The Prophylactic and Protective Effects of *Terfezia Claveryi* Extracts on Ibuprofen Induced Oxidative Stress in Pregnant Rats

*Terfezia Claveryi* Ekstraktların Ibuprofen Verilen Hamile Rat Karaciğer Dokusundaki Oksidatif Stres üzerine Profilaktik ve Koruyucu Etkilerinin Araştırılması

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**ABSTRACT**

**Objective:** This study aimed to investigate the prophylactic and protective roles of *Terfezia Claveryi* extracts on Ibuprofen induced oxidative stress in pregnant albino rats.

**Methods:** Thirty pregnant rats randomly divided in to five experimental groups, each group consist of six pregnant rats. Group 1 (control group): without any treatment. Group 2 (Ibuprofen group): Given Ibuprofen orally by gastric tube at dose of 40 mg/kg/day for 20 days. Group 3 (*Terfezia Claveryi* group): Received *Terfezia Claveryi* extracts via gavage at 250 mg/kg every day dose. Group 4 (Ibuprofen + *Terfezia Claveryi* group): Received with Ibuprofen at dose of 40 mg/kg every day plus *Terfezia Claveryi* extracts 250 mg/kg/day by gastric tube along the experiment. Group 5 (*Terfezia Claveryi* + Ibuprofen): Received with 250 mg/kg every day of *Terfezia Claveryi* via gavage for two weeks before gestation (as a prophylactic) and then treated with Ibuprofen at the above-mentioned dose at zero day of gestation to twenty day of the experiment.

**Results:** Ibuprofen increased serum AST, ALT, ALP activity, total bilirubin concentration, Liver tissue MDA levels and decreased liver tissue Catalase, Superoxide Dismutase and Glutathione Peroxidase. However, this modulation of the biological parameter is significantly ameliorated by the administration of the *Terfezia Claveryi*.

**Conclusion:** In conclusion prophylactic and protective effect of *Terfezia Claveryi* against Ibuprofen induced oxidative stress was reported in pregnant rats.

**Key Words:** Ibuprofen, *Terfezia Claveryi*, Oxidative stress, Pregnant rats.

**Received:** 05.29.2017  **Accepted:** 04.10.2018

**ÖZET**

**Amaç:** Bu çalışmada, hamile albino ratlarında *Terfezia Claveryi* ekstraktlarının ibuprofen kaynaklı oksidatif stres üzerindeki koruyucu ve profilaktik rollerinin araştırılması amaçlandı.

**Hastalar ve Yöntemler:** Otuz gebe rat rastgele beş deney gruba bölündü, her grup altı gebe ratlardan oluşuyordu. Grup 1 (kontrol grubu): herhangi bir tedavi uygulamadı. Grup 2 (Ibuprofen grubu): 20 gün boyunca 40 mg/kg/gün dozunda gastrik tüp ile oral olarak Ibuprofen verildi. Grup 3 (*Terfezia Claveryi* grubu): *Terfezia Claveryi*, her gün 250 mg/kg dozunda gavaj yoluyla verildi. Grup 4 (Ibuprofen + *Terfezia Claveryi* grubu): Her gün 40 mg/kg dozda Ibuprofen ile 250 mg/kg/gün *Terfezia Claveryi*, deney boyunca gastrik tüp ile verildi. Grup 5 (*Terfezia Claveryi* + Ibuprofen): Her gün *Terfezia Claveryi’nin her gün 250 mg/kg gavaj yoluya gebelekti. ancak bağımsızlaştıktan sonra, deneyin ilk gününde 20 gün gebelekti ve daha sonra, belirlenen dozda Ibuprofen ile muamele edildi.

**Bulgular:** Ibuprofen serum AST, ALT, ALP aktivitesi, total bilirubin konstriyasyonu, Karaciğer dokusu MDA düzeyleri ve karaciğer dokusunda Katalaz, Superoxid Dismutaz ve Glutatyon Peroxidaz arttı. Ancak, biyolojik parametrelerin bu modülasyonunun *Terfezia Claveryi’nin uygulaması önemli ölçüde iyileştiğini gösterdi.

