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ORIGINAL ARTICLE

Terfezia claveryi Chatin: Anti-Tumor Effects against Ehrlich Solid Tumor in Mice

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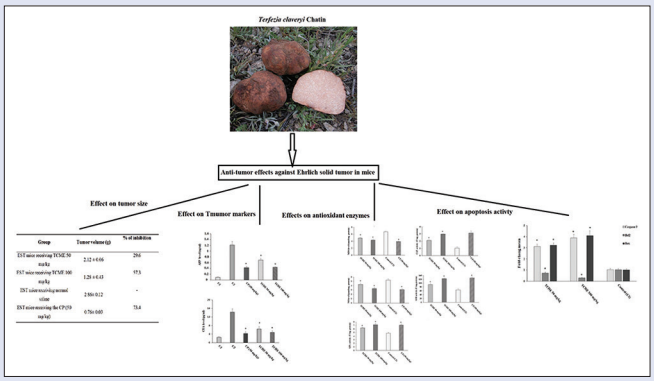
ABSTRACT

Background: Numerous reports on the side effects of anti-cancer drugs have encouraged scientists to search for alternative anti-cancer agents with higher efficacy and fewer side effects. The current investigation was intended to study the anti-tumor efficacy of *Terfezia claveryi* Chatin methanolic extract (TCME) on mice with Ehrlich solid tumors (EST). **Materials and Methods:** EST mice received TCME at doses of 50 and 100 mg/kg orally once a day for two weeks. To study the anti-tumor effects, the rate of tumor growth, weight of the body, the serum level of some tumor markers, enzymes related to liver and kidney function, oxidant and antioxidant enzymes, tumor necrosis factor alpha (TNF-α) level, and some genes related to apoptosis were investigated. **Results:** The results revealed that the growth rate of the tumor, tumor markers, oxidative markers, enzymes related to liver and kidney function, level of TNF-α, and Bcl-2 gene expression were considerably declined in the EST mice receiving the TCME, whereas the enzyme levels related to antioxidant activity as well as Bax and caspase-3 gene expression were considerably elevated ($P < 0.001$). **Conclusion:** We found that *T. claveryi* methanolic extract has relevant anti-tumor efficacy on mice with EST and might be considered as a substitute anti-cancer compound; however, more studies especially in vulnerable humans are mandatory to approve these outcomes.

Key words: Anticancer, cancer, herbal medicines, natural product, truffle

SUMMARY

- We evaluated the anti-tumor effects of *Terfezia claveryi* Chatin (TCME) against the Ehrlich solid tumors (EST) in mice.
- TCME significantly reduced tumor volume tumor markers, serum level of liver and kidney, and the apoptosis enzymes.
- TCME significantly increased the level of antioxidant enzymes.
- TCME has promising anti-tumor activity against EST in mice and might be considered as a substitute anti-cancer agent.



Abbreviations used: EST: Ehrlich solid tumor; **TCME:** *T. claveryi* Chatin methanolic extract; **TGIR:** Tumor growth inhibition rate; **AFP:** Alpha-fetoprotein; **CAE:** Carcinoembryonic antigen tumor; **LPO:** Lipid peroxidation; **NO:** Nitric oxide; **GPx:** Glutathione peroxidase; **CAT:** Catalase enzyme; **SOD:** Superoxide dismutase enzyme activity; **TNF-α:** Tumor necrosis factor alpha; **ALT:** Alanine aminotransferase; **AST:** Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **ALP:** Alkaline phosphatase; **Cr:** Creatinine; **BUN:** Blood urea nitrogen.

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INTRODUCTION

Cancer is described by the growth, incursion, and extent of unregulated cells from the primary organ to other organs of the body.[1] It is one of the most dangerous diseases in the world, which kills nearly 8 million people annually.[2] Previous studies have identified the six main factors in the incidence of cancer, including the ability to produce messages of autonomic growth, escaping growth inhibition messages, avoiding apoptotic cell death, promoting unlimited replication, angiogenesis, invasion, and metastasis.[3,4] Today, various methods are applied for cancer therapy, the most important of which are chemotherapy, radiation therapy, hormone therapy, and surgery.[5] However, existing complications in the usage of chemotherapy and its many side effects such as gastrointestinal disorders, kidney damage, nausea, fatigue, vomiting, and hair loss have prompted scientists to discover novel medications with better efficacy and not as much toxicity.[6,7]

Nature is the valuable origin of promising novel agents with boundless chemical variety found in millions of plant, animal, marine, and microbial species.[8] Mushrooms are one of the important species of the fungi kingdom, and are broadly used in the biosphere, human industry, medicine, and research.[9] Truffles are well-known as a wide family of hypogeous fungi, generally containing the genera of *Picoa*, *Tirmania*, *Tuber* and *Terfezia*.[10] The main genus of truffles is *Terfezia*, belonging to

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the family Tubraceae which have four species including *Terfezia boudieri* Chatin, *T. claveryi* Chatin, *T. leonis* Tul, and *T. Metaxasi* Chatin.[11] Among the *Terfezia* species, *T. claveryi* as a black-colored truffle is the most common and widely used truffle species in Saudi Arabia.[11] This mushroom is rich in phenolic composites, which have been measured as one of the important secondary metabolites with promising preventive and chemotherapeutic efficacy in cancer.[12] In addition to the nutritional benefits of *T. claveryi*, many studies showed different biological and pharmacological activities of this truffle such as antioxidant, anti-angiogenic, anti-diabetic, anti-microbial, and hepatoprotective activities.[11,13] Furthermore, in several studies, the promising anti-cancer effects of *T. claveryi* have been reported against colon adenocarcinoma cell line (HT-29), Ehrlich ascites carcinoma (EAC) cell line, human breast MCF-7 cancer cell line, human brain carcinoma cell line (U-87 MG), and human prostate cancer cell lines (PC-3).[14–16]

Ehrlich tumor originates from impulsive mammary adenocarcinoma of murine and results in an ascites kind through intraperitoneal inoculation.[16] The Ehrlich solid tumor (EST) develops via a subcutaneous injection which makes available a competent tool to study anti-neoplastic agents. Concerning the considerable biological and pharmacological activities of this plant, the current investigation was intended to study the anti-tumor efficacy of *T. claveryi* Chatin methanolic extract (TCME) on mice with EST.

