

# Investigating the effects of fennel (*Foeniculum vulgare*) seed powder on oxidant and antioxidant factors in hepatotoxicity induced by acetaminophen in male rats

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## Original Article

### Abstract

**Introduction:** Acetaminophen is a widely used analgesic and antipyretic drug. Although it is considered safe at therapeutic doses, at higher doses, might produces a centrilobular hepatic necrosis that can be fatal. In the present study, the effect of fennel seed on hepatotoxicity induced by acetaminophen was investigated.

**Methods:** In this experimental study, forty-two adult male Wistar rats, weighing 250 to 280g, were randomly allocated into seven groups (n=6 for each group). After 24 hours of fasting, the control and control+ fennel (600, 1200mg/kg) groups, received normal saline, and the acetaminophen and acetaminophen+fennel (300, 600, 1200mg/kg) groups received acetaminophen 1000mg/kg. After 6 hours, groups control and acetaminophen were given normal saline and groups control+fennel (600, 1200mg/kg) and acetaminophen+fennel (300, 600, 1200mg/kg) were given fennel seed powder in normal saline. Twelve hours later, liver peroxidase, catalase, malondialdehyde (MDA), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed.

**Results:** In acetaminophen group, ALT, AST, MDA and H<sub>2</sub>O<sub>2</sub> increased, and peroxidase and catalase activity decreased significantly compared to control. Fennel seed used in acetaminophen+fennel (600mg/kg) group returned the changes toward control group.

**Conclusion:** The results suggest that fennel seed has a protective role in acetaminophen-induced hepatotoxicity.

**Key words:** Acetaminophen, Fennel Seed, Liver, Oxidative Stress, Rat

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### Introduction:

Acetaminophen (N-acetylcysteine) is widely used as an analgesic and antipyretic (1). It is a safe drug in therapeutic dosage; its analgesic and antipyretic effects are like those of aspirin and ibuprofen, but it has weak anti-inflammatory effect (2). Although acetaminophen is considered safe in

therapeutic doses, it results in hepatic centrilobular necrosis in higher doses which can be deadly (3). In normal adults, regular usage of acetaminophen up to 4000mg/day shows little toxicity, though there is some disagreement in some studies (4). Orally administered acetaminophen is rapidly and almost completely absorbed through gastrointestinal track; major absorption occurs in small intestine (5). In

therapeutic doses, it is metabolized in liver through glucuronidation by UDP-glucuronosyltransferase enzyme (47-62%) and is converted to glucuronide paracetamol, and through sulfation by sulfotransferase (25-36%) to sulfated paracetamol and finally excreted by kidney. Also, one to four percent is eliminated by kidney without any change. About 8-10 percent is oxidized and converted to N-Acetyl-p-benzoquinone imine (NAPQI) (6).

NAPQI is produced by cytochrome P450 through direct two-electron oxidation from acetaminophen (7-9). In human, CYP2E1, CYP1A2 and CYP3A4 contribute to NAPQI production (10), but CYP2E1 is, by far, the primary source (11). In non-toxic doses, produced NAPQI is detoxified with liver glutathione and is converted to conjugated acetaminophen-glutathione (12). In toxic dose, NAPQI causes hepatic glutathione depletion by 80-90% (12,13); then, it binds to proteins covalently. The amount of covalent bonds correlates with hepatic toxicity (14). Glutathione depletion by NAPQI results in H<sub>2</sub>O<sub>2</sub> increase (15). Also, NAPQI binds to mitochondrial and plasma membrane proteins. For example, it results in reduction in CaATPase activity which leads to cytosolic Ca increase. This causes mitochondrial dysfunction and loss of ATP production (16). Medicinal plants have been used all over the world especially in countries like India, China, Egypt and Brazil. Natural medicine originates from different sources such as plants, sea and land microorganisms and invertebrates (17).

Flavonoids and phenolic compounds are widely distributed in plants which have different biological properties like antioxidant and anticancer (18).

For the first time the word *foeniculum vulgare* (Fennel) was introduced by a gardener in 1768 (19). It is widely distributed in many parts of the world especially in dry soil near seas and rivers coasts (20). Fennel is known as a good source of natural antioxidants (21). Wild fennel has higher phenolic and flavonoid compounds and thus free radical protective activity, and Italian fennel shows the most protective level (18). Methanolic extract of fennel seeds contain chlorogenic acid, rosmarinic acid as essential compounds, and quercetin and epigenin as essential flavonoids (22). In this study, we investigated protective effect of fennel seed powder (300, 600, 1200 mg/kg) on some

oxidant/antioxidant factors and liver enzymes aspartate transaminase (AST) and alanine transaminase (ALT) in hepatic toxicity induced by acetaminophen.