**Sonuç:** *Terfezia Claveryi’nin Ibuprofen kaynaklı oksidatif stres eki ve profilaktik etkisi gebe ratlarda bildirilmiştir.

**Anahtar Sözcüklər:** Ibuprofen, Terfezia Claveryi, Oksidatif stres, Hamile ratlar.

**Geliş Tarihi:** 29.05.2017  **Kabul Tarihi:** 10.04.2018
INTRODUCTION

The shift in balance between reactive oxidants and antioxidants is called the oxidative stress. It is produced when the reactive oxygen species (ROS) are more than the antioxidant levels (1,2). Many conditions are associated with oxidative stress shortage of antioxidant vitamin, smoking, diseases, pollution and drug (3).

Ibuprofen, is an example of the non-steroidal anti-inflammatory drugs (NSAIDs) (4,5). It is one of the most commonly used NSAIDs for the relief of pains, fever and treatment of inflammatory conditions. The drug is recorded to be better and preferred for muscle and joint pain than most other NSAIDs and has been employed by patients with arthrits for years (6). The pharmacological actions of ibuprofen, like other NSAIDs, has been reported to be via inhibition of cyclooxygenase (COX) enzyme activity (7). Although, NSAIDs are considered to have high safety, the frequent and employ of ibuprofen and other NSAIDs is likely to increase the prevalence of their adverse effects. Ibuprofen and other NSAIDs are commonly associated with hepato-, nephro and gastrointestinal (GI) toxicity (8,9). Many studies have shown the adverse effects of different NSAIDs to the kidney (10-12).

In addition to that, Lateef, et al. (13) have recorded that NSAIDs may also change liver function, causing elevations of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and necrosis of hepatic cells. Terfezia Claveryi grow naturally in different parts of the world, especially in the Arabian desert (14). Terfezia Claveryi are one of the oldest diets employed by the Arabs peoples (15). The Bedouins in the desert employ Terfezia Claveryi as a replacement for meat in their food (16). In the Iraq and in some the Arabian countries Terfezia Claveryi are employed in Arabian medicine for the treatment of eye diseases. It is reported that Terfezia Claveryi have antimicrobial properties (17) and hepatoprotective effect against carbon tetrachloride (CCL4) toxicity (18). In addition to that it was recorded that Terfezia Claveryi extracts have potent antioxidants due to contain high proportion of vitamin A, C, B-carotene and many phenolic compounds that play an important role scavenger of reactive oxygen species (ROS) and inhibit lipid peroxidation (LPO), which is the cell membrane damage caused by oxidative stress (19).

During our work in the hospital, we noticed the frequent use of ibuprofen by pregnant women, the difference in the metabolites of pregnant women and non-pregnant women, or the difference in the effectiveness of the liver in the drug metabolism of pregnant and non-pregnant women in addition to the difference in the state of oxidant and antioxidant between them and to know about the effect of frequent use of ibuprofen by pregnant women, the aim of this study was to investigate the protective and prophylactic roles of Terfezia Claveryi extracts on Ibuprofen induced oxidative stress in pregnant albino rats.

MATERIALS and METHODS

Chemicals

The chemical materials which used in this study were of highest analytical grade available were obtain from Sigma Chemical Company (St. Louis, MO, USA).

Ibuprofen (Tabufen®) tablets was obtained from the Essential Drug Company (Baghdad, Iraq), the tablets were powdered separately in a glass mortar, mixed with distilled water (DW) and were given as aqueous suspensions at a dose of (40mg/kg) by orally gavage as previously described (20).

