Antioxidant and Anti-Inflammatory Activity of Star Anise (Illicium Verum) in Murine Model

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https://dx.doi.org/10.13005/bpj/2445

(Received: 13 April 2022; accepted: 31 May 2022)

Star anise (Illicium verum) is a medium-sized plant that is native to Asia as well as one of the most important medicinal plants used in Chinese herbal medicine. Star anise has bioactive compounds having antioxidant and anti-inflammatory properties. The antioxidant activity of Star anise (Illicium verum) methanolic extract was studied by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2’azinobis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay. The current study aims to investigate the effect of Star anise (Illicium verum) methanolic extract as anti-inflammatory by reducing the concentration of pro-inflammatory cytokines TNF-a and IL 1beta and reducing the oxidative stress by acting as a scavenger because inflammation and oxidative stress can induce each other. The highest free radical scavenging activities were exerted by the APTS method (95.1±0.33 Trolox/g) whereas, the free radical scavenging activities were exerted by ABTS was 77.7±0.30 Trolox/g. The anti-inflammatory activity of the Star anise (Illicium verum) methanolic extract was studied by its ability to inhibit pro-inflammatory cytokines productions (Tumor necrosis factor alpha (TNF-a) and interleukin-1 beta (IL-1ß)) and reduce oxidative stress at different concentrations. Star anise (Illicium verum) methanolic extract significantly reduce the pro-inflammatory TNF-a and IL-1ß production (p<0.05) compared with negative control which is treated with lipopolysaccharide (LPS) and has a similar effect in reducing pro-inflammatory cytokines production similar to the positive control which treated with ascorbic acid. Star anise (Illicium verum) methanolic extract significantly reduces oxidative stress (p<0.05) by reducing antioxidant enzyme activity catalase and glutathione-peroxidase compared with the LPS treated group. In conclusion Star anise (Illicium verum) methanolic extract act as strong antioxidant and anti-inflammatory medicinal plant.

Keywords: 2,2’azinobis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH); interleukin-1 beta (IL-1ß); (lipopolysaccharide) (LPS); T umor necrosis factor alpha (TNF-a); Star anise.

Inflammation is defined as a normal body response to foreign agents, such as microbial pathogens, allergen particles, microorganisms, viruses, injury, and damage to the tissues. Inflammation has two types either acute or chronic inflammation. Acute inflammation is described as the body’s initial response to harmful stimuli, but chronic inflammation is defined as the inflammatory response that causes the body to deteriorate over time, leading to a range of disorders. Uncontrolled inflammation causes disorders including allergies, cardiovascular problems, metabolic dysfunction, cancer, and autoimmune disorder.1,2 There are many ways for controlling and suppressing
overreaction that resulted from the inflammation process such as using medicinal drugs such as steroids, non-steroid anti-inflammatory drugs NSAID, and immunosuppressant drugs that act to suppress the immune system which is associated with unpropitious effects but the long term uses of these medicinal drugs cause unfavorable side effects and tissue damage that affect all human biological systems. Therefore, now there is a need for found a new safer, potent, and gives less toxic effect to use as an anti-inflammatory drug. The ultimate goal is to find the minimum effective dose by the highest effectiveness with minimum unpropitious effects. Alternative and traditional herbal medicines are the main target for producing new drugs with vital effective sources from herbal medication and advice in modern medicine and must prove this herbal medication through scientific experimental methods before using them in human level as anti-inflammatory drugs.

Medicinal plants represent a unique place in human life, more information about the ability for use of these medicinal plants and active ingredients as medicine and treatment of inflammatory and reduced of oxidative stress. Great attention for using medicinal plants in the traditional medicine based drugs because it is easily available and collected, less expensive compared to other synthetic chemical drugs, and also have minimum side effects and large numbers of compounds that can be used to produce effectively safe drugs to treat the various diseases. Therefore, we have a great potential for using medicinal Plants for producing new safe drugs and used for treating acute and chronic infectious diseases.

