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ORIGINAL ARTICLE

Chemopreventive Strategies of *Teucrium alopecurus* de Noé Water Insoluble Fraction for Hepatocarcinogenesis in Lipopolysaccharide-Induced Mice Models: Pharmacological Attributes

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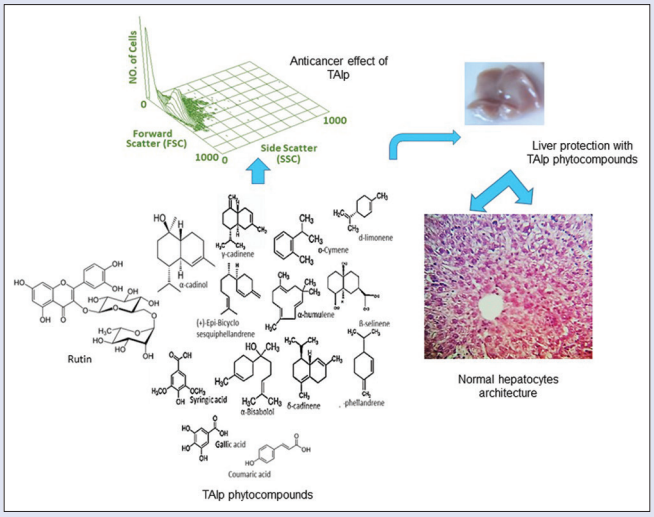
ABSTRACT

Background: In Tunisia, the ethnobotanical study and biological activity of *Teucrium alopecurus* (TAlp), belonging to *Labiatea* family, is poorly recorded. **Objectives:** The goal of this study is to investigate the inhibitory potential of TAlp in lipopolysaccharide (LPS)-induced hepatic damage. **Methods:** Cell viability was measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylbromide (Thiazolyl blue tetrazolium blue) assay. We treated tumor cells with TAlp essential oil (0.005 and 0.01 μg/ml) and curcumin (10 μM). Apoptosis was determined by flow cytometry. Therefore, we carried out the *in vivo* malignant hepatoma induced by LPS and the preventive effect of water-insoluble fraction extracted from aerial parts of TAlp. Moreover, the hepatoprotective effect was investigated by the determination of serum levels of lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase. **Results:** Our results indicated the significant anti-proliferative effects of TAlp on colon tumor cell lines alone or in combination with curcumin. Moreover, hepatocellular inflammation induced by LPS increases metastasis in liver cells and was suppressed by intragastric administration of TAlp essential oil. Inflammation is tightly associated with carcinogenesis at both the initiation and development of tumor. **Conclusion:** Overall, the Tunisian plants showed potency in the prevention of hepatocarcinogenesis.

Key words: 5-Fluorouracil, alanine aminotransferase, aspartate aminotransferase, hepatocarcinogenesis, lactate deshydrogenase, lipopolysaccharide, *Teucrium alopecurus*

SUMMARY

- The pharmacological inhibition of lipopolysaccharide-induced inflammation
- The pathological effects in the liver
- The preventive effect of *Teucrium alopecurus* that attenuates hepatic carcinoma induced by bacterial lipopolysaccharide by triggered apoptotic cell death and this by reduction of serum levels of aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase.



Abbreviations used: DCF-DA: Dichlorodihydrofluorescein diacetate; BSA: Bovine serum albumin; DMSO: Dimethyl sulfoxide; DMEM: Dulbecco's modified Eagle's medium; FBS: Fetal bovine serum; PBS: Phosphate buffered saline; SDS: Sodium dodecyl sulfate; IMDM: Iscove's modified Dulbecco's medium.

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INTRODUCTION

Cancers are heterogeneous populations of cells at multiple differentiation stages that could be the result of acquired mutations and epigenetic differences.[1] Hepatocarcinogenesis (HCC) is a complex multistep process including genetic change accumulation and resulting in altered cancer-related genes expression, such as oncogenes and tumor suppressor genes and their related molecular signaling pathways.[2] Oxidative stress in hepatic tissues significantly influences every of HCC pathology stage in particular the augmentation of tumor cell invasion and metastasis and progression via clonal expansion and.[3] Cell proliferation inhibition and tumor development is one of the primary goals of cancer therapy.

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Biocompounds derived from Mother nature effectively inhibit hepatocellular carcinoma.[4] Cancer worldwide rising burden calls for an alternative treatment therapy.[5] Antioxidants derived from plants prevent progression to HCC.[3] Biocompounds isolated from the plant material are used to induce apoptosis in tumor cells.[5] Curcumin, obtained from *Curcuma longa* plants, has been paid great interest by many scientists in the field of cancer. Strikingly, curcumin is a good alternative to current therapies as a result of its relatively safe profile, even at high doses.[6] It induces BID cleavage, caspase-8, 3, and 9 activation, PARP cleavage, loss of mitochondrial membrane potential in tumor cells, opening of transition poresand cytochrome C release, and inhibitor of caspase-activated deoxyribonuclease.[7]

The essence or volatile oil can be synthesized by several plant parts and stored in glandular trichomes.[8] The species from the *Lamiaceae* family had the same anatomical features.[9] The macro-and–micromorphological observation of different organs of several species are very interested to characterize several taxa of this family. Several *Lamiaceae* species contain high content of essential oils, which are used in perfumery, pharmaceutical preparations, and cosmetics.[10] The essential oil, as a natural metabolic secretion of aromatic plants, is produced by secretory structures of different plant and tree parts including leaf, root, flower, seed, wood, and fruit.[11] Recent reports show that the phytotherapeutic effect and ontogenic feature of this species were of considerable biological importance and that its aromatic parts were distillated to obtain essential oil, used as anti-inflammatory and anticancer agent.[12] Morphological features of all parts of this species were described (Under review).