Terfezia Claveryi extract

Terfezia Claveryi was purchased from local markets of Baghdad ( and identified by Assist. Prof. Dr. Ibrahim Salih Al-Jubori from College of Pharmacy, University of Al-mustansiria). It is brown dark red in color, round in shape and small in size. Terfezia Claveryi was carefully washed, peeled and preserved at -21°C until use. Frozen Iraqi samples of Terfezia Claveryi were homogenised 1:3 (w/v) in cold distilled water employing a household blender on high speed for one minute. The homogenates of Terfezia Claveryi were refrigerated overnight, then filtered through cheesecloth. The filtrates of Terfezia Claveryi were, centrifuged at 4000 rpm for fifteen minute.
At the same time there were significant increases in AST, ALT and ALP activity and total bilirubin levels in the Ibuprofen group as compared to the Ibuprofen + Terfezia Claveryi group. In addition to that, there was no significant change in ALT, AST, ALP activity and total bilirubin concentration in the serum of Terfezia Claveryi treated group as compared to control group as shown in Table 1.

In comparison with control group, MDA increased significantly in the Ibuprofen–treated group. Treating pregnant rats with Ibuprofen + Terfezia Claveryi results in a decrease in MDA in the liver tissue as compared to the Ibuprofen group alone. SOD, GSH, and CAT activities were significantly decreased in pregnant rats treated with Ibuprofen and their values increased significantly after treatment with Ibuprofen + Terfezia Claveryi. No significant differences were reported between control and Terfezia Claveryi groups. In addition to that, there were significant increases in CAT, GSH, and SOD activities in the Terfezia Claveryi + ibuprofen group as compared to the Ibuprofen group (Table 2).

### RESULTS

As compared to control group, treatment of pregnant rats with Ibuprofen for 20 days caused significant increases in the serum AST, ALT and ALP activity and total bilirubin concentration. While these biochemical parameters were restored to near normal values in pregnant rats treated with Ibuprofen plus Terfezia Claveryi.

| Parameters | Control | Ibuprofen | T. claveryi | Ibuprofen + T. claveryi | T. claveryi + ibuprofen |
|------------|---------|-----------|------------|-------------------------|-------------------------|
| AST (U/L)  | 10.816±1.318 | 33.883±2.595*** | 9.512±2.369 | 12.183±1.279 | 11.481±732 |
| ALT (U/L)  | 15.08±1.477 | 45.11±4.152**** | 14.85±1.245 | 16.15±1.245 | 16.23±1.096 |
| TB (mg/dl) | 0.351±0.0366 | 6.271±0.402**** | 0.338±0.0256 | 0.371±0.0285 | 0.376±0.0391 |
| ALP (U/L)  | 71.5±5.999 | 198.58±15.832**** | 70.53±6.032 | 71.85±5.105 | 72.83±7.567 |

Data are shown as means ± SD (standard deviation). Significant differences (***$P<0.001$, ****$P<0.0001$).

| Parameters | Control | Ibuprofen | T. claveryi | Ibuprofen + T. claveryi | T. claveryi + ibuprofen |
|------------|---------|-----------|------------|-------------------------|-------------------------|
| MDA (nmol/g) | 0.933±0.446 | 2.916±0.271*** | 0.865±0.0721 | 1.163±0.167 | 0.953±0.142 |
| SOD (u/g)  | 10.05±1.072 | 3.06±0.186*** | 10.85±0.821 | 8.85±0.8288 | 9.65±1.012 |
| CAT (u/mg) | 0.88±0.0925 | 0.263±0.00967*** | 0.9425±0.0342 | 0.831±0.0972 | 0.841±0.088 |
| GSH (umol/g) | 2.37±0.108 | 0.63±0.0592*** | 2.718±0.0412 | 2.183±0.445 | 2.112±0.3502 |

Data are shown as means ± SD (standard deviation). Significant differences (**$P<0.01$, ***$P<0.001$, ****$P<0.0001$).

Histopathological studies

For histopathological examinations, the liver tissues were removed and fixed in ten percent (10%) formalin. Sections of 5 μm thickness were cut. The sections were stained with haematoxylin and eosin (H&E) (29).