MATERIALS AND METHODS

Plant Material

T. claveryi were provided from a market in Riyadh, Saudi Arabia, and were identified by a mycologist (voucher example No. 2014-2020).

Preparation of the extract

The dried material, weighing 250 g, was extracted using methanol percolation technique for three days at 21°C. After filtering the extract with filter paper, it was evaporated in a vacuum under a turning evaporator and deposited at − 20°C.[17]

Phytochemical analysis and secondary metabolites contents

Preliminary analysis of the TCME was accomplished to prove the presence of tannin, saponin, alkaloid, flavonoid, and glycoside compounds based on the methods described in the previous study.[18] Folin–Ciocâlteu reagent colorimetric technique and aluminium chloride colorimetric assay were applied to measure the total phenolic and flavonoid amount of the TCME, based on the methods explained by Singleton *et al.*[19] and Phuyal *et al.*,[20] respectively. Moreover, we used the technique defined by Broadhurst and Jones[21] to measure the tannin-condensed contents of the TCME.

Animals

Seventy-two female Swiss albino mice (weighing 20–25 g) at the age of four to six weeks were utilized to establish EST murine model. The survey was permitted by the ethical committee of Shaqra University, Saudi Arabia (214-2020). Before and during tests, animals were kept in standardized situations (22 ± 1°C, cycle of 12-h dark and 12-h light cycle and standard air humidity), with open contact to food and water.

The Ehrlich ascites tumor cell line

The cell line of the Ehrlich ascites tumor (EAT) was procured from the American Type Tissue Culture Collection (Manassas, USA) and was then kept as 25×10^5 cells per ml in normal sterilized saline.

Study design

At first, EST was induced in four subgroups of mice via subcutaneous injection of EAT cells (0.2 ml).[22] Five days after inoculation, the animals were randomly allocated into five groups (eight mice per group): mice with no EST and without treatment (C1); mice with EST that received normal saline (C2); mice with EST that received TCME at 50 mg/kg/day orally for 14 days (E1); mice with EST that received TCME at 100 mg/kg/day orally for 14 days (E2); mice with EST that received cyclophosphamide (CP) at 50 mg/kg/day intraperitoneally for 3 days (E3).

Furthermore, to evaluate the toxicity properties of TCME on enzymes associated with liver and kidney function in healthy mice, 32 mice were randomly allocated into four groups comprising eight mice per each: healthy mice that received the normal saline (C3); healthy mice that received TCME at 50 mg/kg/day orally for 14 days (C4); healthy mice that received TCME at 100 mg/kg/day orally for 14 days (C5); healthy mice that received CP once a day for 2 weeks (C6).

Blood sampling

After treatment, the mice underwent deep anesthesia with xylazine (10 mg/kg)–ketamine (100 mg/kg), and blood samples of each animal were collected from the hearts of mice once opening their abdomen. After centrifuging the collected blood samples at 7000 rpm for 15 min, the sera were extracted and held in reserve at − 80°C until testing.

Bodyweight changes

Through the weighing of the mice in all mice on the 5th and 19th days, the rate of obtained weight was calculated using the equation[23]:

$$\%\text{weight gain} = \left[\frac{\text{weight on } 19^{\text{th}} \text{ day}}{\text{weight on day } 0}\right] - 1 \times 100$$

Tumor sampling

After removing the tumors in a sterile manner and measuring their weight, the sizes of the tumors were recorded in mice of E1, E2, E3, and C2 subgroups. Finally, the tumors were in the same way allocated into two parts: the first one was stored at −80°C to use for molecular examinations, and the other part was stored at −20°C for the rest assessments.

Tumor growth inhibition

By determining the tumor volume (TV) and the inhibition rate of tumor growth, we measured the anti-tumor efficacy of TCME. On the 7th day after treatment, TV was measured by Vernier calliper using the equation given below:

$$\text{TV (mm3)} = 4\pi\left(\frac{X}{2}\right)^2 \times \left(\frac{Y}{2}\right)$$

X and Y are the axes of the minor and major tumor.

The inhibition rate of tumor growth (IRTG) was examined according to the technique described by Sharawi,[22] with the below-mentioned formula[14]:

$$\text{IRTG (\%)} = \frac{\text{(The mean tumor weight of control group - the mean tumor weight of treated group)}}{\text{(The mean tumor weight of control group)}} \times 100$$

Evaluating the tumor markers

The serum level of alpha-fetoprotein (AFP) was studied based on automated quantitative enzyme-linked fluorescent assay (ELFA) using mini-VIDAS® AFP (bioMérieux, Marcyl'Etoile, France) according to the manufacturer's instructions. Furthermore, the

serum level of carcinoembryonic antigen tumor (CAE) was determined by quantitative sandwich ELISA using the Mouse Carcinoembryonic Antigen ELISA Kit (MyBio-Source, San Diego, USA).

Evaluation of serum levels of liver enzymes

The enzymes related to liver function in mice with EST receiving TCME, the amount of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in mice sera using Roche's automatic biochemical analyzer and the matching kit (Mannheim, Germany).[24]

Assessment of serum levels of kidney enzymes

To assess the function of the kidneys after treatment with TCME in EST mice as well as healthy mice, the serum level of blood urea nitrogen (BUN) and creatinine (Cr) were investigated using Roche's automatic biochemical analyzer and the matching kit (Mannheim, Germany).[24]

Evaluation of oxidative stress markers

Tumor homogenates (TH) were prepared in phosphate-buffered saline (PBS) at 50 mM pH (7.4) by using a Potter-Elvehjem tissue grinders. Lipid peroxidation was measured by using the malondialdehyde (MDA) colorimetric assay kit (ab118970, Abcam).[25] Moreover, nitric oxide (NO) creation was measured in the tumor homogenate by using the NO colorimetric assay kit (ab65328, Abcam).[26]

Evaluation of the antioxidant enzymes

The level of catalase enzyme (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) enzyme activity as the main enzymes related to antioxidant activity was measured by using the commercial kits (Abcam, USA) and according to the techniques defined by Luck,[27] Weydert and Cullen,[28] and Sun *et al.*,[29] respectively.