The aim of the current report was to evaluate the pharmacological inhibition of inflammation induced by lipopolysaccharide (LPS), with an emphasis on the histopathological effects in the liver. The water-insoluble fractions of TAlp attenuates hepatic carcinoma induced by bacterial LPS by triggered apoptotic cell death and this by reduction of serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate deshydrogenase (LDH).

MATERIALS AND METHODS

Plant materials

Teucrium alopecurus De Noé (n. 1122) was identified and authenticated by Hamdi Lazhar, Engineer and Director of the Natural Park of Bouhedma, Sidi Bouzid, Tunisia. The aerial parts were dried in shade and ground into little pieces. Plant material was subjected to distillation with Clevenger apparatus. For hydrdistillation, plant powder was put in distilled water in a flask, and essential oils were extracted for 3 h. The essences with percent yield of 2.8% were kept in the dark at +4°C in the fridge pending *in vitro* and *in vivo* analysis.

Cell lines

The tumor cell lines HCT116 (colon cancer cell) and KBM-5 (human myeloid leukemia). Cells were purshased from the American Type Culture collection (Manassas, VA, USA). HCT116 cells were cultivated in DMEM supplemented with 10% Fetal Bovine Serum (FBS) and KBM-5 cells were cultured in IMDM supplemented with 15% FBS.

Reagents

Water Insoluble fraction of TAlp aerial parts (100 µg/mL) and curcumin (100 mM) were prepared in dimethyl sulfoxide (DMSO) (≤0.05%), stored at 20°C and then diluted as needed. Bovine serum albumin was obtained from Atlanta Biologicals (Norcross, GA, USA). DMSO, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Sodium dodecyl sulfate (SDS) (20%), 5-FU (purity >99%) and LPS (*Escherichia coli* 055:B5) (10 µg/ml) dissolved in physiological saline were purchased from Sigma-Aldrich Chemicals Co.(St. Louis, MO, USA). Medium (IMDM, RPMI 1640, DMEM), FBS (15%), phosphate-buffer solution PBS (5 mg/ml), Tween 80 (2%), Penicillin and Streptomycin were obtained from Mediatech, Inc.(Herndon, VA, USA).

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylbromide assay

5000 Cells/well were seeded in 96-well plates and challenged the next day with curcumin (100 mM), TAlp (0.5–10 pg/ml) or combination in triplicates, for 24, 48 and 72 h in 96-well plates at 37°C. Cell viability was evaluated after stimulation using MTT staining. MTT solution prepared with PBS was added to each well. Then, extraction buffer solution that contain SDS (20%) and dimethyl formamide (50%) was added after 2 h of incubation. The mixture was incubated overnight at 37°C. OD were read at 570 nm using an MRX Revelation 96-well multiscanner (Dynex Technologies; MRX Revelation) apparatus.

Clonogenic ability

Five hundred colon cancer cells per well were seeded in 6-well plates. After overnight incubation, tumor cells were treated with different doses of TAlp (0–80 pg/ml) and curcumin (0–20 µM) for 24 h. Media was replaced with fresh media and maintained until day 9. The colonies, stained with crystal violet (0.5%), were quantified.

Teucrium alopecurus and curcumin induce production of reactive oxygen species

Twenty micromolar dichlorodihydrofluorescein diacetate were used to label 1×10^6 colon cancer (HCT-116) cell lines which were exposed for 1 h to TAlp and curcumin. ROS production was measured by flow cytometry.

Flow cytometry analysis

To investigate the density of colon cancer cells after various treatment conditions with TAlp (10–200 pg/ml) and Cur (10 µM) we used density plots through the combination of forward scatter versus side scatter.

In vivo hepatoma induced by lipopolysaccharide and protective potential of *Teucrium alopecurus*

Animals

Thirty-six albino mice (20 ± 1.00 g; 5-week-old) housing six per cage were provided by Pasteur Institute, Tunisia (Ethic# LNSP/Pro 152012). Mice were allowed *ad libitum* to access to food pellets and tap water and maintained in cage made with polypropylene under 12 h light/dark cycle and relative humidity of 55%±10% at 20°C–25°C.

