Investigation the antioxidant and anti-inflammatory effects of star anise essential oil and anethole against arsenic induced myocardial ischemia in male rabbits

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Abstract

Star anise (Illicium verum Hook. f.) is a well-known widely used in our daily diet and recently is considered gained importance as a medicinal plant which has important biological properties, especially the phenolic and the volatile constituents, The current study was aimed to assay the protective effects of essential oil of star anise and anethole against deleterious effect in male rabbits exposed to arsenic. 30adult male rabbits were divided equally into five groups (six rabbits in each group): groups one and two (the normal control group) rabbits received normal saline and DMSO, group three rabbits were given sodium arsenate 10mg/kg of rabbit body weight, groups four and five (the protected groups) rabbits received anethole and star anise oil (250mg/kg) for one week and then same grouse received anethole and star anise oil (250mg/kg) simultaneous with sodium arsenate for 3 weeks, the doses once per day respectively. results exhibited that oral exposure of arsenic cause to oxidative stress evidenced by markedly elevation in value of oxidative index (MDA) concomitantly with significantly reduction in total antioxidant capacity value, also results revealed that pronounced elevation in inflammatory biomarkers (TNF-α and CRP) and myocardial function indices(CTnI and CK, LDH) values, these alteration comparison to group without exposure (normal control), while the findings of the study manifested that oral administration of anethole and star anise oil plus arsenic for 3 weeks resulted in decrease the MDA value with along an increase in value of TAC this is accompanied by markedly declined in inflammatory indices and restored myocardial function. Conclusions: it can that Star anise (Illicium verum) administration cause to ameliorative myocardial function evidence by reduction of inflammatory indices and subsequent blockade of the inflammatory response, the Illicium v. may be a useful a potent antioxidant and anti-inflammatory agents.

Keywords: *CTn-I, CK, LDH, MDA, CRP and TNF-α.*

INTRODUCTION

Arsenic, a metalloid, is naturally present in the environment and frequently detected in drinking water worldwide due to various natural and industrial activities, including mining, agriculture, and fuel burning (Chen etal.,2007 and Adeyi etal.,2021). Arsenic is widely distributed in the environment, occurring in both organic and inorganic forms. Exposure to inorganic arsenic compounds, which primarily occurs through occupational or environmental contact, poses a significant health risk. The accumulation of arsenic tends to concentrate in vital organs such as the heart, liver, kidneys, and lungs, with lower levels

present in muscular and neuronal tissues. (Veenema etal.,2019). Arsenite has the ability to interact with amino acids, peptides, and proteins that contain thiol groups, which can cause enzyme inhibition and cellular toxicity. The process of arsenic metabolism can also generate oxygen radicals that have the potential to damage various molecules such as DNA, proteins, and lipid (Kesavan etal.,2014 and Murko etal., 2018). Numerous reports have shown that the primary mechanism of toxicity in sodium arsenite is through oxidative stress and cellular damage caused by the generation of reactive oxygen species (ROS) and lipid peroxidation. These processes can contribute to the development of various health problems and serious diseases. (Tsou etal.,2005 ; Arteel etal.,2008 and Muhammad etal.,2013). Sodium arsenite is commonly used as a pesticide, antiseptic, dye and soap ingredient, and hide preservative. Chronic exposure to inorganic arsenic, mainly through drinking water, has been linked to numerous adverse cardiovascular effects such as peripheral and coronary artery disease, ischemic heart disease, and stroke. Arsenic may increase the incidence of arrhythmic events by directly affecting the atherosclerotic process, which involves endothelial cells, smooth muscle cells, platelets, and macrophages. It may also indirectly impact factors contributing to cardiovascular disease such as hypertension, diabetes mellitus, and hyperlipidemia, while promoting cardiovascular disease (Lemaire etal.,2011; Cosselman etal.,2015 and Batal etal.,2016). IAS (isopropyl alcohol ethoxylate sulfate) is recognized as a compound that can interfere with the endocrine system, leading to changes in the signaling of sex steroids and gene expression mediated by receptors. (Das etal., 2012). Natural antioxidants are frequently utilized to alleviate oxidative harm by inhibiting the generation of free radicals and boosting the body's own antioxidant defense system (Wojdyloa etal.,2007 and Muhammad etal.,2013). Star anise (Illicium verum Hook. f.) is an important traditional Chinese and Asian medicinal herb. It contains bioactive compounds, phenolic and flavonoid compounds, and the most important volatile oil that gives it its characteristic sweet, aromatic flavor is anethole. Previous studies have shown that star anise seeds and essential oil have antioxidant, anti-inflammatory, antidiabetic, antiviral, antispasmodic, anti-rheumatic, chemopreventive, neuroprotective, antiseptic, anticancer, and estrogenic properties, as well as culinary significance. Anethole, the primary component of the essential oil (85%-90%), is a natural bioactive compound with multiple beneficial effects in treating several chronic and neurological diseases in humans. Anethole has been found to be effective in controlling some nonimmune acute inflammation-related diseases, possibly through an inhibitory action on the production or release of PGE2 and NO. Both the seeds and essential oils of anise are promising for safe use as superfood supplements and raw constituents in the pharmaceutical and food industries. The essential oil of star anise is also widely used as a replacement for synthetic antioxidants, helping to prevent the harmful effects of oxidative stress on public health. This study aimed to assay the protective effects of the essential oil of star anise and anethole against deleterious effects in male rabbits exposed to arsenic. (Alhajj et al., 2017; Ding et al., 2017; Padmashree et al., 2007; Singh et al., 2006; Wong et al., 2014).