Measuring the level of tumor necrosis factor alpha

The TNF-α level changes of tumor suspension were measured by the murine TNF-α ELISA kit (ab100747; Abcam) based on the manufacturer's protocol.

Evaluating the apoptosis-regulatory gene expressions

The gene expression of caspase-3, Bcl-2, and Bax were studied via real-time PCR. Briefly, using a RNeasy tissue kit (Qiagen, Germany), total RNA was extracted from an obtained tumor based on the instructions of the manufacturer. The complementary DNA (cDNA) was produced using specific primers via the commercial kits (Qiagen, Germany) according to the manufacturer's instructions and was applied for real-time PCR via SYBR green [Table 1].

The thermal condition of the real-time PCR reaction was 96°C for 7 min, 40 cycles of 96°C for 10 sec, and 57°C for 30 sec, respectively. The fold-change of mRNA expression levels in cells was studied by measuring the $2^{-}\Delta^{Ct}$ via iQTM5 optical system software (Bio-Rad, Hercules, CA).[30]

Statistical analysis

Statistical analysis of the information was accomplished using Statistical Package for the Social Sciences (SPSS) version 26.0. Comparison of variance between groups was performed using one-way analysis of variance (ANOVA) between concentrations and *t*-test between timings in each concentration. Findings were measured significantly with $P < 0.05$.

RESULTS

Phytochemical analysis

This truffle yielded 9.6% methanolic extract. The obtained findings of a primary phytochemical analysis of the TCME confirmed the existence of flavonoids, tannins, glycosides, terpenoids, and deficiency of saponins and alkaloids in TCME [Table 2].

Evaluating the contents of secondary metabolites

The findings of the secondary metabolite contents of TCME revealed that the amount of total flavonoids, phenolics, and tannins was 43.87 (mg QE/g DW), 57.26 (mg GEA/g DW), and 12.6 (mg CE/g DW), respectively.

Tumor growth inhibition

Our findings revealed that in mice with EST that received TCME, the rate of TV was considerably declined in a dose-dependent response; TCME at doses of 500 and 1000 mg/kg declined the weight of the tumor by 2.12 ± 0.06 g and 1.28 ± 0.043 g, respectively. The current study also revealed that tumor inhibition rate was 29.6% and 57.3% after treatment of EST-bearing mice by TCME at the dose of 500 and 1000 mg/kg, respectively [Table 3].

Calculation of body weight

In EST mice cured with TCME at doses of 50 and 100 mg/kg, bodyweight (BW) was considerably declined once it was compared with EST mice that did not receive TCME (C2 group) [Figure 1].

Table 1: Sequence of primers used for real-time PCR

Amplicon	Primers	Sequence (5′–3′)
Bax	F	GGCTGGACACTGGACTTCCT
	R	GGTGAGGACTCCAGCCACAA
Bcl-2	F	CATGCCAAGAGGGAAACACCAGAA
	R	GTGCTTTGCATTCTTGGA TGAGGG
Caspase-3	F	TTCATTATTCAGGCCTGCCGAGG
	R	TTCTGACAGGCCATGTCATCCTCA
β-actin	F	GTGACGTTGACATCCGTAAAGA
	R	GCCGGACTCATCGTACTCC

Table 2: The primary phytochemical analysis of *Terfezia claveryi* Chatin methanolic extract (TCME)

Type of assay	Type of assay	Existence
Flavonoids	Alkaline and Ammonia	Yes
Tannins	$FeCl_3$	Yes
Saponins	Frothing	No
Alkaloids	Mayer's and Dragendorff's	No
Glycosides	Nitroprusside	Yes
Terpenoids	Salkowski	Yes

Table 3: Effect of various doses of *Terfezia claveryi* hexane extract (TCME) of tumor volume and tumor inhibition rate in the tested mice. Results are displayed as the mean±SD (*n*=8)

Group	Tumor volume (g)	% of inhibition
EST mice receiving TCME 50 mg/kg	2.12±0.06	29.6
EST mice receiving TCME 100 mg/kg	1.28±0.43	57.3*
EST mice receiving normal saline	2.86±0.12	-
EST mice receiving the CP (50 mg/kg)	0.76±0.03	73.4*

* $P<0.001$ Findings were considered significant

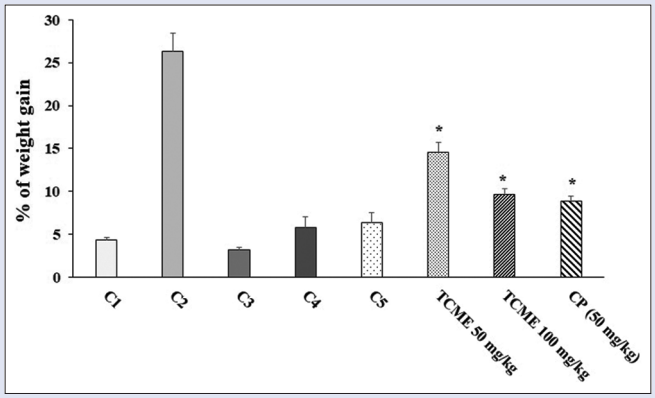

Figure 1: The mean of % of weight gains in EST mice that received with TCME at doses of 50 and 100 mg/kg when compared with mice with no EST and without treatment (C1); mice with EST that received normal saline (C2); healthy mice that received normal saline (C3); healthy mice that received TCME 50 mg/kg/day orally for 14 days (C4); and healthy mice that received TCME 100 mg/kg/day orally for 14 days (C5). Data are displayed as mean ± SD (n = 8). * P < 0.001 Findings were considered significant

Evaluating tumor markers

The evaluation of serum level tumor markers exhibited that in mice of the C2 group, the amount of CEA and AFP was meaningfully raised compared with of C1 mice; while in mice present in groups E1, E2, and E3, the amount of CEA and AFP significantly decreased (P < 0.001) in comparison with mice with EST that received the normal saline [Figure 2].

Evaluation of enzymes related to liver function

The serum amount of AST and ALT increased in mice of C2 group; while in mice present in groups E1, E2, and E3, the amount of AST and ALT significantly declined (P < 0.001) in comparison with mice with EST that received the normal saline [Figure 3]. The results also showed that in mice present in groups C4 and C5, the serum amount of AST and ALT had not shown any significant change once compared with mice in C1 and C3 groups.