Cytokine is a chemical or hormones like material produced by the active immune cell, they have a major role in the development and control of inflammatory processes by a cascade very sensitive complex system. Cytokines have two types’ pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines act in the first period of inflammation by induction and progression of the inflammatory process, such as Tumor Necrosis Factor-alpha (TNF-á) and Interleukin-1á and â. Pro-inflammatory cytokines responsible for the modulation of the immune system and play pleiotropic effects on a variety of human cells and play major critical roles in acute and chronic inflammation and abnormal increase in its concentration can lead to autoimmune disease and other inflammatory disorders. Medicinal plants have great effective roles in inhibiting Pro-inflammatory cytokines and decreasing activity with lesser side or no effects such as curcumin, shogaol, paradol, and equol.

Star anise (Illicium verum) is a medium-sized plant that is native to Asia. In traditional medicine, it is used commonly as a spice and to treat gastrointestinal symptoms such as colic and flatulence as well as for spasmodic pain. On the other hand, essential oil of star anise is applied to treat rheumatism, antioxidant, antibacterial, antiviral, antifungal, and anticancer activities.

The current study aims to investigate the antioxidant and anti-inflammatory activities of Star anise methanolic extracts. The levels of pro-inflammatory mediators Tumor Necrosis Factor-alpha (TNF-á) and Interleukin-1á (IL-1á) and the antioxidant enzymes catalase and glutathione peroxidase were measured in Balb-C mice in vivo.

**MATERIAL AND METHODS**

**Extraction of star anise**

The dry fruits of Illicium verum (Star anise) were obtained from a local herb market in Karak, Jordan. A fine powder of dry fruits was prepared using a grinder. Then, 100g of powder was soaked in 500 mL of methanol (99%) for 24 h at room temperature. After 24h, the supernatant was separated and filtered using Whatman filter paper No. 1. The solvent was removed and the dried extract was collected and stored as a liquates in a refrigerator until use.

**Antioxidant activity**

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The total antioxidant of Illicium verum (Star anise) methanolic extract was investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The antioxidant activity of the extract was measured by bleaching a purple-colored DPPH methanol solution. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) acts as an oxidizing agent that can donate an electron to inhibit the free radicals. Thus, the purple color diminishes quickly indicating the potential the antioxidant activity.

In this test, different concentrations of Star anise methanol extract were mixed with 4 ml of DPPH methanol solution (0.004%). The prepared
solutions were incubated at room temperature for 30 min. Then the absorbance of the tested samples was measured at 517 nm. The percentage of free radical’s inhibition by DPPH was calculated using the following equation:

\[
I\% = \frac{[\text{A blank} - \text{A sample}]}{\text{A blank}} \times 100
\]

Where A blank is the absorbance of blank (solution contains all reagents except the Star anise) methanolic extract, and A Sample is the absorbance of the Star anise methanolic extract. The IC 50 was calculated using the linear equation of different concentrations of Trolox [([6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid]. The results were expressed as μg Trolox equivalents per gram of Star anise methanolic extract.

2.22 azinobis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) activity

The 2,2 azinobis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay was used to evaluate the antioxidant activity of Star anise methanolic extract (Re R., 1999). To prepare the ABTS stock solution, 5 ml of ABTS (7 mM) was mixed with 88 ml of potassium persulfate (140 mM) and incubated in dark at room temperature for 16 h. Then 22 ml of ethanol was added to 250 l of the prepared stock solution. 50 l of Star anise methanolic extract was applied to 1 ml of ABTS solution. Trolox was used as the positive control. After 3 min, the absorbance was measured using a spectrophotometer and the ABTS radical scavenging capacity was measured using the following equation.

\[
\text{ABTS radical scavenging activity} (\%) = \left(\frac{\text{A control} - \text{A sample}}{\text{A control}}\right) \times 100
\]

Ac is the absorbance of the control, As is the absorbance of the sample.