Materials and methods

Extraction of essential oil : dried fruit of star anise were ground to a fine powder using an electric grinder, the essential oil was carried out by steam distillation method using a Clevenger

apparatus for 3 hours, the oil was dried over anhydrous sodium sulfate and filtered essential oil, and stored at -4 °C until the chromatographic analysis and evaluation the protective effect on the heart function.(Al-Hilphy etal .,2015).

Care of experimental Animals :Thirty adult male rabbits (1350-1400 g) were obtained from animal house of the faculty of veterinary medicine , They were kept under observation for one weeks before the start of the experiment to exclude any infection. Animals were housed in plastic cages under good ventilation and temperature (25 ± 2 °C), 12 hours light-dark cycle. Animals were received standard diet and tap water ad libitum throughout experimental period (4 weeks plus one week acclimatization) , male rabbits were divided equally into five groups (six rabbits in each group) as following: group one animals were received (1ml) normal saline, group two rabbits were received (0.5ml) of dimethyl sulphoxide (DMSO) groups one and two as normal control, group three rabbits were given sodium arsenite 10mg/kg of rabbit body weight, groups four animals were received anethole (250mg/kg) dissolved in DMSO for one week and then this group received same dose of anethole simultaneous with sodium arsenite for 3 weeks, group five rabbits received star anise oil (250mg/kg) dissolved in DMSO for one week and then this group received same dose of star anise oil (250mg/kg) simultaneous with sodium arsenite for 3 weeks (four and five protected groups), the doses once per day respectively.

Blood sampling: At the end of the experimental period, blood samples were drawn from marginal vein of the ear , then put into non-heparinized tubes, blood separated by centrifugation at 3000 rpm for15 min, serum was kept at-20C until used for estimation oxidative , inflammatory and heart function biomarkers.

Investigation of biochemical: estimate the concentration of malondialdehyde (MDA) according to method described by(Ohkawa etal.,1979),Total antioxidant capacity (TAC) level was assessed to method of (Bartosz ,2003 and Alwan et al., 2021), while, the inflammatory indices Tumor necrosis factor-alpha (TNF-alpha) and C-reactive protein concentrations were determined by using method of (Alwan and Al-Saeed, 2023, Naz etal.,1995 and Pradhan etal., 2001(, the heart function biomarker cardiac troponin- I, creatine kinase and lactate dehydrogenase (CTnI, CK, LDH) were measurement by method of(Storrow etal.2007, Abd Maan etal.2022, Dawson etal.1965, AlSaad etal.2020 and Kaplan etal.1984).

Statistical analysis

The data obtained were presented as mean ± SD. The statistical analysis involved the use of the computerized SPSS program (Statistical Program for Social Sciences) for one-way analysis of variance (ANOVA). A p-value of less than 0.05 was considered significant. The least significant difference (LSD) test was used to assess the differences between the means of the groups analyzed using ANOVA. The statistical analysis was carried out using the SPSS software (Version 19.0, SPSS Inc., Chicago, Illinois, USA, 2010). (SPSS, 2010)

