who showed that implantation of EAC cells resulted in local inflammation, high vascular permeability, an intensive oedema, cellular migration and a progressive ascitic fluid formation. Induction of EAC lead to morohological and metabolic changes involving structural deterioration as decreasing in the number of mitochondria, mutation in DNA and RNA synthesis, loss of intracellular purin, pyrimidine, a decline of ATP concentration and decrease in protein synthesis. In addition, these tumor cells probably depend on the ascetic fluid as a direct source of nutrition for their growth and proliferation. Concerning to the decreasing in the tumour volume in EAC- treated mice probably resulted from the reduction of the ascitic nutritional fluid volume and vascular permeability (Farhadul et al., 2014; Hertweck and Dasgupta, (2017).

The data demonstrated in table (1) revealed that experimental animals with EAC developed significant hypoglycaemia when compared to the control normal mice; on the other hand, treatment of the animals with Lettuce previously inoculated with EAC cells resulted in return to the glucose level towads the normal when compared to EAC untreated mice. Our findings came in a harmony with Rasida et al., (2012) and Farhadul et al., (2014) who found that the glucose content was reduced from normal level in EAC cells bearing mice. Likewise, Hanahan and Weinberg, (2011) who suggested that tumour cells undergo a complex metabolic reprogramming to satisfy the increased demands of macromolecules and energy for proliferation.

EAC cells induction resulted in conversion of glucose into lactate even under aerobic conditions (aerobic glycolysis) which was considered as one of the three major components of the metabolic transformation of cancer cells (Sciacovelli et al., 2014). Furthermore, normal cells might support the proliferation of cancer cells by fully oxidizing of glucose in mitochondria due to mitochondrial oxidative stress (Levine and Puzio-Kuter, 2010). Besides, aerobic glycolysis may act as a universal metabolic phenotype of proliferating cells. As a result of its recording in non-transformed cells such as activated lymphocytes and embryonic stem cells (Wang and Green, 2012 and Zhang et al., 2012).

Concerning to the improvement effect of Lettuce to the glucose level, our results agreed with Farhadul et al., (2014) who found that petroleum ether extract of bacterial metabolites altered a parameter like glucose towards normal level as a result of its antioxidant and anti infilamatory propertiesin comparison to those of untreated EAC carcinoma bearing mice. Parallel to this suggestion, Lettuce may exert an inhibatory effect to oxidative stress, free radicals formation as well as infilamation due to EAC inoculation by the help of its phytoconstituents; involving:1) high levels of (ascorbic acid, α-tocopherol, carotenoids and other lipid-soluble antioxidants as lutein(Komaki et al., 2014) which increase the plasma antioxidant capacity within 2 hours of consumption, preventing aerobic glycolysis, decreasing tumor growth and inhibiting malignant cells prolifraton. 2) Flavonoids and phenolic compounds exerted free radical scavenging abilities, anti-inflmmatory, anti-carcinogenic and anxiolytic properties (Seeram et al., 2008; Harsha and Anilakumar, 2012). 3) Polyphenols and chlorogenic acid,

vanillin, epicatechin, caffic acid, rutin hydrate, sinapic acid, quercetin-3-rhamnoside, p-coumeric acid and quercitin act as functional groups delayed phases of inflammation and ameliorate of damaged structure caused byEAC inoculation. Besides, having of immune modulatory activity (Harsha et al., 2013).

Data recorded in table (1) revealed that EAC-induced tumour in female mice developed dramatic alterations in the lipid profile, there were significant elevation in serum concentrations of Tg; TC and LDL-c accompanied with marked decrease in serum value of HDL-c when compared to the control normal mice. Our results came in agreement with Rasida et al., (2012); Rodrigues dos Santos et al., (2014); Sciacovelli et al., (2014) and Samiron et al., (2018) who reported that cancer cells exhibit profound metabolic alterations.