Experimental design

To test whether treatment with TAlp suppressed LPS-induced liver inflammation in mouse, both sexes of Swiss albino mice, randomized into six groups (6 mice/group), were orally treated with drugs through 7 days. Group 1 (normal control), received an orally treatment of saline vehicle; Group 2 (tumor control), received an orally treatment of 10 µg/ml LPS (*E. coli* 055:B5); Group 3 and Group 4, served as drug control, treated solely by gavage of TAlp (20 and 50 µg/kg/day, respectively). Group 5 (comparator control), were intragastrically treated by 5-FU (20 mg/kg/day). For Group 6 and Group 7, mice were intragastrically treated by both TAlp (20 and 50 µg/kg/day) and LPS (10 µg/ml) respectively. Group 8 were intragastrically treated by LPS (10

μg/ml) and 5-Fu (20 mg/kg/day). Orally treatments were given daily, 1 h before LPS administration. Hydrophobic fractions were diluted with 2% Tween 80. Food intake, water consumption and body weight of experimental mice were recorded daily before the intragastric treatments. The animal experimentation process were done according to Guide for the Care and Use of Laboratory Animals approved by the Ethics Committee of Animals.

Histopathological analysis

After either 1 week, the animals were placed under a bell jar with a sponge soaked in ether for anesthesia. Mice were sacrificed and lying on its back hepatic tissues were collected to be analyzed macroscopically and liver injury were photographed (LP_N-50).

Liver sampling were fixaed in phosphate-buffered paraformaldehyde (10%). The samples were then dehydrated with graded alcohol (30°–100°). Thereafter, traces of alcohol were removed and hepatic portions were impregnated with molten paraffin (58°C) and included in special paraffin molds and blocking that will be debited on a knife or razor blade. The resulting paraffin sections (5 μm) were sticked together and placed on a glass slide. Sample rehydratation allows the elimination of intracellular paraffin by immersing the slides in alcohol baths of decreasing degrees (100° to 50°), then in distilled water. Finally, sections were stained with h and e. Liver sections were embedded in synthetic resin and observed microscopically between slide and coverslip using a light microscope (Motic SFC-28 SERIES (Mains voltage 220–240V, 50–60HZ; Lamp 12V, 20W)) through various objectives to obtain different magnifications.

Biochemical studies

Blood samples of all mice groups were collected using a syringe and centrifuged to separate serum to measure liver function biochemical parameters (AST, ALT, LDH). The levels of aminotransferases (AST, ALT) that catalyze the transfer of the group α-amino to the group α-keto and LDH were evaluated using automatic biochemical analyzer (Hitachi, Ltd.).

Statistical analysis

Statistical analysis was performed by GraphPad Prism 4.00. Data presented are the mean ± standard error of the mean of experiments done in triplicate. To compare different groups with each other we use a one-way analysis of variance and Tukey's test. $P < 0.05$ was significant.

RESULTS

Teucrium alopecurus and curcumin suppress tumor cell growth

To determine the cell viability inhibition of TAlp phytocompounds [Figure 1a] on various tumor cells and the combination of TAlp and Curcumin on colon cancer cells (HCT116), we used MTT