Evaluation of Anti-inflammatory effects

Animals

Female BALB/c mice weighing around 25 grams were obtained from Applied Science University animal house Amman/Jordan. BALB/c mice were allowed to receive water and a standard rodent diet.

Acute toxicity study and dose selection

The dose of the Star anise (Illicium verum) methanolic extract selected in this study was based on the LD50 dose. This was performed using 30 BALB/C mice (divided into six groups, each of mice) that were treated interperitonially with five different doses (100, 200, 400, 500, 1000, and 2000 mg/kg) of Star anise (Illicium verum) methanolic extract Star anise (Illicium verum) methanolic extract. During 24 h upon administration of the extracts, the animals were monitored for any abnormal clinical signs. After 24h, the LD50 was calculated for each group as the number of live mice/ total number of mice) ×100% based on Reed–Muench method (1938).

Study design

In this study, 30 balb/c mice were divided into 5 groups of 6 mice each. Group 1: given distilled water (intraperitoneal). Group 2: given only lipopolysaccharide (LPS) as a negative control (at a dose of 2.5 mg/kg i.p.). Group 3: given lipopolysaccharide (2.5 mg/kg i.p) and after 24 h treated with ascorbic acid as positive control (500 mg/kg i.p.). Group 4: given lipopolysaccharide (2.5 mg/kg i.p) and after 24 h treated with Star anise (Illicium verum) methanolic extract (100 mg/kg i.p.). Group 5: given lipopolysaccharide (2.5 mg/kg i.p) and after 24 h treated with Star anise (Illicium verum) methanolic extract (200 mg/kg i.p).

Biochemical analysis

Mice injected with LPS and after 24 treated peritoneal with Star anise (Illicium verum) methanolic extract. After 24 h, 1 milliliter of blood samples were collected and allowed to clot at room temperature. After 10 min, the collected blood samples were centrifuged (2500 rpm for 10 min) and the serum was transferred to a proper tube and stored at -21 °C until used. These samples were used to determine the levels of TNF- alpha, IL-1 â, CAT, and GP-X.

IL-1 â and TNF-â concentrations

The serum concentration of cytokines, IL-1 â, and TNF-â were measured using enzyme-linked immunoassay (ELISA) kits according to the manufacturer’s instructions (Abcam, Cambridge, MA, USA).

Superoxide dismutase and catalase concentrations

The serum concentration of total superoxide dismutase and catalase were determined enzyme-linked immunoassay (ELISA) kits according to the manufacturer instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Statistical analysis

To find any significant differences between different groups, a one-way analysis
of variance (ANOVA) was used followed by Dunnett’s post hoc test. The data was analyzed by SPSS© 22 (SPSS, Inc., USA). The rest of the results were presented as means± standard deviation (SD) of 3-4 independent experiments. Statistical differences between control and different treatment groups were determined using Graph Pad Prism ANOVA followed by Dunnett’s post hoc test.

RESULTS AND DISCUSSION

Antioxidant activity

Antioxidant activities of Star anise (*Illicium verum*) methanolic extract were examined using DPPH, and ABTS radical scavenging activity methods.

ABTS and DPPH

In the current study Star anise (*Illicium verum*) methanolic extract show high antioxidant activity in TEAC_DPPH and TEAC_ABTs results, Star anise (*Illicium verum*) contained a high amount of phenolics TEAC_DPPH (77.7±0.30 Trolox/g) and TEAC_ABTs (95.1±0.33 Trolox/g) as shown in the table (1).

<table>
<thead>
<tr>
<th>Test</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEAC_DPPH</td>
<td>77.7±0.30 Trolox/g</td>
</tr>
<tr>
<td>TEAC_ABTs</td>
<td>95.1±0.33 Trolox/g</td>
</tr>
</tbody>
</table>

The standard curve of TEAC_ABTs and TEAC_DPPH were shown in Figures 1 and 2, respectively.