Evaluation of enzymes related to kidney function

The serum amount of BUN and Cr increased in mice of the C2 group, while in mice present in groups E1, E2, and E3, the amount of BUN and Cr significantly declined (p < 0.001) in comparison with mice with EST that received the normal saline [Figure 4]. The results also showed that in mice present in groups C4 and C5, the serum amount of BUN and Cr had not shown any significant change once compared with mice in groups C1 and C3.

Evaluating the oxidant/antioxidant factors

Figure 5 shows the amount of oxidative stress and NO in tumors. The study results revealed that the amount of MDA and NO significantly increased, whereas the amount of some antioxidant enzymes such as GPx, CAT, and SOD exhibited a considerable decrease in the EST-bearing mice treated with no drug (C2). However, in mice of groups E1 and E2, the level of MDO and NO significantly declined (P < 0.05), while they exhibited a significant increase (P < 0.05) in the amount of GPx, CAT, and SOD.

Measuring the TNF-α level

The study findings demonstrated that the level of TNF-α in mice with EST that received normal saline (C2) remarkably increased, but in EST mice

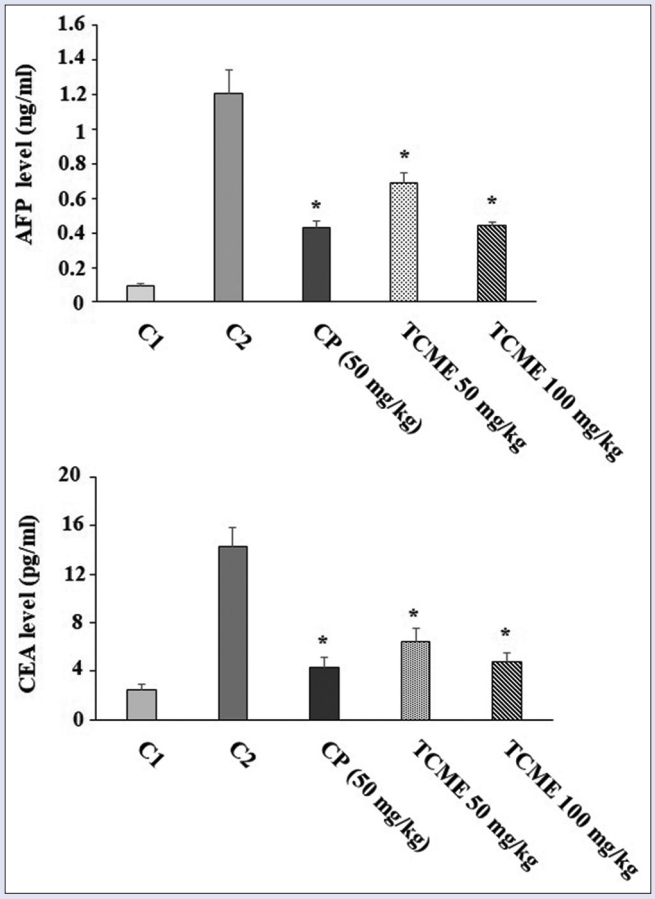

Figure 2: The serum level of alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) tumor in EST-bearing mice treated with TCME at doses of 50 and 100 mg/kg when compared with mice with no EST and without treatment (C1) and mice with EST that received normal saline (C2). Results are displayed as the mean ± SD (n = 8). * P < 0.001 Findings were considered significant

that received TCME at doses of 500 and 1000 mg/kg/day, a significant decrease (P < 0.05) was observed in the TNF-α level [Figure 6].

Evaluating the apoptosis-regulatory genes expression

The expression rate of the caspase-3 gene significantly increased (P < 0.001) by 2.48- and 3.21-fold in mice of the E1 and E2 group, respectively. Similarly, the expression rate of the Bax gene was significantly improved (P < 0.001) by 2.56- and 3.33-fold in mice of the E1 and E2 groups, respectively. Conversely, a significant down-regulation was detected in the expression rate of the Bcl-2 gene in mice of the E1 and E2 groups [Figure 7].

DISCUSSION

The current investigation intended to study the anti-tumor efficacy of TCME on mice with EST. We found that in mice with EST that received TCME, there was significant decline in the tumor weight, TV, and BW once compared with EST mice that received normal saline.

To the best of our knowledge, there are no *in vivo* anti-cancer effects of *T. claveryi*; however, several studies have reported the *in vitro* anti-cancer effects of TCME. For example, Dahham *et al.*[14] reported

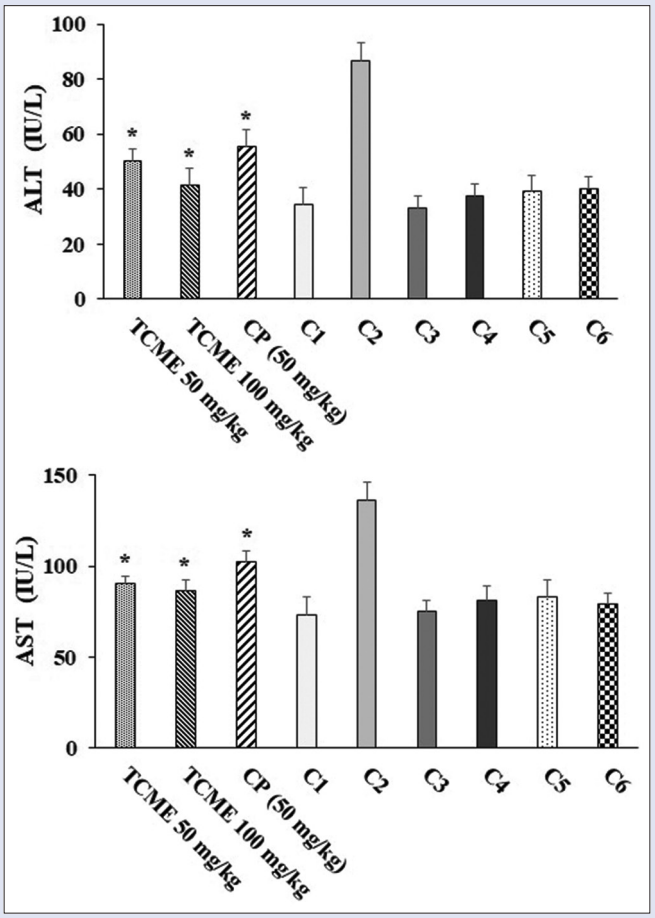

Figure 3: The level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in mice with EST that received with TCME at doses of 50 and 100 mg/kg when compared with mice with no EST and without treatment (C1) and mice with EST that received normal saline (C2); healthy mice that received normal saline (C3); healthy mice that received TCME 50 mg/kg/day orally for 14 days (C4); healthy mice that received TCME 100 mg/kg/day orally for 14 days (C5); and healthy mice treated with CP once a day for two weeks (C6). Results are displayed as the mean ± SD ($n = 8$). * $P < 0.001$ Findings were considered significant