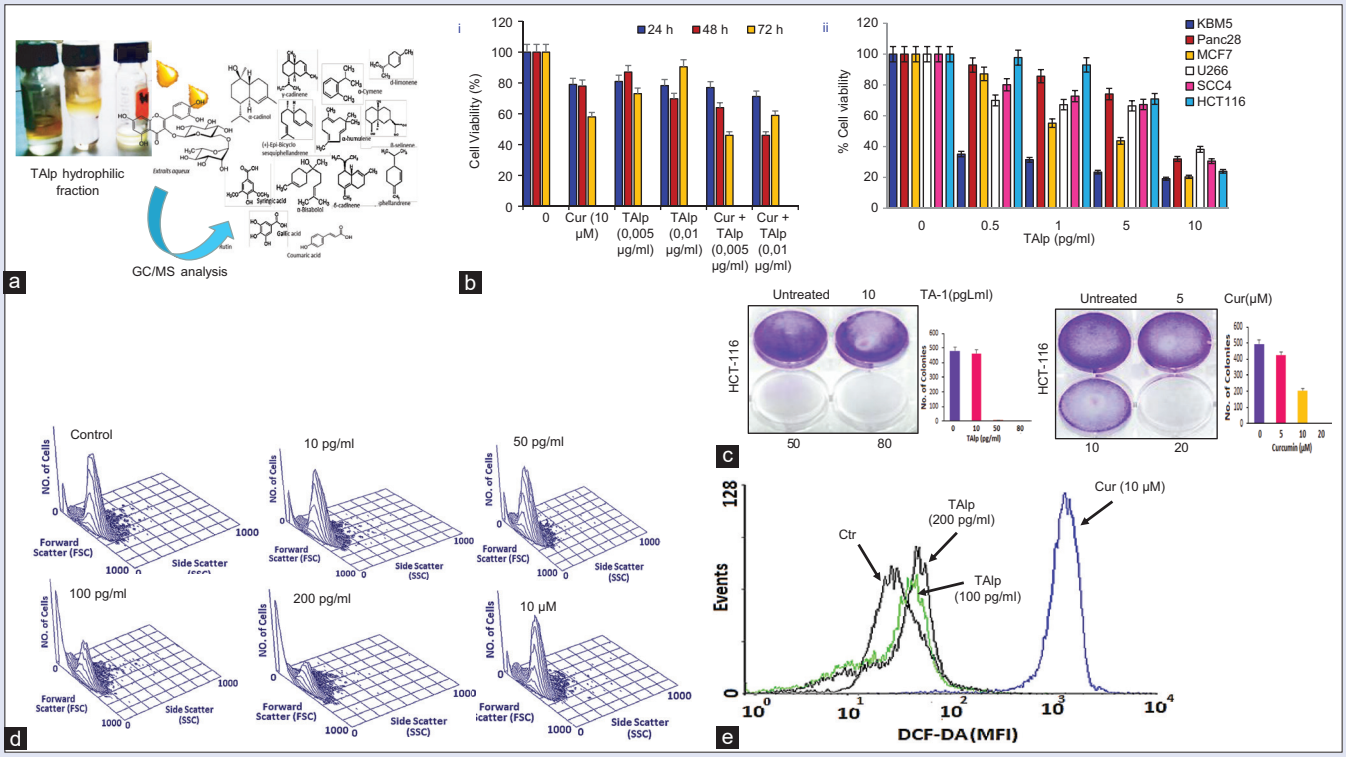

Figure 1: (a) Structure of terpenic compounds identified in *Teucrium alopecurus*. (b) *Teucrium alopecurus* induces cell survival inhibition (i) and enhances Curcumin-induced cytotoxicity (ii). Colon cancer cells (HCT116) were pretreated with indicated dosesof *Teucrium alopecurus* and Curcumin for 72 h. Cell viability was analyzed by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylbromide assay. (c) *Teucrium alopecurus* and curcumin inhibit clonogenic potential of HCT-116. (d) Density plots analysis. *Teucrium alopecurus* and Curcumin significantly decrease the KBM cell numbers in dose-dependent manner. Human myeloid leukemia (KBM-5) cells (1×10^6/ml) were incubated in the presence or absence of different concentrations of *Teucrium alopecurus* and with curcumin. After that, cancer cells were washed, fixed and analyzed for cell number by flow cytometric analyses. Assays were done three times and cell numbers were graphically represented using the combination forward scatter versus Side scatter. (e) *Teucrium alopecurus* and Curcumin induce production of ROS. HCT116 (1×10^6 cells) were treated with *Teucrium alopecurus* (200 pg/ml) and curcumin (10 μM) for 1 h, labeled with dichlorodihydrofluorescein diacetate and then examined for ROS production by flow cytometer

assay. Figure 1bi shows that TAlp exhibited dose-dependent cytotoxicity of tumor cell lines. As Figure 1bii shows, the indicated doses of curcumin and TAlp induce the reduction of cell survival and a significant ($P < 0.05$) cytotoxic effect of HCT116 cells in a time-dependent manner. KBM-5 cell line was the most sensitive at doses as low as 5 pg/ml of Teucrium that inhibited cell viability, whereas at 10 pg/ml, essential oil was required to suppress other cell lines growth.

Reduction of colony-forming ability

Teucrium essential oil induced the reduction of the ability of HCT116 cell lines to form colonies. As indicated in Figure 1c, we found that treatment with TAlp and curcumin induce dose-dependent repression of colony formation. TAlp had the most pronounced reduction of colony formation in comparison with the curcumin and the control, even at 50 and 80 pg/ml.

Density plots analyses

TAlp and curcumin significantly decrease the KBM cell numbers in dose-dependent manner [Figure 1d].

Flow cytometry analysis with fluorescence-activated cell sorting

Phytocompounds of Teucrium oily fraction and curcumin induce apoptosis through ROS production in HCT116 [Figure 1e]. ROS levels increased in HCT116 cells treated with curcumin (10 μM) compared to the TAlp (200 pg/ml) and control.

Body weight, food intake and histopathologic appearance of liver tumorigenesis

The scheme represented in Figure 2a depicts the protective effect of T. alopecurus essential oil. As seen in Figure 2bi, control and TAlp-treated mice gained weight during the experimental duration, whereas 10 μg/ml LPS-treated mice significantly ($P < 0.05$) lost the body weight.

The gain in food intake and water consumption in LPS treated groups was significantly ($P < 0.05$) lower than that in the control group. Administration of positive (tumor) control (LPS) at10 μg/ml resulted in significant decrease in the food intake ($P < 0.05$) [Figure 2bi, iii].

As shown in Figure 3, oral administration of LPS to mice leads to liver carcinoma and pretreatment with TAlp protect liver tissues from inflammation and damage. Macroscopic analysis taken from control and experimental-treated liver tissues are presented in Figure 3a. Normal control group shows normal lobular architecture [Figure 3bi]. Administration of TAlp protects mice from LPS-induced liver injury and inflammation. The photomicrograph overview of normal hepatocytes was detected in TAlp-treated mice [Figure 3bii, iii and 3c]. Hepatocellular degeneration was observed for LPS-treated mice. Notably, intragastric administration of LPS to mice triggers tumor formation and lead to HCC; otherwise, we detected altered hepatocytes and abnormal mononuclear cells [Figure 3bv]. Furthermore, less protective effect was observed in 5-Fu-pretreated mice [Figure 3bvi]. In fact, TAlp-pretreated mice protect from developing liver inflammation and carcinoma [Figure 3bvii, viii].