## Methods:

In this experimental study, 42 male Wistar rats, weighing 250-280g, were obtained from the animal house of Shahid Bahonar University of Kerman and were stored in the same center. The rats were stored under standard light, temperature (20-23°C) and humidity and were given sufficient food and water a few days before the experiment. The rats were randomly divided into seven groups of six rats:

**Control group (C):** The rats received normal saline via gavage after 24 hours of fasting. Six hours later, normal saline was administered via gavage.

**C+Fennel 600mg/kg group (C+F600):** After 24 hours of fasting, the rats received normal saline. Six hours later, fennel seed powder (600 mg/kg) was administered via gavage.

**C+F group 1200mg/kg (C+F1200):** After 24 hours of fasting, the rats received normal saline. Six hours later, fennel seed powder (1200 mg/kg) was administered via gavage.

**Acetaminophen group (A):** After 24 hours of fasting, the rats received 1000 mg/kg of acetaminophen via gavage. Six hours later, normal saline was administered.

**A+F group (A+F300):** The rats received acetaminophen after 24 hours of fasting via gavage.

Six hours later, **A+F group (A+F600):** The rats received acetaminophen after 24 hours of fasting via gavage. Six hours later, fennel seed powder (600 mg/kg) was administered via gavage.

**A+F group (A+F1200):** The rats received acetaminophen after 24 hours of fasting via gavage. Six hours later, fennel seed powder (1200 mg/kg) was administered via gavage.

Fennel seeds were purchased from an apothecary shop and approved by a botanist. Pure acetaminophen powder, which was provided by Darou Pakhsh Co. (Tehran, Iran), was dissolved in normal saline and administered via gavage to induce hepatic injury.

Twelve hours after the second gavage, the rats were anesthetized with CO<sub>2</sub> and sacrificed. Blood was collected and after 20 min, it was centrifuged to separate serum for measuring ALT and AST. One part of the liver was removed and washed with normal saline and frozen with Nitrogen gas and finally stored at - 80 degrees Celsius for assay of oxidant and antioxidant factors.

**Evaluation of antioxidant factors:** In this study, Bradford assay was applied to prepare the tissue extracts in order to measure the activities of catalase, peroxidase and proteins

**Measurement of catalase activity:** Catalase activity was measured by calculating H<sub>2</sub>O<sub>2</sub> reduced absorption (H<sub>2</sub>O<sub>2</sub> reduction) at 240nm, using Dhindsa and Motowe method (23).

**Evaluation of peroxidase activity:** Peroxidase activity was measured by using guaiacol and measuring the absorbance rate of tetra-guaiacol, composed of guaiacol (produced by peroxidase activity) at 470nm using the Plewa method (24).

**Measurement of total protein concentration:** About 0.1 ml of the tissue extract and 5 ml of the biuret reagent were added to the test tube and were immediately vortexed to measure the protein concentration. After 25 minutes,

The absorbance was determined at 595nm (25).

**Measurement of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>):** Measurement of H<sub>2</sub>O<sub>2</sub> was performed by using the method proposed by Velikova (26).

**Measurement of malondialdehyde (MDA):** The measurement of MDA concentration was performed by using Heath and Packer method (27).

**Measurement of serum ALT and AST:** They were assayed with Pars Azmun kit.

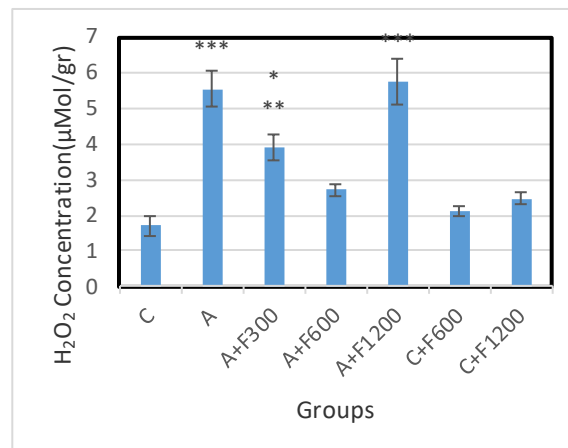
**Data analysis:** For data analysis, one-way ANOVA and Tukey's post-hoc test was performed, using SPSS. P<0.05 was considered statistically significant. Data are presented as mean±S.E.M.

