
**POTENTIAL PROTECTIVE EFFECTS OF LEMON VERBENA (*ALOYSIA CITRIODORA*)
LEAVES AGAINST GASTRIC ULCER IN RATS: BIOLOGICAL, BIOCHEMICAL AND
TECHNOLOGICAL STUDIES**

By

Basma R.M.khateib

Mohammed R.Elkabary

*Department of Nutrition and Food Science,
Faculty of Home Economics, Menoufia University, Shebin El-kom, Egypt.*

Corresponding author : Email :

Basmaelkhateeb9@gmail.com

M_elkabary@yahoo.com

Research Journal Specific Education

Faculty of Specific Education

Mansoura University

ISSUE NO. 69, JULY , 2022

POTENTIAL PROTECTIVE EFFECTS OF LEMON VERBENA (*ALOYSIA CITRIODORA*) LEAVES AGAINST GASTRIC ULCER IN RATS: BIOLOGICAL, BIOCHEMICAL AND TECHNOLOGICAL STUDIES

*Basma R.M.khateib**

*Mohammed R.Elkabary**

Abstract:

Lemon verbena (*Aloysia citriodora*) is an herb characterized by containing a large amount of phenols such as flavonoids and phenylpropanoids, which have been found to play an effective role in controlling digestive disorders. So that, this study sought to evaluate the gastroprotective properties of lemon verbena against stomach ulcers in rats induced by ethanol. Randomly, thirty male Albino rats were divided into five groups (6/each). Rats were injected with ethanol to induce stomach ulcers. The antiulcer activity of lemon verbena leaves ethanol extract (LVEE) were estimated at different doses (50 and 100 mg/kg body weight) and compared with omeprazole (OMZ) which used as a standard drug at dose (40mg/kg body weight). To find out the possible mechanisms of action, data of the present study estimated the levels of oxidative stress parameters. Gastric juice volume, pH, and other stomach ulcer examinations as well as histological examination of gastric ulcers were done. Rats that were given ethanol only (positive group) showed significant elevations in ulcer score, ulcer index (UI) and decreases antioxidant enzymes. Moreover, the levels of malonaldehyde (MDA) remarkably increased in the positive group compared to the negative group. The pretreatment of rats with LVEE at different dose levels not only improved stomach ulcer measurements and the activities of antioxidants but also significantly reduced the concentration of MDA in stomach tissues. These results confirm that LVEE has gastroprotective properties by stimulating antioxidant enzymes. Finally, lemon verbena leaves powder has been used in bread flour with the goal of producing a fortified bread that can be recommended for stomach ulcer patients.

Keywords: Peptic ulcer, aloysia, antioxidant activity, bread, ulcer index, omeprazole.

* Department of Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Shebin El-kom, Egypt.

Introduction

Stomach ulcers are one of the most popular diseases of the gastrointestinal tract . These disease occurs as a result of several factors resulting from a defect in the preventive factors of the gastric mucosa (pepsin) and factors that destroy the gastric mucosa (acid). The most important other factors causing ulcers are infection, smoking , stress, consumption of non-steroidal anti-inflammatory drugs for a long time and excessive alcohol consumption (**Park *et al.*, 2020**) .Thus, the experimental model of ethanol-induced gastric ulcers is often used to estimate anti-ulcer components (**Kadasah *et al.*, 2021**).

Ethanol is a harmful substance that causes many diseases and can be used orally to cause stomach ulcers in experimental animals. Gastric ulcers caused by ethanol appear as a result of rupture of the gastric mucosa, which leads to increased bleeding and mucosal permeability. White blood cells (WBC) , like neutrophils, go to the location of ulcers in the stomach and increase production of inflammation mediating compounds and reactive oxygen species (ROS) so oxidative and cellular damage occurs (**Shin *et al.*, 2020**). Also, a precedent study demonstrated that oxidative stress during ethanol-induced gastric ulcers increases lipid peroxidation, which is demonstrated by elevated malondialdehyde concentrations in gastric tissues (**Tureyen and Ince 2021**).