Liver biomarkers in serum

The results showed that intragastric administration of TAlp (50 μg/ml) for 1 week exhibited a significant ($P < 0.05$) decrease in serum

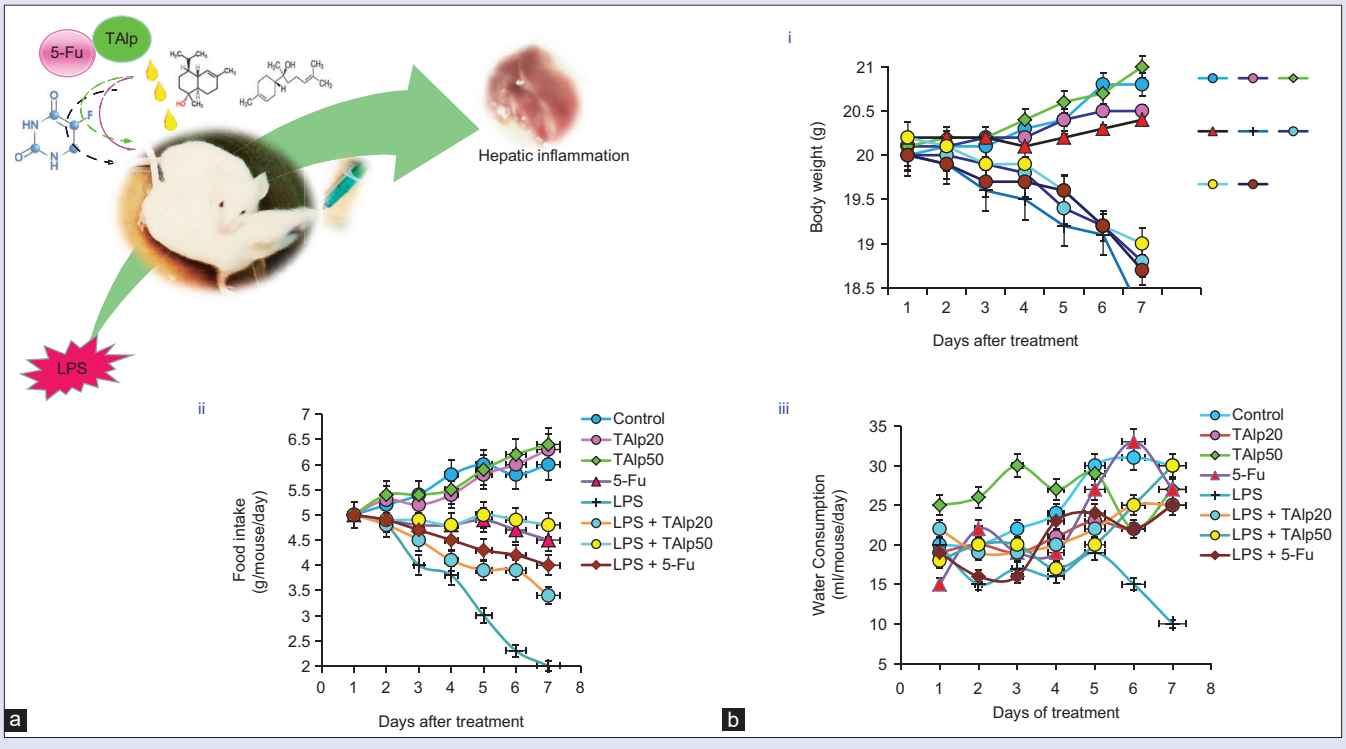

Figure 2: (a) Experimental design of *in vivo* study using mice model and proposed strategy of therapeutic effect of *Teucrium alopecurus* essential oil and 5-Fu. (b) Change of body weight (i), food intake (ii) and water consumption (iii) of mice with the gavage of *Teucrium alopecurus* in LPS-induced liver cancer during 1 week of treatment. Body weight was each days. There were significant differences in body weight between the groups