**Lethal dose (LD<sub>50</sub>)**

The LD<sub>50</sub> value of the Star anise (*Illicium verum*) methanolic extract was 1100mg/kg. for the in vivo study, a concentration of 100 mg/kg and 200 mg/kg as a therapeutic dose were used to be safer and to avoid any toxicity resulting from Star anise (*Illicium verum*) methanolic extract. for all selected doses, no lethality was observed during the experiment.

**Table 1. Antioxidant activity of the methanolic extract**

![Fig. 1. Represents the Trolox® [(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] standard curve. This was performed using 10 concentrations of Trolox (0 to 1.5μg/ml)
Effect of Star anise (*Illicium verum*) methanolic extract on Serum pro-inflammatory Cytokine Levels

Figure (3) and Figure (4) show the TNF alpha concentration and IL1 beta concentration in different groups respectively. LPS which is used as a negative control group significantly induces the cell to produce pro-inflammatory cytokines TNF and IL1 beta compared with the normal untreated group. Ascorbic acid was used as positive control which highly significantly reduce pro-inflammatory cytokines TNF and IL1 beta production level compared with LPS negative group. In the current study Star anise (*Illicium verum*) methanolic extract at concentration 100 mg/k.g and 200 mg/k.g highly significant decreases, the production of pro-inflammatory cytokines TNF and IL1 beta as the concentration of extract increases compared with negative groups and have a similar effect in reducing pro-inflammatory cytokines in ascorbic acid positive control.

Figure (5) and figure (6) represents the standard curves of different known TNF and IL1 â concentrations respectively, from this curve we calculate the different experiment serum TNF and IL1 â concentration.

Effect of Star anise (*Illicium verum*) methanolic extract on the Oxidation-Related Serum Levels (catalase and glutathione-peroxidase enzyme)

Figure (7) and Figure (8) shows catalase and glutathione-peroxidase enzyme concentration level respectively. LPS which is used as negative control significantly induces oxidative stress by increasing the level of oxidative enzymes catalase and glutathione-peroxidase compared with the normal nontreated group. Ascorbic acid which is used as positive control highly significantly reduces the level of oxidative enzymes compared with the LPS negative group. In the current study Star anise (*Illicium verum*) methanolic extract at concentration 100 mg/k.g and 200 mg/k.g highly significant decreases the production level of oxidative enzymes as concentration increase compared with LPS negative groups and have a similar effect compared with ascorbic acid positive control.

Figure (9) and figure (10) represent the standard curve of different known catalase and glutathione-peroxidase enzymes respectively. From these curves, we calculate the different experiment serum catalase enzyme concentrations.

\[ y = 1.1321x \]
\[ R^2 = 0.9817 \]

Fig. 2. Represents the Trolox® [(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] was used as standard antioxidant. A Trolox® calibration curve was established using ten Trolox® standard solutions ranging from 0 to 1.5 μg/ml
DISCUSSION

Recently, all researchers’ efforts around the world have been to investigate and found new miracle medicinal plants with unusual high activity as antioxidant and anti-inflammatory.

It was observed that the activity of medicinal plant extracts varies from one plant to another and from a different country of the world in different research’s, and this may result due to many factors such as different of climate, soil composition, age, and vegetation cycle stage on the quality, quantity, and composition of the extracted product. 

In the current study, the antioxidants and anti-inflammatory effects of Star anise (Illicium verum) methanolic extract was studied.