The increased isoprenoid derivatives promote tumor cell growth, differentiation, migration and intracellular traffiking (Mullen et al., 2016). In addition, the transformed malignant cells may be highly dependent on the mevalonate pathway for the synthesis of lipid moieties critical for cell proliferation, membrane integrity, cell cycle progression and cell signaling (Pisanti et al., 2014). Likewise, a positive feedback caused by pro-inflammation nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling might trigger via enhancing cholesterol accumulation in cancer cells.

On the other hand, the increasing in fatty acids biosynthesis and the frequent containing of higher lipid accumulation in the form of lipid droplets in cancer cells leading to cell proliferation and malignancy, might result from not only upregulation of ATP-citrate lyase (ACLY) which acts as a rate-limiting enzyme and converts mitochondria-derived citrate into acetyl-CoA, which serves as an important precursor for de novo FA synthesis (Beckner et al.,2010 and Zhou et al., 2013).

Data represented in table (1) revealed that feeding of EAC-induced tumor female mice on a ration mixed with L. sativa and its milky juice extract powder in the two different doses showed significant reduction in serum values of Tg, TC and LDL-c, accompanied with marked increase in serum level of HDL-c in comparing with EAC-induced tumored female mice. Our findings came in harmony with Chu et al., (2002); Sayyah et al., (2004) and Nallamilli et al., (2018) who reported that lettuce have the ability to prevent chronic diseases related to oxidative stress, such as cancer and in preventing its progression.

Lettuce can exert its beneficial effects on lipid metabolism and tissue oxidation by acting on several mechanisms, Lettuce intake may increase total cholesterol end-product excretion, lettuce may act as lipid lowering drug by inducing antiinfilamatory and immune modulatory activities, due to the plant is rich in polyphenols and other secondary metabolites including chlorogenic acid, vanillin, epicatechin, caffic acid, rutin hydrate, sinapic acid, quercetin-3-rhamnoside, p-coumeric acid and quercitin (Harsha et al., 2013). Furthermore, Lettuce plant is rich inflavonoids and phenolic compounds which might exert free radical scavenging abilities, anti-inflammatory, anticarcinogenic, and anxiolytic properties (Schointuchet al., 2014; Greenaway et al., 2016 and Stine et al., 2016).

Data represented in table (1) revealed marked elevation in ALT, AST, LDH and ALP enzyme activities accompanied with significant decrease in albumin concentration in EAC bearing untreated mice when compared to normal mice. Meanwhile, activities of these enzymes were found to be significantly decreased accompanied with significant

increase in albumin concentration in EAC bearing untreated mice groups fed on lettuce juice extract powder in comparing to EAC bearing untreated mice.

Our findings are came in accordance with the recorded data of Farhadul et al., (2014) who found significant elevations in the levels of serum GPT, GOT and ALP; moreover, major pathological abnormities including high infiltration with central vein dilation and fat accumulation or nodules formation in EAC bearing untreated mice when compared to that of normal mice and attributed their findings to EAC induced acute and permanent hepatotoxicity leading to destruction and malfunction of the liver (the main drug detoxifying organ). Our results agreed with Muhammad et al., (2010) who reported that significant elevations in the levels of serum GPT, GOT and ALP in liver diseases and disorders in hepatocellular damage caused by a number of agents including cancer and myocardial infarction.

On the other hand, the inhibitory effect of lettuce extract in the previous hepatic enzymatic activities may be due to its acting as a hepatoprotective agent against the permanent damage caused by EAC inoculation, depending on its phytochemical constituents including antioxidants, free radical scavenging ability and anti-inflammatory properties preventing autoxidation and deleterious destruction of hepatic tissue (Harsha and Anilakumar, 2012; Komaki et al., 2014 and Nallamilli et al., 2018).

5. Conclusion

The present study revealed the potent antitumour efficacy of lettuce extract supplement as a natural plant by attenuating the metabolic and hepatic enzymes disorders. As well as the cellular damages induced by EAC.

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