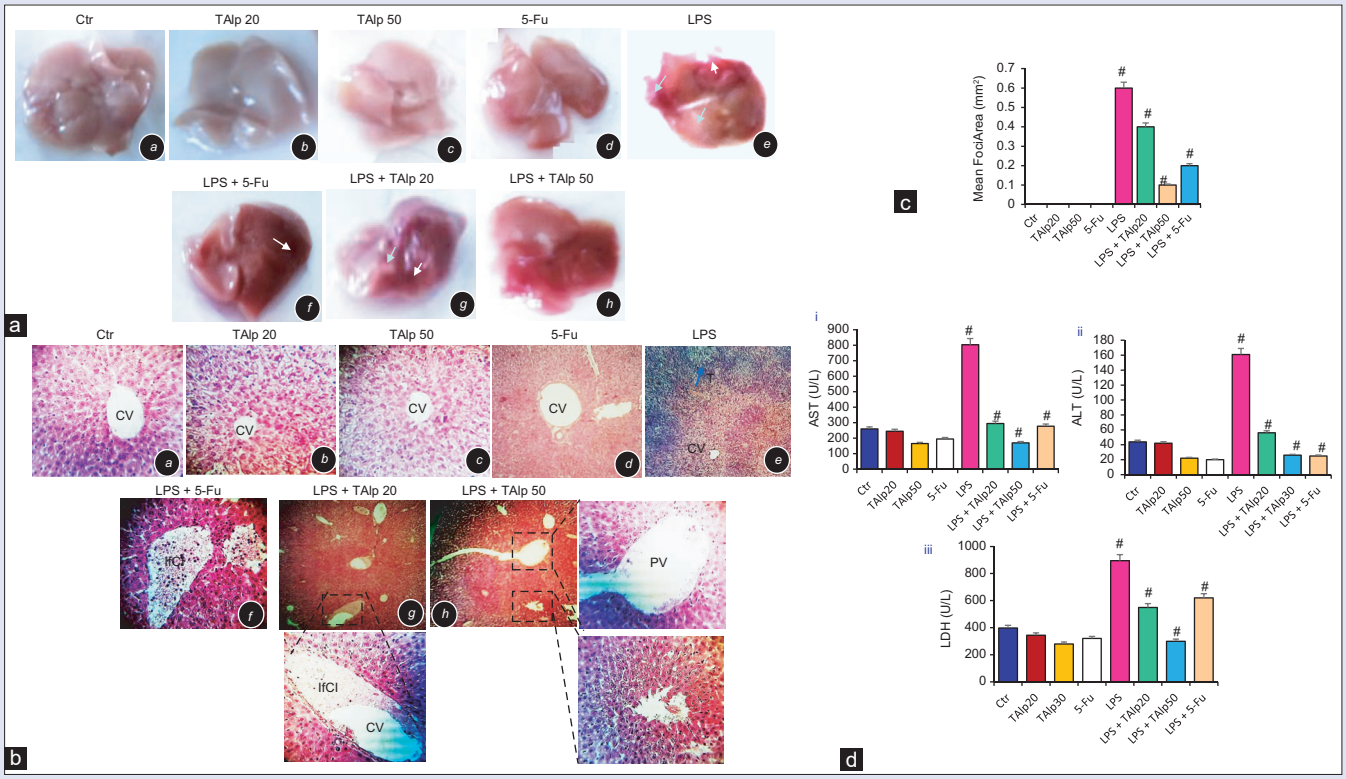

Figure 3: (a) Macroscopical view of liver resected from mice. (b) Histological appearance of H and E stained liver sections of control mice (i), mice treated with different doses of TAlp essential oil (ii and iii), 5-Fu (iv), LPS (v), LPS + TAlp (vi and vii) and LPS + 5-Fu (viii). (c) Mean area (mm²) of liver foci. (d) Effect of TAlp on biochemical parameters. CV: Central veine; PV: Portal veine, IfCI: Inflammatory cellular infiltration; AMC: Abnormal mononuclear cells; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LDH: Lactate dehydrogenase. $n \geq 5$ animals/group, $P < 0.05$ Ctr versus LPS, TAlp versus LPS, LPS + TAlp and LPS + TAlp in mice models. Values are presented as mean ± SEM, #LPS versus LPS + TAlp20, LPS + TAlp50 and LPS + 5-Fu. Significant data was at $P < 0.05$

AST [Figure 3di], ALT [Figure 3dii] and LDH [Figure 3diii] levels as compared to the LPS-administered mice. However, treatment of mice with low dose of TAlp and LPS induced moderate effects on the enzyme levels compared to mice treated with antitumor drug 5-Fu.

DISCUSSION

Uncontrolled cell proliferation and tumor growth are the main characteristics of cancer.[4] Liver tumor develops from the pre-cancerous stages due to the accumulation of a series of genetic and cellular alterations.[13] LPS, the major endotoxin of the Gram-negative bacteria cell wall, is considered as a key stimulator of pathogenicity for the dysfunctions.[14] It is one of the most effective triggers of host inflammatory responses and LPS-induced production of cytokine is regulated at both the transcriptional and post-transcriptional levels. Transcription of cytokine genes is controlled by NF-κB, C/EBPδ and ATF-3.[15] Drugs with chemical ingredients used to treat liver diseases have shown life threatening situations and therefore, the preference is being shifted to either natural products or their derivatives.[16] Medicinal herbs promise a huge anticancer potential.[5]

Microscopic analysis showed that livers of LPS-treated mice contain massive cell infiltration and severe injury.[17] LPS-mediated responses including changes in inflammatory cytokines, electrophysiological properties, cell-injury, necrosis and apoptosis.[14] Inhibition of metastasis is linked to a decrease in global chromatin accessibility and reduced expression of genes that inhibit cancer metastasis.[2]