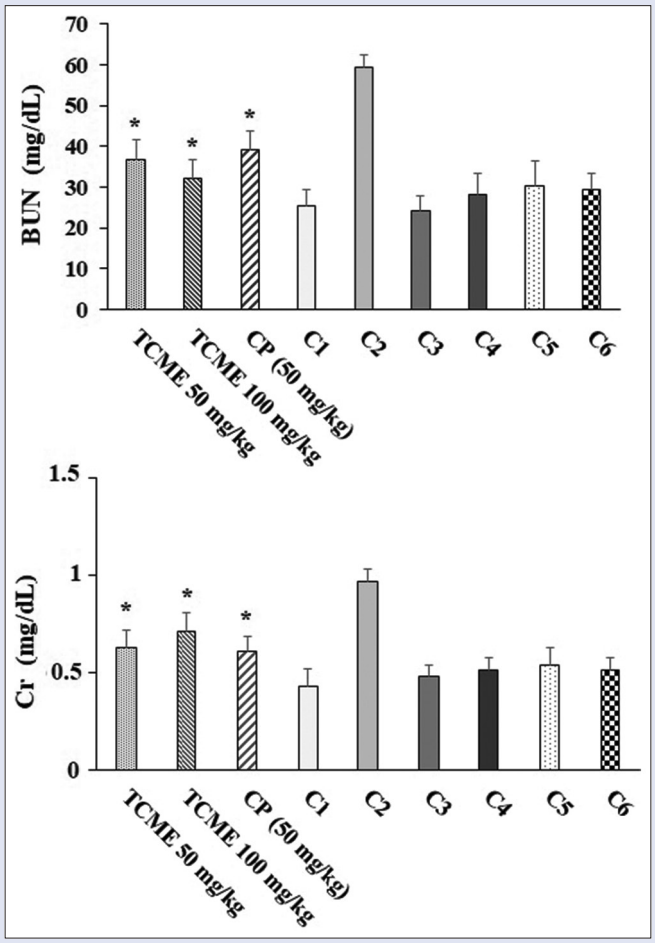

Figure 4: The level of blood urea nitrogen (BUN) and creatinine (Cr) in mice with EST that received TCME at doses of 50 and 100 mg/kg when compared with mice with no EST and without treatment (C1) and mice with EST that received the normal saline (C2); non-EST mice that received the normal saline (C3); non-EST mice that received TCME at 50 mg/kg/day orally for 14 days (C4); non-EST mice that received TCME at 100 mg/kg/day orally for 14 days (C5); and non-EST mice treated with CP once a day for 2 weeks (C6). Findings are displayed as mean ± SD ($n = 8$). * $P < 0.001$ Findings were considered significant

on the anti-cancer effects of ethanol, methanol, ethyl acetate, hexane, and water extracts of *T. claveryi* against some cancer cell lines such as U-87 MG, HT29 (CCL-247), and MCF-7 (HTB-22) using MTT assay with IC_{50} values ranging from 50.3–389.6 µg/ml.[14] They also concluded that among the extraction solvents, hexane extract displayed the higher anti-cancer, antioxidant, and anti-angiogenic activity. In addition, Attia *et al.* also reported the *in vitro* anti-cancer properties of polysaccharides extracted from ascomata of *T. claveryi* against EAC cells with IC_{50} value of 77 and 47 µg/mL after one and two days of incubation.[15]

The preliminary analysis confirmed the existence of flavonoids, tannins, glycosides, terpenoids and the absence of alkaloids and saponins in TCME. Reviews have demonstrated that flavonoid compounds and their main derivatives (e.g., flavanones, isoflavonoids, flavanols, flavones, flavonols, and anthocyanidins), which are mostly found in a wide range of plants, display their anti-cancer effects through induction of apoptosis, control of the activities of reactive oxygen species (ROS)-scavenging enzymes, suppressing the cell cycle, hang-up of the cell proliferation, etc.; instead, it has been proven that flavonoids are considered as antioxidants during common situations and are effective pro-oxidants in cancer cells activating the pathways of apoptotic and reducing the pro-inflammatory mediators.[16,31] Phenolic compounds in plants displayed their anti-cancer effects through inhibiting the angiogenesis and inducing apoptosis in cancer cells, inhibiting tumor growth and development, inhibiting oncogenic proteins, controlling the ROS level in cells, inhibiting cell proliferation, etc.[32] Therefore, we propose that the anti-tumor effects of TCME are due to the presence of these phytosterols and triterpene constituents.

Nowadays, the serum levels of AFP and CEA are measured as the key tumor factors that elevated in various type of cancers, which indicate the liver and renal damage induced by the invasion of cancer cells. Subsequently, we found that the amount of these tumor factors in mice present in groups E1 and E2 considerably declined in comparison with EST mice that received the normal saline. Cancer cells through impairing the metabolism of common liver cells, increase the activity of some functional enzymes. In addition, the damage to the hepatocytes through the attack of cancer cells cause the emancipation of AST and ALT across the serum and then the rise of their serum level.[33] Similar to