The liver tissues of Terfezia Claveryi group treated pregnant rats did not reveal any pathological changes (necrosis, inflammation or fibrosis). The histology of liver tissue from Ibuprofen + Terfezia Claveryi, Terfezia Claveryi + Ibuprofen groups showed less necrosis (black indicator), severe congestion (yellow indicator) and disarrangement of normal hepatic cells were showed (figure 1 B).

The liver tissues of Terfezia Claveryi group treated pregnant rats did not reveal any pathological changes (necrosis, inflammation or fibrosis). The histology of liver tissue from Ibuprofen + Terfezia Claveryi, Terfezia Claveryi + Ibuprofen groups showed less necrosis (black indicator), severe congestion (yellow indicator) and disarrangement of normal hepatic cells were showed (figure 1 D,E).
Histopathological study of liver tissue. Microphotograph of the liver tissue of the different treatment groups. (A) Normal control (H&E, X400) (B) Ibuprofen induced liver toxicity (H&E, X400) (C) treated with Terfezia claveryi (H&E, X400) (D) Ibuprofen+ Terfezia claveryi (H&E, X200) (E) Terfezia claveryi + Ibuprofen (H&E, X400)

DISCUSSION

In this study we aimed to investigate the prophylactic and protective roles of Terfezia claveryi against Ibuprofen-induced oxidative stress in albino pregnant rats. This study evaluated liver function by assaying serum ALT, AST, ALP activities, and total bilirubin levels. In addition to that we evaluated oxidative stress in pregnant rats by assaying malondialdehyde (MDA) levels and glutathione peroxidase (GSH-Px), Catalase (CAT), Superoxide Dismutase (SOD) activities.

Liver is one of the largest and vital important organ in the human body. It plays a very important role in regulating homeostasis within the body by many functions, such as metabolism, secretion and storage. Liver damage caused by toxic chemicals and some drugs has been identified as a toxic problem. Hepatotoxicity is one of the most common factors leading to serious complications ranging from severe metabolic disorders to even fatalities. Most hepatic toxic chemicals infect liver cells mainly by stimulating lipid peroxidation (LPO) and other oxidative damage (30-33). Ibuprofen treatment causes significant increase in the serum activity of liver function tests such as (ALT), (AST), (ALP) and total bilirubin concentration as compared to control group, indicating hepatic dysfunction. These defects in liver functions may be due to the production of free radicals and involvement of oxidative stress (OS) to hepatic toxicity caused by ibuprofen treatment. The results from this study confirmed that Ibuprofen at a dose of 40 mg/kg/day for 20 days produces significant hepatotoxicity as evidenced by increase in serum AST and ALT, ALP activity and total bilirubin concentration. The increase in liver function tests were well directly correlated with them liver histological damage. In the humans and in the experimental animals, ibuprofen (at high doses) is well known to be the cause of hepatotoxicity. In the liver ibuprofen is metabolized to sulphate conjugates and extractable glucuronide. However, hepatotoxicity produced by ibuprofen may be due to formation of toxic metabolites. High doses of ibuprofen results to mitochondrial disorders followed by liver necrosis. All these changes mentioned above culminate in functional and morphological alterations resulting to loss of integrity of cell membranes which is manifested by the increase in the activity of serum marker enzymes (ALT and AST activity). Transaminase were secreted to blood in hepatocellular damage and their levels increased (34). This changes occurs because of hepatocyte damage due to the decreased activity of the antioxidant enzymes (SOD, CAT and disturbance of calcium (Ca²⁺) homeostasis(35). ALP is an enzymes derived from the liver and it is considered one of the liver function tests. It is plasma activity rise in cholestatic liver disorder because ALP synthesis is elevated and the enzyme within the biliary tract is regurgitated into the blood (36). As compared to control groups, there was significant increase in serum bilirubin concentration in ibuprofen treated groups, and this increase may be linked to regurgitation of bile due to obstruction within the liver as a result of inflammation or injury caused by Ibuprofen. This results reported in this study are agree with other studies showing elevations of these parameters (AST, ALP, ALT and total bilirubin) in experimental animals exposed to Ibuprofen (20,35). However, administration of Terfezia claveryi along with ibuprofen ameliorated the histological alterations induced in the liver by ibuprofen. Liver functions were also ameliorated as evidenced by significant restoration of serum AST, ALT, ALP activity and Bilirubin levels.
According to these results, Janakat et al., recorded that T. claveryi has a hepatoprotective effect on CC14-induced liver toxicity in wistar albino rats. Giving ibuprofen leads to a significant increase in liver content from MDA suggesting an increase in LPO that refers to oxidative stress (37). LPO is one of the basic mechanisms of damage to liver tissue caused by free radicals (38). Moreover, ibuprofen caused a significant reduction in SOD, CAT and GSH-Px activities. Antioxidants act as a radical tonic and inhibit LPO, thus protecting animal and human tissues from various diseases. There is a dynamic balance between the output of free radicals produced in the body and the antioxidant defense system that scavenges them and thereby protecting the human and animal body against pathogenesis. Antioxidant enzymes, such as SOD, GSH-Px and CAT are considered the first line of defense mechanism on free radical induced oxidative stress. SOD catalyzed the dismutation of the highly reactive superoxide anion to oxygen and to the less reactive species hydrogen peroxide (H$_2$O$_2$). H$_2$O$_2$ can be destroyed by GPx or CAT reactions (39-41). And CAT is responsible for the degradation of H$_2$O$_2$ to O$_2$ and H$_2$O. It is a protective antioxidant enzyme found in nearly all animal cells (42). A decrease in the activity of antioxidant enzymes and an increase in LPO level were reported after ibuprofen intoxication (20,35). Damage of liver tissue seen in this study may be resulted from the increase in LPO level and decrease of antioxidant enzymes activity in the liver following exposure to Ibuprofen.