Results and Discussion

Arsenic (As) exposure can induce alteration in physiological functions of the body and is associated with many disease such as cardiovascular disorders due to immunemodulation, oxidative and inflammatory mechanisms(Naujokas etal.,2013). The importance of natural

antioxidants lies in the fact that there are numerous medicinal plants that contain various bioactive compounds, which are capable of reinforcing the endogenous antioxidant defenses against reactive oxygen species (ROS). These natural antioxidants are able to neutralize the reactive species and help to restore the optimal balance, thereby playing a critical role in the prevention of various diseases.(Wojdyloa etal.,2007 and Ulaç etal.,2019). In current study, the observed data in table (1) manifested there is a significant rise of MDA value in concurrently with significantly lower TAO value in group exposed to sodium arsenite compared to group without exposure , this is an indication of lipid peroxidation provoked by exposed to arsenic, this reflects greater depletion of endogenous antioxidant and increasing ROS result in cellular damage through lipid peroxidation, is the major pathway for the arsenic toxicity(Sun etal.,2014). The findings in this work are similar with outcomes those described by (Muhammad etal.,2013) who demonstrated that increased MDA value due to the a decrease in the GPX activity that scavenges hydroperoxides and lipid peroxides or as is result to diminished antioxidant GSH and CAT activities, this attributed to the deactivation of their isoenzymes by oxidation of a cysteine. Also (Chen etal.,2007) who noticed that low level of TAC resulted from arsenic metabolism and its compounds which stimulates more- production of ROS thereby increased formation of by -products MDA concomitant with increased the lipid peroxidation process that occurs mainly through mitochondrial destabilization and imbalance of oxidant - antioxidant, hence leading to DNA damage, membrane loss and the death of cell when exposure to arsenic and other environmental pollutants. Results also seen in same table (1) revealed that the oral administration of star anise oil and anethol plus sodium arsenite cause to remarkable raise of TAC level in concomitantly with declined MDA content observably in studied rabbits comparison with arsenic group alone. These findings may be attribute to inhibits lipid peroxidation and decrease in MDA formation by its powerful free-radical scavenging properties of either star oil extracted and anethol (Yang etal.,2012). The outcomes in present study are agreement with results described by(Ding et al., 2017) who exhibited that the Supplementation of SAO to the diet of broilers 200 mg/kg of diet for 21 and 42 days cause to increased activities of antioxidant enzymes(SOD, and GSH-PX) and diminished MDA value in serum and liver, this indicated that dietary supplementation of SAO may probably be restored the antioxidant defense of broilers by elevated antioxidant status. These findings are in accordance with results in present study (Domiciano et al., 2013 and Alhajj et al., 2017) who also observed that the laying hens fed the diets contained 200 to 600 mg/kg of SAO for 28 and 56 days produce increasing activity of TAC and reduced MDA content in serum and liver as well as the SAO may extend the time of store of eggs by enhancing the antioxidant status of yolk, this indicated that SAO has higher capacity in removing ROS by elevated the activities of SOD and GSH-PX and declined MDA level.

Furthermore, arsenic is a harmful substance that is foreign to the body and can elicit immune responses upon entering the biological system. One such response is an elevated level of cytokines, which can promote the production of reactive oxygen species (ROS) and mutagenesis, thereby contributing to the development of diseases induced by toxicants. (Arteel etal.,2008). according the outcomes in table (2) illustrated that the oral arsenic exposure induced markedly elevation in TNF-