The total antioxidant activity of Star anise (Illicium verum) methanolic extract was measured by using two commonly accepted assays, DPPH and ABTS, and high antioxidant activity TEACDPPH and TEACABTS were indicated. These results are in agreement with Aly et al., (2016) results, who reported that the extracts of plants with high amounts of phenolic compounds have high potential as protecting agents against the lethal effects of oxidative stress and protection of DNA from damage. Also, these observations agreed with several previous findings. The present study showed a strong correlation between the mean values of TEACDPPH and TEACABTS which indicated that compounds present in the Star anise (Illicium verum) methanolic extract capable of reducing DPPH radicals were also able to reduce ABTS. Also, many other studies are in agreement with the current study, Mosaffa-Jahromi et al., (2017) reported that Star anise (Illicium verum) have anti-oxidative and anti-diabetic activity,
Cai et al., (2013) revealed that Star anise (*Illicium verum*) constitute to have a novel source of natural antioxidants, Yadav and Bhatnagar (2007) reported that the treatment with star anise reduced the oxidative stress. So far, many other studies have been performed on *Illicium verum* was revealed as an antioxidant plant \(^{24,25}\). Star anise exhibited antioxidant ability against \(\text{H}_2\text{O}_2\) which induced cell death and DNA protection, and these activities may due to the presence of polyphenols, proteins, and flavonoids in star anise extracts \(^{28}\).

Luis et al.2019 studied the antioxidant activity of *Illicium verum* essential oil using the DPPH free-radical-scavenging assay and found

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**Fig. 5.** TNF standard curve

**Fig. 6.** IL1\(\beta\) standard curve.
that the essential oil activity of Illicium verum gives a strong antioxidant activity, which was related to the high content of phenylpropanoids including trans-anethole. They assumed that the double bonds of trans-anethole and the synergistic effect of the different components of Illicium verum essential oil contributed to the observed antioxidant activity. These results are in accordance with the results of the current study.

Pro-inflammatory cytokines production at a high concentration level accelerate chronic inflammations, which leads to more activations of the immune system, and continuous activation leads to tissue destruction and cancer development. TNF-α and IL-1 considerers as commonly pro-inflammatory mediator produced by the different immune cell and acts in the promotion and progression of the inflammatory process, but these mediators have a direct effect on the progression and formation of multiple inflammatory and immunological disorders when expressed and released in high level and inhibition of its production or effects may have the therapeutic benefit. Ullah et al. (2014) also reported that star anise extract has analgesic and anti-inflammatory effects on mice intestinal smooth muscles. This study confirmed the effective anti-inflammatory property of Illicium verum. Illicium verum has anti-inflammatory activity by reducing serum levels of IL-1 and TNF-α.

Ullah Zaman (2014) found that the anethole natural bioactive compound of Illicium verum has been effective in controlling some immune acute inflammation-related disease, probably by an inhibitory action on production or release of pro-inflammatory mediators. These results are in accordance with the results of the current study.

![Graph](image1)

**Fig. 7.** Effect of Star anise (Illicium verum) methanolic extract on the expression of catalase enzyme concentration. The result represents the concentration of catalase enzyme after 24h exposure to LPS in the negative group then treated with ascorbic acid in the positive control group and treated with 100, 200 mg/kg of Star anise (Illicium verum) methanolic extract after 24 hours. *: p<0.05, ** p<0.01, ***: p<0.001 LPS group compared to normal group, +: p<0.05, ++ p<0.01, +++: p<0.001 compared to LPS group.

![Graph](image2)

**Fig. 8.** Effect of Star anise (Illicium verum) methanolic extract on the expression on peroxidase enzyme concentration. The result represents the concentration of glutathione-peroxidase enzyme after 24h exposure to LPS in the negative group then treated with ascorbic acid in the positive control group and treated with 100, 200 mg/kg of Star anise (Illicium verum) methanolic extract after 24 hours. *: p<0.05, ** p<0.01, ***: p<0.001 LPS group compared to normal group, +: p<0.05, ++ p<0.01, +++: p<0.001 compared to LPS group.
Sung et al. 2017 were studied the anti-inflammatory activity of Illicium verum extract and anethole which its main compound in *Illicium verum* fruit in mice. Anethole was orally administered by gavages methods to the mice. The results indicate that anethole was reduced the inflammation by decrease inflammatory cell infiltrates and fibrosis in the airways. Trans-anethole also decrease IL-4 and IFN-α expression. Also, Sung et al. 2012 investigated the anti-inflammatory effect of Illicium verum extract and its main compound anethole in the human keratinocyte cell line. They observed that anethole exhibited anti-inflammatory activity in the human keratinocyte cell line, which was evidenced by the reduced protein expression of IL-4, and IL-1α without any cytotoxic effect. Moradi et al. 2014 also studied the anti-inflammatory effect of anethole in rats by inducing inflammation by injection of LPS for 10 days. Anethole was intraperitoneally administered before 20 min of LPS injection. The results showed that the mice