Apoptosis is one of the most frequently studied cell processes for elucidating the effective mechanisms of herbal compounds against HCC.[4] The antitumor potential of curcumin on human breast cancer cell lines (MCF-7) were investigated through MTT and lactate dehydrogenase assays to assess cell viability and cytotoxicity, respectively.[5]

This report investigated a significant effect of oily fractions of TAlp and 5-Fu on the treatment of malignant hepatoma development in animals after consumption of LPS. In the comparator group (5-FU), the histopathological changes were consistent with slightly active hepatitis. Consistent with these observations, the biochemical data obtained after treating mice with 20 and 50 µg/kg/day of TAlp alone or the combination with bacterial LPS suggest a significant reduction in the values of AST, ALT and LDH. Another phytotherapeutic effect of wild edible plant terpenes and hydrolate of TAlp was undertaken to explore the antioxidant and antibacterial potential of essential oil and to trigger apoptosis and suppress inflammation and tumorigenesis in human colon cancer cells and animal models (Guesmi *et al.* 2018).

Additionally, we found that TAlp alone reduced human colon cancer (HCT116) cells proliferation *in vitro*. We also found that liver inflammation and enzyme levels were elevated in the serum and hepatic tissues, respectively, of 5-Fu-treated mice in comparison with LPS treated control animals.

Moreover, TAlp can inhibit tumorigenesis and cytotoxicity *in vivo* by reducing the enzyme levels (AST, ALT and LDH). Our study, however, is the first to show the potent effect of TAlp in inhibiting the progression of liver tumor in a mouse model. The data corroborate with the literature done by Meireles *et al.*[18] who shows inflammation within the portal spaces, peri-septal necrosis featuring discrete (piecemeal) areas of hepatic cytolysis, in the liver of animals treated with 5-FU, peri-portalinflammation, and focal hepatocyte necrosis surrounded by

hepatocellular polyploidy and lymphohistiocytic aggregates phenomena. Apoptosis induced by blocking NFκB sensitizes cancer cells to treatment with 5-FU.[19] Upon stimulation by TNF or LPS, transforming growth factor-β-activated kinase 1 plays an important role in the NF-κB activation and prevents tumor through preventing apoptosis of cholangiocyte and Caspase-3-dependent hepatocyte.[2] Consumption of Chronic ethanol mediates liver inflammation through increased gastrointestinal permeability and subsequent translocation of bacterial endotoxin (LPS) to the liver.[3]

Multistep process of hepatocarcinoma has been recapitulated using genetically engineered mouse models to understand it and to design experimental therapeutics. Whereas, heterogeneity of human liver cancer was highly detected at molecular levels that enable HCC mouse models to recapitulate only subsets of molecular events occurring inpatients with HCC.[2] Through the study of Rabachini *et al.*[20] inhibition of apoptosis and, consequently, compensatory hepatocellular proliferation and inflammation is highly related to reduced hepatic cancer growth.

Our findings suggests that essential oil administration protects mice from LPS-induced liver inflammation *in vivo*, further supporting the anticancer effect of Teucrium. Thus, the underlying mechanism by which the aromatic therapy may be a potent strategy for preventing associated liver inflammation seems to be through phytocompounds. It is interesting to note that the preventive effect of TAlp could be related to the richness of essential oil with terpenes. The biocompound limonene represses liver carcinogenesis through inhibition of the association of Ras plasma membrane.[21] Furthermore, Chirumbolo[22] reported that alpha-bisabolol, a small, plant-derived, reduces the mass of mammary cancer in mice and promotes the response of natural killer cells.

The known polyphenolic compound Curcumin isolated from *Curcuma longa* specie inhibit proliferation of several tumor cells, down-regulate transcription factors (AP-1, NF-κB, Egr-1); the expression of chemokines (COX2, LOX, NOS, MMP-9, uPA, TNF), cyclin D1 and cell surface adhesion molecules; down-regulate growth factor receptors (EGFR and HER2); and inhibit c-Jun N-terminal kinase, protein tyrosine kinases and protein serine/threonine kinases activities.[7] A recent study suggested the potent effects of curcumin on the activation of NF-κB and STAT-3, NF-κB-and STAT-3-regulated gene products and cell growth and the induction of apoptosis and the inhibition of proliferation in human biliary cancer cells.[23] Our data are consistent with the report done by Gupta *et al.*[24] who found that, curcumin was shown to inhibit the gastric cancer cell transition from the G1 to S phase.