There are many modern drugs, such as antacids, antibiotics, H2 receptor antagonists and proton pump inhibitors (PPIs) , which are largely used to reduce stomach infections (**Zhou *et al.*, 2020**) . Omeprazole (OMZ) is one of the most important drugs that have shown an effective therapeutic effect for stomach ulcers in several published studies (**Guzman-Gomez *et al.*, (2018)** and **Zhou *et al.*, 2020**). Despite this, treatment with modern medicines for a long period of time can cause many other health problems (**Raesi *et al.*, 2019**)

Through previous studies , it was found that natural herbs with broad biological properties, better performance and safe features are essential as an alternative to chemical treatments. Thus, there is a great need to analyze herbal products that are characterized by their pharmacological properties in order to identify alternative plant components with biological activity (**Asnaashari *et al.*, 2018**). One of the most famous herbs is lemon verbena which is scientifically called *Aloysia citrodora* and it is one of the plants known for its medicinal properties. *Aloysia* is one of the most important aromatic plants high in phenolic and phenylpropanoids substances, which

are considered one of the most powerful antioxidants. Verbena is used in traditional medicine to treat digestive disorders , such as indigestion, flatulence and acidity (**Rashidet al., 2022**). Lemon verbena leaves extracts are characterized by its high concentration of actioside, also called verbascoside . Studies have shown that it has important therapeutic effects such as its role as an antioxidant, sedative, relaxing, anticancer, anesthetic and antimicrobial (**Ghasempour et al., 2016 and Razavi et al., 2017**).Alcoholic and aqueous extracts of verbena leaves act as antipyretic, anti-inflammatory, antioxidant and analgesic (**Dubey, 2014**).

Since there is insufficient evidence for the intestinal effects of ethanolic lemon verbena extract, in this study, ethanolic extract of lemon verbena was prepared to study its gastroprotective effect in rats exposed to ethanol and comparing that effect with the therapeutic effect of omeprazole (OMZ) as one of the drugs recommended for gastric ulcers.

Material and methods

Plants: Lemon verbena leaves have been obtained from Harraz for Food Industry and Natural Products, Bab Alkhalq, Cairo, Egypt. The plant part was confirmed by the faculty staff in the plant department at faculty of agriculture, menoufia university.

Chemicals: Ethyl alcohol (95%) and all other chemicals were purchased from El- Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments. Omeprazole was purchased from local pharmacy

Diet: The basal diet was prepared according to the method developed by **AIN (1993)**. As for the vitamin mixture and salts mixture used were added according to **Reeves et al., (1993)** . Diet contents were obtained from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments.

Preparation of LVEE

Lemon verbena leaves were milled to be a fine powder. 100 g of verbena powder was mixed in 1000 ml of 96% ethanol for 48 hours. The mixture was then filtered using a filtered paper. The alcohol was evaporated and the extract was kept at -20 °C until use (**Mashayekhi-Sardoo et al., 2020**).

Determination of total phenolic contents, flavonoid , flavones , flavonols and the antioxidant activity of LVEE.

The level of antioxidant activity of LVEE was measured by spectrophotometric estimation based on the decreasing of methanol extract of DPPH according to (Okeet *et al.*, 2009). Flavonols and flavones were estimated according to Popova *et al.*, (2004) and The results were presented as mg quercetin per gram of dry weight of the sample (Popova *et al.*, 2004). Total flavonoid content was estimated according to Menichini *et al.*, (2009) quercetin was taken as the standard and the total phenolic contents were estimated according to Wojdyło *et al.*, (2007) and gallic acid was taken as the standard.

Experimental groups

Thirty male albino rats were used in the study and divided into five groups (6 rats each). The first and second groups fed on Basel diet, The third and fourth groups fed on standered diet and received LVEE (50 and 100 mg / kg body weight) , respectively while the fifth group fed on basel diet and received omeprazole 40 mg / kg body weight (Motawi *et al.*, 2012) orally, for 28 day as an experiment period.