## Results:

Figure 1 shows H<sub>2</sub>O<sub>2</sub> changes in hepatic tissue in experimental groups. H<sub>2</sub>O<sub>2</sub> concentration in group A and A+F1200 show a significant increase (p<0.001) compared to groups C, C+F600, C+F1200 and A+F600. Also, group A+F300 shows a significant increase compared to group C and C+F600 (P<0.01 and P<0.05 respectively),

and a significant decrease (P<0.05) compared to group A and A+F1200.

Figure 2 shows MDA changes in hepatic tissue in experimental groups. MDA concentration in group A shows a significant increase (P<0.001) compared to all groups.



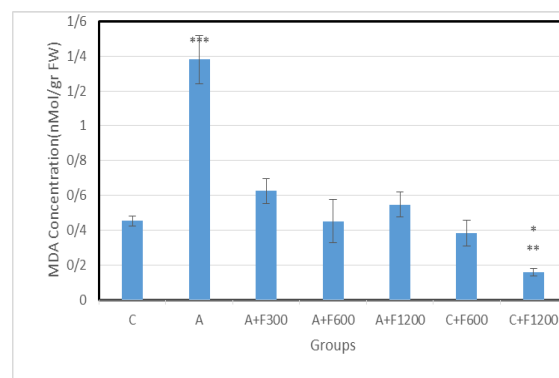
**Figure 1. H<sub>2</sub>O<sub>2</sub> changes in hepatic tissue in experimental groups. Each column shows mean±S.E.M. n=6**

\*\*\* P<0.001 significant difference with group C, C+F600, C+F1200, A+F600.

\*\* P<0.01 significant difference with group C.

\* P<0.05 significant difference with group C+F600, A, A+F1200.

C = Control, A = Acetaminophen, C+F600 = Control+Fennel 600, C+F1200 = Control+Fennel 1200, A+F300 = Acetaminophen + Fennel 300, A+F600 = Acetaminophen + Fennel 600, A+F1200 = Acetaminophen + Fennel 1200



**Figure 2. MDA changes in hepatic tissue in experimental groups. Each column shows mean±S.E.M. n=6**

\*\*\* P<0.001 significant difference with all groups.

\*\* P<0.01 significant difference with group A+F300.

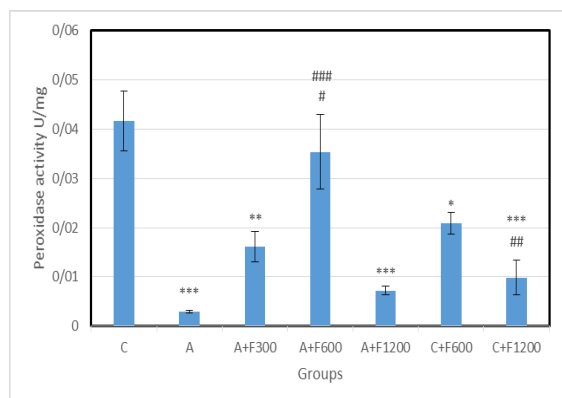
\* P<0.05 significant difference with group A+F1200.

C = Control, A = Acetaminophen, C+F600 = Control+Fennel 600, C+F1200 = Control+Fennel 1200, A+F300 = Acetaminophen +

Fennel 300, A+F600= Acetaminophen + Fennel 600, A+F1200= Acetaminophen+Fennel 1200.

Also, group C+F1200 shows a significant decrease compared to A+F300 and A+F1200 ( $P < 0.01$  and  $P < 0.05$  respectively).

Figure 3 shows peroxidase activity changes in hepatic tissue in experimental groups. Peroxidase activity in groups A, A+F1200 and C+F1200 show a significant decrease ( $P < 0.001$ ) compared to group C. Also, group A+F600 shows a significant increase ( $P < 0.001$ ) compared to groups A and A+F1200, and also a significant increase ( $P < 0.05$ ) compared to group A+F300. Group A+F300 shows a significant decrease compared to group C ( $P < 0.01$ ). Group C+F1200 shows a significant decrease ( $P < 0.01$ ) compared to A+F600. Group C+F600 shows a significant decrease compared to group C ( $P < 0.05$ ).



**Figure 3. Peroxidase activity changes in hepatic tissue in experimental groups. Each column shows mean  $\pm$  S.E.M. n=6**

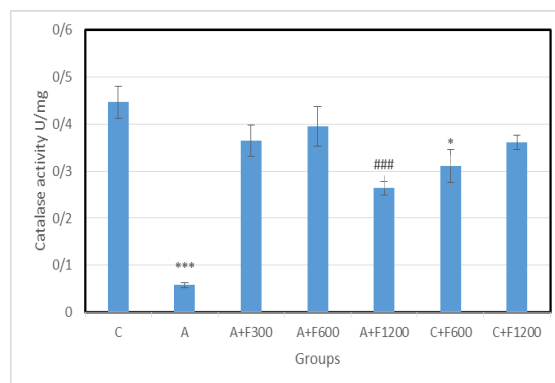
\*\*\*  $P < 0.001$  significant difference with group C. ###  $P < 0.001$  significant difference with groups A and A+F1200.