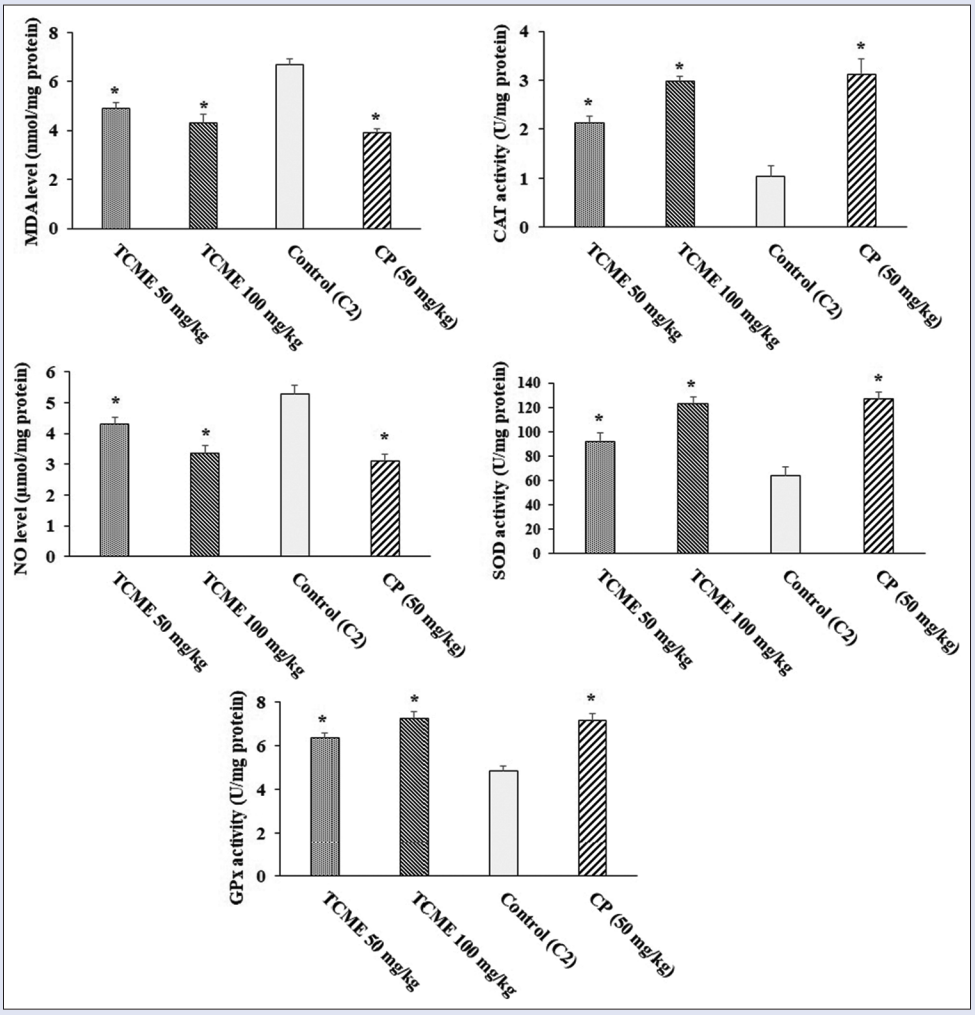

Figure 5: The level of the oxidant (malondialdehyde (MDA) and nitric oxide (NO)) and antioxidant (catalase enzyme (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) enzyme activity) factors in mice with EST that received TCME at doses of 500 and 1000 mg/kg once compared with EST mice that received normal saline (C2). Findings are expressed as mean ± SD ($n = 8$). * $P < 0.001$ Findings were considered significant

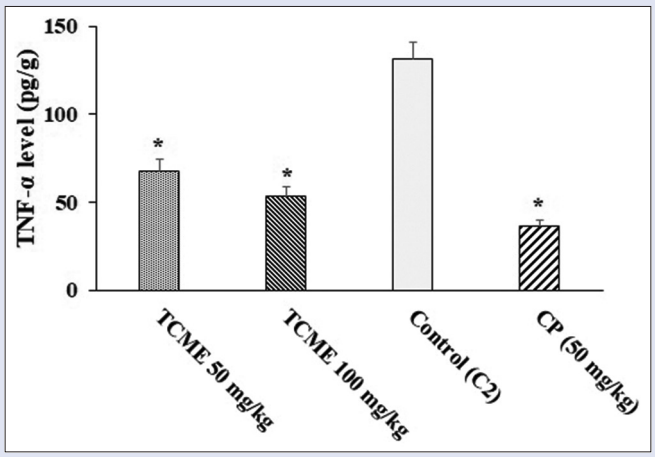

Figure 6: The tumor level of the TNF-α in EST-bearing mice that received TCME at doses of 50 and 100 mg/kg once compared with EST mice that received normal saline (C2). Results are displayed as the mean ± SD ($n = 8$). * $P < 0.001$ Findings were considered significant

previous studies,[34,35] our results showed that the serum amount of AST and ALT in mice present in groups E1 and E2 considerably increased in comparison with mice with EST that received the normal saline. According to the previous studies, EST impairs the function of the kidneys and causes the rise of serum BUN and Cr.[35,36] In line with these findings, our results revealed that in mice present in groups E1 and E2, the amount of BUN and Cr considerably increased in comparison with mice with EST that received the normal saline.

Among the many important mechanisms in the progression of cancer, oxidative stress (OS) has been considered as an important therapeutic target.[37] It has been proven that antioxidant agents isolated from medicinal plants are theoretically able to keep human beings from compensations of OS.[38] We found that the level of tumor MDA and NO considerably increased in the EST-bearing mice treated with no drug (C2); in contrast, TCME at the tested doses decreased the level of MDA and NO. TCME also significantly decreased ($P < 0.05$) the level of the LPO and NO as well as increased the level of GPx, CAT, and SOD.