alpha and CRP values corresponding with health rabbits this May be explained that the metabolism of arsenic is positive correlated to production of ROS thereby triggering inflammatory response since oxidative stress and inflammation are companied by a complex feedback cycle as that ROS trigger transcription factors that up-regulate expressions of pro-inflammatory cytokines(Tsou etal.,2005). The current outcomes are agree to those results obtained by (SW etal.,2017) who exhibited that the toxic material mediate and progressive of the inflammatory response through stimulation of neutrophil and macrophages and induction of pro-inflammatory mediators, as IL-6 and IL-1, Inflammatory reactions are mediated by TNF-α and IL-1β, these results also are similar with reported by(Manna etal.,2008) According to the author, oxidative stress is responsible for inflammation through activation of redox-sensitive transcription factors that regulate the expression of pro-inflammatory mediators and antioxidants. The study found that the use of star anise oil led to a significant decrease in inflammatory markers such as TNF-α and CRP. This could be attributed to the presence of methyl eugenol in the oil, which has antioxidant and anti-inflammatory properties. Additionally, the major components found in star anise oil, including trans-anethole, limonene, and estragole, may contribute to the oil's ability to enhance antioxidant status and block inflammatory responses (Padmashree et al., 2007). These results were confirmed with those report of (Freire etal.,2005 and Aly etal.,2016) who demonstrated that the star anise oil cause to suppression in inflammatory response This might be due to star anise oil contains natural products ascorbic acid, sterols and squalene, which could reduce the membrane peroxidation and subsequent restored inflammatory indices. The data observed in table (3) revealed that the oral administration of sodium arsenite caused to increased in increased in troponine-I concentration was parallel to the creatine kinase and lactate dehydrogenase concentrations compared corresponding control this may due to the heart is primarily targeted for accumulation of arsenic result in cardio- toxicity could be a secondary event following arsenic -induced lipid peroxidation of cardiac membranes with the consequent increase in the troponine and increased leakage of LDH and CK enzymes from cardiac myocytes (Yang etal.,2007 and Adil etal.,2016).The data of this study are consistent with those observed by (Veenema etal.,2019). The administration of both star anise oil and anethole resulted in a significant decrease in the aforementioned values when compared to the arsenic group. These findings suggest that star anise oil can provide protection to injured myocytes from toxins, thereby improving heart function (Dinesha etal.,2014 and . Díaz etal.,2014). these outcomes are agree with other studies by (Yadav and Bhatnagar,2007 and Huang etal.,2010) have shown that Star anise is rich sources of flavonoids which have various biological properties related to antioxidant mechanisms that were responsible for their protective effects. Also (Deng etal.,2014) According to the author, the protective effects of star anise oil are attributed to its antioxidative, anti-fibrotic, anti-lipid peroxidative, anti-inflammatory, membrane stabilizing, and organs regenerating properties. These results are in agreement with previous studies.(Domiciano et al., 2013 and Wang etal.,2011) who demonstrated that anise oil or anethole oil prevents oxidative damage due to its intrinsic antioxidant properties or which possibly related to have estrogenic activity

Table (1) the effect of star anise oil and anethole on malondialdehyde and total

antioxidant capacity levels in male rabbits Exposed to arsenic.

Treatment groups (n=6)	MDA µmol/L	TAC µmol/L
Control (n.s)	0.59± 0.06 B	1.73 ± 0.03 A
Arsenic 10mg/kg	1.69±0.02 A	0.78±0.03 B
Control DMSO	0.57±0.03 B	1.71±0.01 A
Arsenic + anethol 10mg+250mg\kg	0.64±0.04 B	1.68±0.02 A
Arsenic + SAO 10mg+250mg\kg	0.61±0.02 B	1.65±0.03 A

Different letters means a significant different at (P< 0.05) level

SAO: Star anise oil

Table (2) the effect of star anise oil and anethole on tumor necrosis factor -alpha and C- reactive protein levels in male rabbits exposed to arsenic

Groups (n=6)	TNF-α pg/ml	CRP mg/dl
Control (n.s)	24.77+2.23 B	1.87 ±0.07 B
Arsenic 10mg/kg	39.04±1.72 A	3.58±0.61 A
DMSO	24.69 ±1.76 B	1.86±0.05 B
Arsenic + anethol 10mg+250mg/kg	25.92±1.21 B	2.04 ±0.03 B
Arsenic + SAO 10mg+250mg/kg	25.75 ±2.29 B	1.96±0.20 B

Table (3) the effect of star anise oil and anethole on heart function biomarkers (CTn-I, CK and LDH) levels in male rabbits exposed to arsenic.

Groups (n=6)	CTIn pg\ml	CK-MB ng\ml	LDH U/L
Control (n.s)	0.43± 0.03 C	76.62±2.80 B	86.86±3.57 B
Arsenic 10mg/kg	1.86± 0.03 A	146.48±3.38 A	214.64±5.68 A
DMSO Control	0.42± 0.04 C	76.58±2.24 B	86.80±4.37 B
Arsenic +anethol 10mg+250mg/kg	0.02 0.59± B	82.55±1.78 B	92.36±1.81 B
Arsenic + SAO 10mg+250mg/kg	0.57±0.02 B	81.84±2.57 B	90.44±2.24 B

Different letters means a significant different at (P< 0.05) level.

SAO: Star anise oil

Conclusions

It can be that Star anise (Illicium verum) administration causes ameliorative myocardial function evidence by reduction of inflammatory indices and subsequent blockade of the inflammatory response ,the Illicium verum. may be useful as potent antioxidant and antiflammatory agents.

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