Study the Potential Effect of Rheum Palmatum Root Extract Against the Toxicity of A. fumigatus in Adult Male Rabbits

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Abstract
The present study was conducted to find the correlation between the potential effects of Rheum palmatum root extract against the toxicity of A. fumigatus. 16 male rabbits were used and divided into four groups (each group contain 4 rabbits). Control group; infected group; infected group and treated with (50mg) root extract and infected group and treated with (100mg) root extract. MDA levels in infected rabbits show high significant increased (P < 0.05) compared with normal rabbits. GSH and catalase levels in infected rabbits show significant decreased (P < 0.05) compared with normal rabbits. While, MDA, GSH and catalase levels in groups treated with root extract (50mg & 100mg) show non-significant changes (P < 0.05) compared with normal rabbits. It was concluded from this study that the Rheum palmatum root extract has potential effects against the toxicity of A. fumigatus.

Keywords: A. fumigatus; Rheum palmatum; MDA; GSH; Catalase.

Introduction
The Rheum palmatum (R. palmatum) L. is the root or stem of R. palmatum of Polygonaceae family. The R. palmatum L. is bitter in taste and cold in nature [1-2]. The genus Rheum consists of approximately 60 perennial species distribution around the world; several species are used in medicine, some for culinary purpose and few others are grown as ornament; about ten species occur in India [3-4]. Rheum palmatum contains Rhein which is a lipophilic anthraquinone have been used medicinally in China for more than 1,000 years. The most common side effect was diarrhea, is well tolerated in humans. Rhein exhibits linear pharmacokinetics between 50 and 200 mg [5]. Rheum palmatum has many pharmacological effects, including: antimicrobial activities, antioxidant, hepatoprotective [5-6], diabetes [7], atherosclerosis [8] and various cancers [9-10]. Aspergillus fumigatus is ubiquitous in the environment and its conidia are inhaled frequently by many normal, healthy people, yet only rarely does invasive aspergillosis develop [11]. Aspergillus fumigatus, the major causative agent of aspergillosis, is a ubiquitous and opportunistic fungus that elicits respiratory infections, such as sinusitis, aspergilloma and invasive aspergillosis [12]. So the aim of study was study the potential effect of Rheum palmatum root extract against the toxicity of A.fumigatus.
Materials & Methods

Aspergillus samples
A. fumigatus isolate from external ear of patients attending Al-Jomhory hospital – Kirkuk. Conidia suspension was prepared according to the method of Faraj (1990). The number of conidia was calculated under a light microscope, using the following equation. Concentration of conidia = Z x 4 x 10^6 / n. Where Z= conidia total number, n= small squares total number [13].

Plant extract
50g of root powder add to 500ml distils water and the mixture was put in shaking incubator at 37 C0 for 24 hour. Then, mixture was filtered by filter paper and centrifuged at 4000 cycle/10 min. supernatant transferring to petri dishes and incubated at 45 C0 until the obtain the extract that storage until to use [14].

Animal model
16 adult male were collected locally (Kirkuk city markets). The weights (1.5kg) and age range (7-11 Mon). All animals were received free food and water. The animals were observed to avoid any possibility for infection.

Experimental design
Male rabbits were used and divided to four groups as follow (each group consist four rabbits):
Control: rabbits received normal diet and used as control.
Infected group: rabbits injected (intraperitonel) conidia of A.fumigatus. After infection, they killed.
Treated group (50 mg): rabbits injected (intraperitonel) conidia of A.fumigatus and treated with root extract (for 3 weeks) and then killed.
Treated group (100 mg): rats injected (intraperitonel) conidia of A.fumigatus and treated with root extract (for 3 weeks) and then killed.

Samples collection
The blood samples were collected by cardiac puncture under anesthesia and put in test tubes. After clotting, the blood sample tubes were centrifuged (5000 cycle/min for 10 min) to isolate blood serum. The lung was removed immediately and homogenized with NaCl2. After that, supernatant and serum were taken and stored by deep freezing until use.

Oxidative stress factors
MDA (malonedialdehyde) by thiobarbituric acid (TBA) according to method [15], and Glutathione (GSH) by using DTNB, with estimate catalase according to method [16].

Statistical analysis
Data were analyzed using a statistical Minitab program, using Analysis of Variance (ANOVA) test, in order to evaluate the significance of variability between treated and control groups.