![catalase graph](image1)

**Fig. 9.** Represent the standard curve of the catalase enzyme

![peroxidase graph](image2)

**Fig. 10.** Represents the standard curve of the glutathione-peroxidase enzyme
that treated with anethole showed a significantly anti-inflammatory effect by a decrease in IL-1β and TNF-α concentration \(^{31}\). These results are in accordance with the results of the current study.

Bacterial endotoxin lipopolysaccharide LPS was able to activate the immune cell to produce pro-inflammatory cytokines \(^{32}\). Therefore, in the current study, we used LPS as negative control and we did not measure the level of pro-inflammatory mediators and antioxidant enzyme in response to Star anise (\textit{Illicium verum}) methanolic extract only without LPS because we aim to investigate if Star anise (\textit{Illicium verum}) methanolic extract could exert a protective effect as an anti-inflammatory by reducing a pro-inflammatory mediators level and reducing the oxidative stress resulted by LPS.

In current study inbred female Balb/c mice were used and Balb/c males were not used since the become aggressive at around 8 weeks of age and this might affect the oxidative stress and inflammatory response.

In the current study ascorbic acid was used as an anti-inflammatory positive control because ascorbic acid is considered as one of the main supplements that increase the activity of the immune system by enhancing T-lymphocyte proliferation in response to inflammation and increasing immunoglobulin production from B-lymphocytes. Previous studies reported that depletion of ascorbic acids levels as compared to healthy controls was seen in many conditions associated with chronic inflammation and oxidative stress \(^{8}\).

It is important to highlight that the activity of Star anise (\textit{Illicium verum}) methanolic extract as an anti-inflammatory by reducing the pro-inflammatory level and reducing the antioxidant enzyme level was not due to its cytotoxicity because the data obtained from the LD\(_{50}\) of the Star anise (\textit{Illicium verum}) plant indicated that the Star anise (\textit{Illicium verum}) methanolic extract did not cause any death or toxicity during the experiment at any concentration 100 and 200 mg/kg used in the experiment and we used two safe concentration 100 and 200 mg/kg under the LD\(_{50}\) to avoid any toxicity. Therefore, it can be hypothesized that the anti-inflammatory effect of Star anise (\textit{Illicium verum}) methanolic extract by reducing the level of pro-inflammatory cytokines could result in the modulation of inflammatory pathways.

It has been assumed that the pro-inflammatory reducing activity of Star anise (\textit{Illicium verum}) methanolic extract can be by one of the followings mechanisms, inhibiting the binding pro-inflammatory mediators to its specific receptors, lowering the activation of transcriptional factors that lowered the pro-inflammatory mediator’s production at the gene level, lowering the activation signaling cascade that is responsible for pro-inflammatory mediator’s production, and pro-inflammatory mediator’s degradation.

CONCLUSION

Star anise (\textit{Illicium verum}) methanolic extract act as strong antioxidant and anti-inflammatory medicinal plant. More studies were needed to investigate and detect the target compounds that have the highly activity than other.

ACKNOWLEDGMENT

I thank all Doctors in MLS department in mutah university. Many thanks to Doctor khaled khleifat and Doctor Haitham Qaraleh for his help in preparation of the paper.

Funding

The research was not funded.

REFERENCES

5. Rayyan WA, Alshammarri SA, ALSammary AM, AL-Shammari MS, Seder N and Abu-Qatouseh LF. The phytochemical analysis and