Induction of gastric ulcer

On the last day of the experiment, which lasted for 28 consecutive days, rats were fasted for 24 hours, but water was available. The rats of the second, third, fourth and fifth groups were given a single oral dose of ethanol at a dose of 10 ml/kg of body weight (Huang *et al.*, 2014) to induce stomach ulcers for two hours. While normal rats group received a single oral dose of saline.

Biochemical analysis

After two hours of administration of ethyl alcohol to rats, the rats were anesthetized by diethyl ether, then the abdominal wall of the rats was opened, the pyloric opening was accurately identified and then the stomach was carefully tied from the esophageal opening, then removed and opened, gastric juice was collected and centrifuged to estimate gastric secretion . ulcer index (U.I) was carried out by multiplying ulcer score x 100 according to Radwan *et al.*, (2003) while ulcer score was estimated according to Robert *et al.*, (1968). The ulceration (%) and the preventive index were calculated according to Ohara *et al.*, (1992) and Hano *et al.* (1976) , respectively. The pH value of stomach juice was estimated by pH meter and the total acidity was determined by titration of 1ml gastric juice in

10ml of distilled water with 0.01N NaOH using two drops of phenolphthalein as an indicator (Bakr, 2020). Malondialdehyde (MDA), Superoxide dismutase (SOD) and Catalase enzyme (CAT) were assayed in stomach tissue according to Ohkawa *et al.*, (1979), Nishikimi *et al.*, (1972) and Aebi (1984), respectively.

Histopathology examinations of the stomach

Specimens from stomach were collected directly after scarification of animals at the end of experimental period (28days), fixed in 10% neutral buffered formalin, dehydrated in ethyl alcohol, cleared in xylene and embedded in paraffin 4 - 6 thick sections were prepared and stained with hematoxylin and eosin according to Carleton, (1976)

Technological methods

Baladi bread was prepared according to Shroubaet *al.*, (2009), in which wheat flour was replaced by 1–1.5% lemon verbena leaves. A set of sensory tests were performed to evaluate the product after the addition of verbena by 20 judges according to Watts *et al.*, (1989).

Statistical Analysis

Data was statistically analyzed using the Costat program, and the results were presented in the form of mean \pm SD according to (Steel and Torrie, 1980).

Results and Discussion

Total phenolic contents, total flavonoid, flavones and flavonols content and the antioxidant activity of LVEE

Data in Table (1) showed total phenolic contents, total flavonoid, flavones and flavonols content and the antioxidant activity of LVEE. Regarding to total phenolic contents, data indicated that the mean value of total phenolic compounds in LVEE was 52.5 ± 0.12 mg / g. In this context, Mohtashami *et al.*, (2013) reported that there are many factors that affect the medicinal plant's content of phenolic substances, one of them is growing factors and the phenological phase. Also, the method of extraction may affect the plant content of measured phenolic substances. The findings of the present research which showed the percentage of total phenolic components in LVEE are the same former results showed by NaserAldeen *et al.*, (2015), they reported that the mean value of total phenolic components in verbena leaves extract starting from 22.83 ± 0.76 to 48.21 ± 1.35 mg/g as gallic acid. While, the amount of total phenolic contents in the present study appeared to be higher than those reported by Choupani *et al.*, (2014). The difference is may be due to the phenological phase, time of

harvest and nutrition of soil (Moradi *et al.*, 2014). The mean values of total flavones and total flavonoids were 2.23 ± 0.08 and 16.45 ± 0.66 mg/g quercetin, respectively. These results are in agreement with HematianSourki *et al.*, (2021) they found that the amount of flavone and flavonols content and total flavonoids in the LVEE were 2.23 ± 0.08 and 14.54 ± 0.67 mg/g quercetin, respectively.