\*\*  $P < 0.01$  significant difference with group C. ##  $P < 0.01$  significant difference with group A+F600.

\*  $P < 0.05$  significant difference with group C. #  $P < 0.05$  significant difference with group A+F300.

C= Control, A= Acetaminophen, C+F600= Control+Fennel 600, C+F1200= Control+Fennel 1200, A+F300= Acetaminophen+Fennel 300, A+F600= Acetaminophen+Fennel 600, A+F1200= Acetaminophen+Fennel 1200.

Figure 4 shows catalase activity changes in hepatic tissue in experimental groups. Catalase activity in group A shows a significant decrease ( $P < 0.001$ ) compared to all groups. Also groups C+F600 and A+F1200 show a significant decrease compared to group C ( $P < 0.05$  and  $P < 0.001$  respectively).



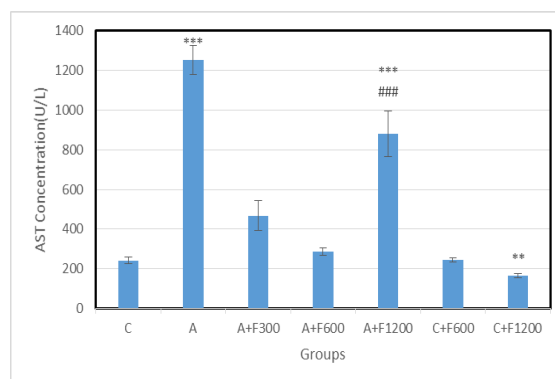
**Figure 4. Catalase activity changes in hepatic tissue in experimental groups. Each column shows mean  $\pm$  S.E.M. n=6**

\*\*\*  $P < 0.001$  significant difference with all groups.

###  $P < 0.001$  significant difference with groups C.

\*  $P < 0.05$  significant difference with group C.

C= Control, A= Acetaminophen, C+F600= Control+Fennel 600, C+F1200= Control+Fennel 1200, A+F300= Acetaminophen + Fennel 300, A+F600= Acetaminophen + Fennel 600, A+F1200= Acetaminophen + Fennel 1200.



**Figure 5: serum AST changes in experimental groups. Each column shows mean  $\pm$  S.E.M. n=6**

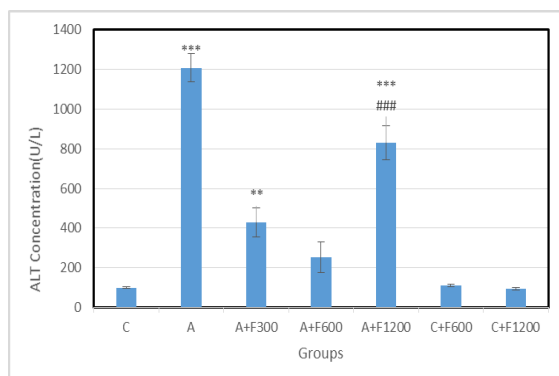
\*\*\*  $P < 0.001$  significant difference with all groups. ###  $P < 0.001$  significant difference with group A.

\*\*  $P < 0.01$  significant difference with group A+F300.

C= Control, A= Acetaminophen, C+F600= Control+Fennel 600, C+F1200= Control+Fennel 1200, A+F300= Acetaminophen + Fennel 300, A+F600= Acetaminophen + Fennel 600, A+F1200= Acetaminophen + Fennel 1200.

Figure 5 shows serum AST changes in experimental groups. AST concentration in groups A and A+F1200 show a significant increase ( $p < 0.001$ ) compared to all other groups. Also group A+F1200 shows a significant decrease ( $P < 0.001$ ) to group A. Group C+F1200 shows a significant decrease ( $P < 0.01$ ) compared to A+F300.

Figure 6 shows serum ALT changes in experimental groups. ALT concentration in groups A and A+F1200 show a significant increase ( $p < 0.001$ ) compared to all other groups. Also group A+F1200 shows a significant decrease ( $P < 0.001$ ) to group A, and group A+F300 show a significant increase ( $P < 0.01$ ) compared to groups C, C+F600 and C+F1200.



**Figure 6. Serum ALT changes in experimental groups. Each column shows mean  $\pm$  S.E.M. n=6**

\*\*\*  $P < 0.001$  significant difference with all groups. ###  $P < 0.001$  significant difference with group A.