Similarly, in previous investigations, the antioxidant effects of *T. claveryi* have been proven by DPPH-based radical scavenging (with IC_{50} values ranging from 52 to 121.84 µg/ml), Ferric reducing antioxidant

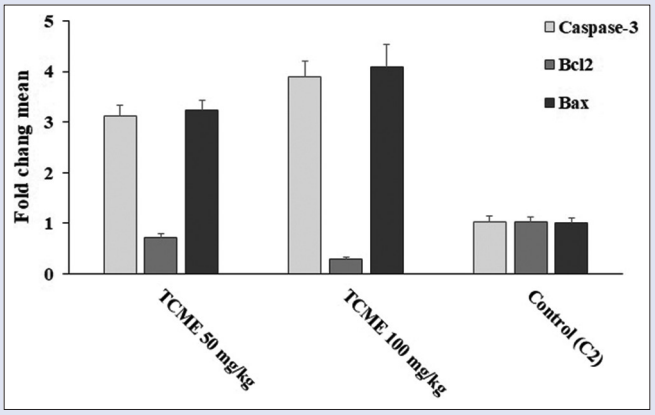

Figure 7: The level of the caspase-3, Bcl-2, and Bax genes in EST-bearing mice that received TCME at doses of 50 and 100 mg/kg once compared with EST mice that received normal saline (C2). Findings are expressed as mean ± SD ($n = 8$). * $P < 0.001$ Findings were considered significant

power assay (with IC_{50} value ranging from 48.53 to 105.8 μg/ml), ABTS cation radical scavenging assay (with the values ranging from 64.76 to 133.71 μg/ml), etc.[14,39] Previous studies have demonstrated that phytosterols and some of their constituents such as stigmasterol, beta-sitosterol, qualene, etc., have potent antioxidant and radical scavenger properties.[40,41]

Today, it has been proven that cancer cells damage normal tissues and cause hypoxia through two main ways, that is, physical damage of the tumor, and promoting the production of various pro-inflammatory mediators.[42] Consistent with other studies,[43,44] we found that in EST mice that received TCME at doses of 500 and 1000 mg/kg/day, a considerable decrease was observed in the TNF-α level. This might be associated with the scavenging of free radicals and also the anti-inflammatory ability of TCME.

Apoptosis is the programed death of a cell that occurs in response to various stressors in the body. This process is an active and energy-dependent phenomenon in which genetic mechanisms and factors play a role in controlling and implementing it with special programs.[45] Because of the essential role of apoptosis and programed cell death in monitoring cell division, the recommended novel agents must preferably be able to promote apoptosis in cancer cells.

The results of the real-time PCR demonstrated that the expression rate of caspase-3 and Bax genes notably increased in mice of groups E1 and E2, whereas a significant down-regulation was observed in the expression rate of Bcl-2 gene in mice of groups E1 and E2. Previously, Dahham *et al.* demonstrated that *T. claveryi* extract provoked apoptosis in cells through the mitochondrial processes.[14]

Considering the toxicity of TCME, our findings demonstrated that non-EST mice that received TCME for 14 days did not show any significant change in AST, ALT, BUN, and Cr once compared with mice in groups C1 and C3. Inconsistent with our results, Dyari *at el.*, in a study in 2020, demonstrated that oral administration of *T. claveryi* extract at the doses of 200, 400, and 800 mg/kg for two weeks had no notable toxicity on body and organ weight, hematological parameters, serum liver and kidney parameters, with no pathological lesions in vital organs e.g., lungs, liver, brain, and heart in Sprague Dawley rats.[46]

CONCLUSION

The obtained findings showed that TCME, at doses of 50 and 100 mg/kg, shows considerable anti-tumor activity against EST in mice. Although the accurate mechanisms are not clear, this could be related to the stimulation of apoptosis (as displayed by increase in caspase-3 and Bax gene, and decrease in Bcl-2 gene expressions), anti-inflammatory effects (as demonstrated by the decline of TNF-α levels), as well as antioxidant effects (as exhibited by reducing the LPO and NO). Therefore, TCME might be considered as a substitute anti-cancer agent against the tumor, after clarifying the clinical aspects and possible mechanisms. However, there are limitations in this study, such as identifying and isolating the effective compounds of this truffle and studying its other possible mechanisms and its effective compounds. We hope that in the future, by removing these limitations and conducting additional studies, especially in the clinical setting, we can develop a new anti-cancer drug.

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Authors' contribution

Study conception or design: NAA, RHA and MNB; data processing, collection, perform experiment: NAA, AA and IA; draft manuscript preparation: NAA, RHA and AA; Critical revision of the paper: NAA,RHA, MNB, AA and IA; final approval of the version to be published: NAA, RHA, MNB, AA, and IA.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011;144:646-74.
2. Mattiuzzi C, Lippi G. Current cancer epidemiology. J Epidemiol Glob Health 2019;9:217-22.
3. Golemis EA, Scheet P, Beck TN, Scolnick EM, Hunter DJ, Hawk E, *et al.* Molecular mechanisms of the preventable causes of cancer in the United States. Genes Dev 2018;32:868-902.
4. Figueroa-González G, Pérez-Plasencia C. Strategies for the evaluation of DNA damage and repair mechanisms in cancer. Oncol Lett 2017;13:3982-8.
5. Nguyen A, Ho L, Wan Y. Chemotherapy and oncolytic virotherapy: Advanced tactics in the war against cancer. Front Oncol 2014;4:145.
6. Diaby V, Tawk R, Sanogo V, Xiao H, Montero AJ. A review of systematic reviews of the cost-effectiveness of hormone therapy, chemotherapy, and targeted therapy for breast cancer. Breast Cancer Res Treat 2015;151:27-40.
7. Valenti RB. Chemotherapy education for patients with cancer: A literature review. Clin J Oncol Nurs 2014;18:637-40.
8. Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochim Biophys Acta 2013;1830:3670-95.
9. Patel S, Goyal A. Recent developments in mushrooms as anti-cancer therapeutics: A review. 3 Biotech 2012;2:1-15.
10. Patel S, Rauf A, Khan H, Khalid S, Mubarak MS. Potential health benefits of natural products derived from truffles: A review. Trends Food Sci Technol 2017;70:1-8.
11. Veeraraghavan VP, Hussain S, Papayya Balakrishna J, Dhawale L, Kullappan M, Mallavarapu Ambrose J, *et al.* A comprehensive and critical review on ethnopharmacological importance of desert truffles: *Terfezia claveryi, Terfezia boudieri,* and *Tirmania nivea.* Food Rev Int 2021:1-20.
12. Jafari S, Saeidnia S, Abdollahi M. Role of natural phenolic compounds in cancer chemoprevention via regulation of the cell cycle. Curr Pharm Biotechnol 2014;15:409-21.
13. Farag MA, Fathi D, Shamma S, Shawkat MSA, Shalabi SM, El Seedi HR, *et al.* Comparative metabolome classification of desert truffles *Terfezia claveryi* and *Terfezia boudieri* via its aroma and nutrients profile. LWT 2021;142:111046.
14. Dahham SS, Al-Rawi SS, Ibrahim AH, Abdul Majid ASA, Abdul Majid AMS. Antioxidant, anticancer, apoptosis properties and chemical composition of black truffle *Terfezia claveryi.*

Saudi J Biol Sci 2018;25:1524-34.