CONCLUSION

Taken together, these data indicate that TAlp essential oil has been shown to have promising antitumor potential upon several tumor cell lines and a therapeutic use to treat liver inflammation. It appears that the terpenic content of this specie has been reported to inhibit carcinogenesis and induced tumor cell death. In addition, the safety and the absence of side effects of Teucrium essential oil may lead to its use for human therapy. Study limitations include the lack of clinical strategies on the field of hepatoprotective effect of several species in the african Mother nature including TAlp.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression and resemble stages in neurogenesis. Cancer Cell 2006;9:157-73.
2. Yim SY, Lee JS. Genomic perspective on mouse liver cancer models. Cancers 2019;11:1648.
3. Brandon-Warner E, Eheim AL, Foureau DM, Walling TL, Schrum LW, McKillop IH. Silibinin (Milk Thistle) potentiates ethanol-dependent hepatocellular carcinoma progression in male mice. Cancer Lett 2012;326:88-95.
4. Hu B, An HM, Wang SS, Chen JJ, Xu L. Preventive and therapeutic effects of Chinese herbal compounds against hepatocellular carcinoma. Molecules 2016;21:142.
5. Khan T, Ali M, Khan A, Nisar P, Jan SA, Afridi S, *et al.* Anticancer plants: A review of the active phytochemicals, applications in animal models and regulatory aspects. Biomolecules 2020;10:47.
6. Iqbal B, Ghildiyal A, Singh S, Siddiqui S, Kumari P, Arshad M, et al. Combinatorial effect of curcumin and tumor necrosis factor-α-related apoptosis-inducing ligand (TRAIL) in induction of apoptosis via inhibition of nuclear factor kappa activity and enhancement of caspase-3 activity in chronic myeloid cells: An *in-vitro* study. J Can Res Ther 2018;14:S1193-200.
7. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: Preclinical and clinical studies. Anticancer Res 2003;23:363-98.
8. Russo R, Corasaniti MT, Bagetta G, Morrone LA. Exploitation of cytotoxicity of some essential oils for translation in cancer therapy. Evid Based Complement Altern Med 2015;2015:397821.
9. Dinç M, Doğu S. Anatomical and micromorphological studies on *Teucrium* sect. *Isotriodon* (*Lamiaceae*) in Turkey with a taxonomic note. Biologia 2012;67:663-72.
10. Ascensão L, Mota L, Castro M De M. Glandular trichomes on the leaves and flowers of *Plectranthus ornatus*: Morphology, distribution and histochemistry. Ann Bot 1999;84:437-47.
11. Mejri J, Aydi A, Abderrabba M, Mejri M. Emerging extraction processes of essential oils: A review. Asian J Green Chem 2018;2:246-67.
12. Guesmi F, Tyagi AK, Prasad S and Landoulsi L. Terpenes from essential oils and hydrolate of *Teucrium alopecurus* triggered apoptotic events dependent on caspases activation and PARP cleavage in human colon cancer cells through decreased protein expressions. Oncotarget 2018;9:32305-20.
13. Mathew S, Faheem M, Suhail M, Fatima K, Archunan G, Begum N, *et al.* Updates on traditional medicinal plants for hepatocellular carcinoma. Pharmacogn J 2016;8:203-14.
14. Yücel G, Zhao Z, El-Battrawy I, Lan H, Lang S, Li X, *et al.* Lipopolysaccharides induced inflammatory responses and electrophysiological dysfunctions in human-induced pluripotent stem cell derived cardiomyocytes. Sci Rep 2017;7:2935.
15. Li Y, Huang X, Huang S, He H, Lei T, Saaoud F, *et al.* Central role of myeloid MCPIP1 in protecting against LPS induced inflammation and lung injury. Signal Transduct Targeted Ther 2017;2:e17066.
16. Latief U, Ahmad R. Herbal remedies for liver fibrosis: A review on the mode of action of fifty herbs. J Tradit Complement Med 2018;8:352-60.
17. Kawaguchi K, Kuchi S, Hasegawa H, Maruyama H, Morita H, Kumazawa Y. Suppression of lipopolysaccharide-induced tumor necrosis factor-release and liver injury in mice by naringin. Eur J Pharmacol 1999;368:245-50.
18. Meireles DR, Fernandes HM, Rolim TL, Batista TM, Mangueira VM, de Sousa TK, *et al.* Toxicity and antitumor efficacy of *Croton polyandrus* oil against Ehrlich ascites carcinoma cells. Rev Bras Farmacogn 2016;26:751-8.
19. Huang CY, Yu LC. Pathophysiological mechanisms of death resistance in colorectal carcinoma. World J Gastroenterol 2015;21:11777-92.
20. Rabachini T, Fernandez-Marrero Y, Montani M, Loforese G, Sladky V, He Z, *et al.* BOK promotes chemical-induced hepatocarcinogenesis in mice. Cell Death Dif 2018;25:706-18.

21. Kaji I, Tatsuta M, Iishi H, Baba M, Inoue A, Kasugai H. Inhibition by d-limonene of experimental hepatocarcinogenesis in Sprague-Dawley rats does not involve P21Ras plasma membrane association. Int J Cancer 2001;93:441-4.
22. Chirumbolo S. Alpha-bisabolol, not a matter for cancer therapy. Commentary: "Research on the immunosuppressive activity of ingredients contained in sunscreens". Front Pharmacol 2015;6:96.
23. Prakobwong S, Gupta SC, Kim JH, Sung B, Pinlaor P, Hiraku Y, *et al.* Curcumin suppresses proliferation and induces apoptosis in human biliary cancer cells through modulation of multiple cell signaling pathways. Carcinogenesis 2011;32:1372-80.
24. Gupta SC, Kim JH, Prasad S, Aggarwal BB. Regulation of survival, proliferation, invasion, angiogenesis and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastasis Rev 2010;29:405-34.