\*\*  $P < 0.01$  significant difference with groups C, C+F600 and C+F1200.

C= Control, A= Acetaminophen, C+F600= Control+Fennel 600, C+F1200= Control+Fennel 1200, A+F300= Acetaminophen + Fennel 300, A+F600= Acetaminophen + Fennel 600, A+F1200= Acetaminophen + Fennel 1200.

## Conclusion:

In this study, Fennel in doses 300, 600, and 1200 mg/kg was able to reduce hepatic injury (increased liver enzymes ALT & AST in serum) and also prevented Oxidant factor increase (MDA and  $H_2O_2$ ) and antioxidant factor decrease (catalase and peroxidase enzymes) in hepatic tissue.

When used in large amount for suicide or accidentally, Acetaminophen causes severe hepatic necrosis (28). Cytochrome p450 metabolism is the first step in toxicity which leads to the formation of the reactive metabolite NAPQI (29). Superoxide anion produced by P450 enzyme, leads to the formation of  $H_2O_2$  which by fenton reaction results in hydroxyl radical formation (30).

In this study there was an increase in hepatic tissue  $H_2O_2$  in rats treated with acetaminophen which is in agreement with Rasha et al (31).

Lipid peroxidation is one of the most important destructive effect of free radicals which destroys cell membrane. Unsaturated fatty oxidation leads to decreased membrane fluidity and loss of its structure and function (32).

This results in many disease pathogenesis. Lipid peroxidation causes a loss in membrane integrity and a change in related enzymes.

MDA is the final product of lipid per oxidation (33). In the present study, there was an increased in MDA in rats treated with acetaminophen compared to control group which is in agreement with Chandrasekaran et al. In their study, acetaminophen was administered orally (1000 mg/kg), and after 24h, significant increase in hepatic MDA was seen compared with normal group (34).

We saw a significant decrease in hepatic enzymes catalase and peroxidase in rats treated with acetaminophen which is in agreement with Simeonova et al (35).

In our study, different doses of fennel seed powder were able to prevent changes in oxidative stress factors; these effects were prominent in dose 600 mg/kg so that it could bring the variables more toward control group that doses 300 and 600mg/kg.

These findings are in agreement with Farouk et al who showed methanolic extract of fennel has protective effect against  $CCl_4$  induced hepatic toxicity by decrease and increase of hepatic MDA and catalase activity (36).

We measured ALT and AST as functional assessment of liver. These enzymes are normally present within hepatic cells and during injury, due to plasma membrane disintegrity and or lysis of these cells, they enter in circulation and result in increased serum level of them (37). So, serum increase level of these two enzymes is used as an index of hepatic injury.

We observed a serum increase of ALT & AST in rats receiving acetaminophen which is in agreement with Chandrasekaran et al (34).

The results of our study show that fennel seed administration causes a significant reduction in acute increase of serum ALT & AST induced by acetaminophen which is in and agreement with Devik et al and in dose 600mg/kg it had the most effectivity. Although the dose 300mg/kg had some hepatic protective effect, but it was the dose 600

mg/kg which was most effective and could take the changes toward control group. Devika et al showed methanolic extract of fennel protects liver from toxicity induced by acetaminophen and this effect was more prominent with the dose 400 mg/kg compared to a dose less than that (38).

In the present study a reduction in peroxidase activity was seen in groups receiving fennel seed. Also, the group receiving fennel seed with dose of 600 mg/kg showed a reduction in catalase activity. That why the plant alone caused a reduction in activity of the enzymes is not clear.

May be there is a direct inhibitory effect of one component of the plant which needs further investigation. In this study dose of 1200mg/kg was not able to prevent acetaminophen induced hepatic injury as effective as dose of 600mg/kg in some variables. It leaves a question for us. However, it should be mentioned that Antioxidants (such as flavonoids) in high doses can show prooxidant effect (39), but overall, using the plant alone did not have this effect. So, less effectiveness of 1200mg/kg to 600mg/kg in acetaminophen induced injury needs more investigation.

Fennel contains quercetin (a flavonoid) in high amount which has a powerful inhibition against ROS and protection against lipid and protein oxidation. EL-Shafey et al showed that quercetin reduces hepatic injury induced by acetaminophen in rats. Rats receiving acetaminophen+quercetin compared to ones receiving acetaminophen showed a significant increase in catalase and peroxidase activity and a significant decrease in serum, ALT, AST, and ALP (40).

Quercetin prevents tissue damage resulted from free radicals by removing ROS. It also inhibits xanthine oxidase enzyme and therefore, decreases oxidative stress (41).

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