Results and Discussion

Oxidative stress factors
MDA levels (sera: 2.72 ± 0.63 & lungs: 2.03 ± 0.15) in rabbits injected with A. fumigatus show high significant increased (P < 0.05) compared with normal rabbits (sera: 1.43 ± 0.15 & lungs: 1.1 ± 0.21). MDA levels in groups treated with root extract (50mg & 100mg) show non-significant changes (P < 0.05) compared with normal rabbits. GSH levels (sera: 0.213 ± 0.043 & lungs: 0.154 ± 0.026) in rabbits injected with A. fumigatus show high significant increased (P < 0.05) compared with normal rabbits (sera: 0.75 ± 0.2 & lungs: 0.62 ± 0.13). GSH levels in groups treated with root extract (50mg & 100mg) show non-significant changes (P < 0.05) compared with normal rabbits. Catalase levels (sera: 0.68 ± 0.17 & lungs: 0.79 ± 0.062) in rabbits injected with A. fumigatus show high significant increased (P < 0.05) compared with normal rabbits (sera: 1.27 ± 0.14 & lungs: 1.09 ± 0.082). Catalase levels in groups treated with root extract (50mg & 100mg) show non-significant changes (P < 0.05) compared with normal rabbits as shown in Figures 1 and 2.

The Discussion
A.fumigatus spreads within the environment by means of conidia, and the small diameter of spores (2 to 3 m) enables them to reach the lung alveoli [17]. If the immune defense system of the lung is weakened, then conidia germinate and produce hyphae that invade the surrounding lung tissues [18], leading to the de-
Development of invasive pulmonary aspergillosis (IPA) [17]. In this study the oxidative stress factors in serum and lungs tissue of infected group show significant changes compare with control group. Fahmy et al. (2014) referred that mice which infected by A. fumigatus suffering infiltration of mononucleated inflammatory cells and hemorrhage with increased of MDA levels ($8.59 \pm 0.26$) and decreased of GSH levels ($74.75 \pm 7.44$) compare with control mice [19], that is in agreement with results of present study.

Figure 1: Oxidative stress factors levels in groups of study.

![Figure 1](image1.png)

Also, Nawada et al. (1996) that A. fumigatus lead to different histopathological changes including: necrosis of lung parenchyma around the bronchi and infiltration of lymphocytes with degenerative changes [20]. R. palmatum has various bioactive compounds as flaviniods, tannins, phenolic acids, anthraquinones and oxalic acid [21]. The root extract of R. palmatum has strong antibacterial activities [22]. The present study show the antioxidant and antimicrobial activity of R. palmatum root extract both invtro and invivo. Aly and Nehad (2011) study the Antimicrobial efficacy of R. palmatum extracts against some pathogenic microorganisms. They found the inhibition zones that caused by R. palmatum extracts against E. coli, P. aeruginosa, S. dysenterae, K. pneumonia, B. subtilis and S. aureus reached to 20, 21, 20, 22, 17 and 16mm respectively. Also, they study the antifungal activity of R. palmatum extracts against C. albicans, C. trobicals, A. solani, F. oxosporium and A. niger. Where, in-

![Figure 2](image2.png)

Figure 2: Oxidative stress factors levels in groups of study.
hilation zone diameters reached to 10, 11, 17, 19 and 24mm respectively [23]. The mechanisms of extract that responsible for this antimicrobial activity against microorganisms depend on its compounds. The mechanism of actions may include enzyme inhibition [24]. The present study also show an antioxidant effects of root extract against the toxicity of A. fumigatus in rabbits that lead to increased MDA and decreased GSH and catalase. Chen & Wang (2010) referred that R. franzenschuh has been antioxidant effects in mice with diabetes mellitus. They found that the levels of MDA, GSH and catalase back to normal ranges in mice with diabetes mellitus after using R. palmatum extract. They suggest that the extract of R. franzenschuh has strong antioxidant effects [25].

Conclusions
In our study, Rheum palmatum root extract has antioxidant activity and free radical scavenger with its ability to decrease the levels of oxidative stress factors (MDA) and attack A. fumigatus with remove its effects. So that concluded Rheum palmatum root extract has been potential activity against the toxicity of A. fumigatus in adult male rabbits.

References