However, administration of Terfezia Claveryi along with Ibuprofen caused significant decrease in MDA levels and significant increase in CAT, SOD and GSH-Px, suggested the protective role of Terfezia Claveryi. This protection offered by Terfezia Claveryi may be related to its free radical scavenging property. It is a very rich source of flavonoids which have been shown to possess different biological properties attributed to antioxidant mechanisms. Some studies recorded that the antioxidant capacity (ADC) of Terfezia Claveryi can be attributed to various chemical components of T. claveryi such as vitamin A, B-carotenoids, C and a large amount of phenolic compounds, which have an extremely strong antioxidant activity with high ability to search for peroxo roots, prevent cell membrane protrusions and reduce LPO (43). Thus, the protective effect of Terfezia Claveryi against Ibuprofen toxicity could be the result of direct free radical scavenger and antioxidant properties. There was a significant decrease in serum AST, ALT, ALP activity, total bilirubin levels, in addition to that there was significant decrease in liver tissue MDA level and significant increase in liver tissue SOD, CAT activity in Terfezia claveryi + Ibuprofen group as compared to Ibuprofen group. These results suggested that Terfezia claveryi and Ocimum basilicum has a prophylactic effect against liver toxicity caused by Ibuprofen.

We concluded, that addition of ibuprofen to pregnant rats increased LPO. Terfezia claveryi decreased the MDA levels in ibuprofen treated pregnant animals. Terfezia claveryi improved CAT, SOD and GSH-Px activities in liver tissues. We reported according to data application with Terfezia claveryi against oxidative injury in the liver which has the potential protective and prophylactic effect of Terfezia claveryi and can be said.

Conflict of interest
No conflict of interest was declared by the authors.

Acknowledgement
The author are very much thankfull to the College of Pharmacy, University of Kerbala / Kerbala / Iraq for their constant help, encouragement and support to the research. At the same time we acknowledge Dr. Nezar metab from Hussein hospital teaching/Kerbala/Iraq for histopathological examination.

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