15. Attia WY, El-Naggar RE, Bawadekji A, Al Ali M. Evaluation of some *in vitro* anti-carcinogenic activities of polysaccharides extracted from ascomata of the desert truffle *Terfezia claveryi* Chatin. J Appl Environ Biol Sci 2018;8:152-9.
16. Frajacomo FT, de Souza Padilha C, Marinello PC, Guarnier FA, Cecchini R, Duarte JA, *et al.* Solid Ehrlich carcinoma reproduces functional and biological characteristics of cancer cachexia. Life Sci 2016;162:47-53.
17. Ezatpour B, Saedi Dezaki E, Mahmoudvand H, Azadpour M, Ezzatkhah F. *In vitro* and *in vivo* antileishmanial effects of Pistacia khinjuk against Leishmania tropica and Leishmania major. Evid Based Complement Alternat Med 2015;2015:1-6.
18. Nazer MR, Jahanbakhsh S, Ebrahimi K, Niazi M, Sepahvand M, Khatami M, *et al.* Cytotoxic and antileishmanial effects of various extracts of *Capparis spinosa L.* Turk J Pharm Sci 2021;18:146-50.
19. Singleton VL, Orthofer R, Lamuela-Raventós RM.[14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol 1999;299:152-78.
20. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. ScientificWorldJournal 2020;2020:8780704.
21. Broadhurst RB, Jones WT. Analysis of condensed tannins using acidified vanillin. J Sci Food Agric 1978;29:788-94.
22. Sharawi ZW. Therapeutic effect of Arthrocnemum machrostachyum methanolic extract on Ehrlich solid tumor in mice. BMC Complement Med Ther 2020;20:153.
23. Metwally FM, El-Mezayen HA, Abdel Moneim AE, Sharaf NE. Anti-tumor effect of Azadirachta indica (Neem) on murine solid Ehrlich carcinoma. Acad J Cancer Res 2014;7:38-45.
24. Shakibaie M, Shahverdi AR, Faramarzi MA, Hassanzadeh GR, Rahimi HR, Sabzevari O. Acute and subacute toxicity of novel biogenic selenium nanoparticles in mice. Pharm Biol 2013;51:58-63.
25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
26. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 1982;126:131-8.
27. Luck H. Catalase. *In: Bergmeyer HU, editor.* Methods of Enzymatic Analysis. New York: Academic Press; 1965. p. 855-88.
28. Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc 2010;5:51-66.
29. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497-500.
30. Selim NM, Elgazar AA, Abdel-Hamid NM, El-Magd MRA, Yasri A, Hefnawy HME, *et al.* Chrysophanol, physcion, hesperidin and curcumin modulate the gene expression of pro-inflammatory mediators induced by LPS in hepg2: *In silico* and molecular studies. Antioxidants (Basel) 2019;8:371.
31. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as anticancer agents. Nutrients 2020;12:457.
32. Anantharaju PG, Gowda PC, Vimalambike MG, Madhunapantula SV. An overview on the role of dietary phenolics for the treatment of cancers. Nutr J 2016;15:99.
33. Saravanan R, Viswanathan P, Pugalendi KV. Protective effect of ursolic acid on ethanol-mediated experimental liver damage in rats. Life Sci 2006;78:713-8.
34. Gupta M, Mazumder UK, Kumar RS, Sivakumar T, Vamsi ML. Antitumor activity and antioxidant status of Caesalpinia bonducella against Ehrlich ascites carcinoma in Swiss albino mice. J Pharmacol Sci 2004;94:177-84.
35. Abou Zaid OA, Hassanein MR, El-Senosi YA, El-Shiekha MF. Biochemical effect of some antioxidant on metabolic changes in experimentally induced tumor in female mice. Benha Vet Med J 2011;1:52-60.
36. Hussein SA, Azab ME. Effect of insulin treatment on some metabolic changes on experimentally induced tumor in female mice. Egypt J Biochem Mol Biol 1997;15:61-80.
37. Chen W, Jia Z, Pan MH, Anandh Babu PV. Natural products for the prevention of oxidative stress-related diseases: Mechanisms and strategies. Oxid Med Cell Longev 2016;2016:4628502.
38. Neggaz S, Fortas Z, Chenni M, El Abed D, Ramli B, Kambouche N. *In vitro* evaluation of antioxidant, antibacterial and antifungal activities of Terfeziaclaveryi Chatin. Phytothérapie 2018;16:20-6.
39. Yoshida Y, Niki E. Antioxidant effects of phytosterol and its components. J Nutr Sci Vitaminol (Tokyo) 2003;49:277-80.
40. Kim SK, Karadeniz F. Biological importance and applications of squalene and squalane. Adv Food Nutr Res 2012;65:223-33.
41. Balkwill F. TNF-α in promotion and progression of cancer. Cancer Metastasis Rev 2006;25:409-16.
42. Aldubayan MA, Elgharabawy RM, Ahmed AS, Tousson E. Antineoplastic activity and curative role of avenanthramides against the growth of Ehrlich solid tumors in mice. Oxid Med Cell Longev 2019;2019:5162687.
43. El-Dayem SM, Fouda FM, Ali EH, Motelp BA. The antitumor effects of tetrodotoxin and/or doxorubicin on Ehrlich ascites carcinoma-bearing female mice. Toxicol Ind Health 2013;29:404-17.
44. Sankari SL, Masthan KM, Babu NA, Bhattacharjee T, Elumalai M. Apoptosis in cancer–An update. Asian Pac J Cancer Prev 2012;13:4873-8.
45. Koff JL, Ramachandiran S, Bernal-Mizrachi L. A time to kill: Targeting apoptosis in cancer. Int J Mol Sci 2015;16:2942-55.
46. Dyary HO. Subacute toxicity of brown truffle (*Terfezia claveryi*) on Sprague-Dawley rats. Iraqi J Vet Med 2020;44:103-12.