According to the data, the inhibitory force against DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radicals in LVEE was 79.33 ± 0.21 . In similar studies, Farahmandfar *et al.*, (2018) illustrated that raising the amount of verbena essential oil of from 200 to 3,200 ppm in sunflower oil led to improve the free radical scavenging activity of DPPH from 10% to 56%. Also, the present study is in accordance with Choupani *et al.*, (2014) they showed that the amount of DPPH radical scavenging activity in LVEE was 85% and this percentage was greater than the acetone and aqueous extract. In this regard also, Bilia *et al.*, (2008) and Ismail *et al.*, (2010) reported that LVEE showed a higher ability to restore organic compounds compared to the aqueous extract of lemon verbena.

Table (1): Total phenolic contents, total flavonoid, Flavones and flavonols content and the antioxidant activity of LVEE

Component	Mean+SD
Total phenols content (mg Gallic acid/g)	52.5 ± 0.12
Antioxidant activity (DPPH) (%)	79.33 ± 0.21
Flavones and flavonols content (mg Quercetin/g)	2.23 ± 0.08
Total flavonoid content (mg Quercetin/g)	16.45 ± 0.66

Effect of OMZ and LVEE on gastric lesions

Data in Table (2) showed the effect of *Lemon verbena* leaves extract on gastric juice volume (ml), gastric ulcer (GU) length (mm.), pH of gastric juice and total acidity (%) in negative control and gastric ulcer groups. It is known that alcohol rapidly permeates the gastric mucosa leading to smash the plasma membrane, this increases the permeability of the membrane to sodium and water (AL-Yahya and Asad, 2016). The positive group was significantly lower ($P \leq 0.05$) in pH and higher in gastric juice volume, ulcer length and total acidity compared to the negative group. Oral treatment with ethanol in positive control group led to reduce pH and raise gastric juice volume, ulcer length and total acidity (ENO *et al.*, 2004). The

group which treated with 100 mg / kg body weight LVEE showed more efficient in reducing the amount of total acidity and gastric juice and increasing pH than groups treated with 50 mg / kg LVE and omeprazole. Treatment of rats with LVEE (100 mg/kg body weight) for 28 days increased gastric mucosal protection resulting in gastric juice volume, pH and pH levels reaching the negative control level. **Enyew *et al.*, (2014)** reported that lemon verbena leaves used in the treatment of gastrointestinal disorders, such as stomach pain, intestinal worms, abdominal colic, jaundice, and cholecystitis. There are many of the proposed mechanisms for the anti-ulcer activity of polysaccharides, this may be due to their capacity to link to the mucosal surface and act as a preventive layer, by reducing acid secretion as well as free radical production. (**Ciprianiet *al.*, 2006**). Also, **Romano *et al.*, (2013)** point out that flavonoids have a good anti-ulcer effect because of their antioxidant influence. These results are consistent with **Kubicaet *al.*, (2020)** showing that lemon verbena leaves has a gastric preventive influence, and the mechanism of action may be due to the antioxidant properties related to anti-ulcer activity, as free radicals are developed in gastric mucosal lesions. Ethanolic extracts of lemon verbena leaves as a result of containing verbascoside, the main active compound in lemon verbena, showed more gastrointestinal protection than aqueous extract against indomethacin which caused stomach ulcers(**Mashayekhi-Sardo *et al.*, 2020**). On the other hand, **Speroniet *al.*, (2007)** reported that there were no significant changes observed in the value of gastric pH and the volume of gastric juice in the rats treated with verbena extracts comparison to positive group.

Table (2): Effect of OMZ and LVEE on gastric lesions

Groups	Gastric juice volume (ml)	PH value	Total acidity (%)	GU Length (mm)
control (-)	1.26 ^d ± 0.21	4.9 ^a ± 0.2	0.024 ^d ± 0.01	0 ^c ± 0
control (+)	5.06 ^a ± .49	1.23 ^d ± 0.15	0.133 ^a ± 0.02	5.9 ^a ± 0.2
LVEE (50 mg/kg B.Wt.)	4.26 ^b ± 0.25	2.13 ^c ± 2.08	0.092 ^b ± 0.01	4.76 ^b ± 0.25
LVEE (100 mg/kg B.Wt.)	1.67 ^d ± 0.16	5.2 ^a ± 0.20	0.041 ^{cd} ± 0.01	1.13 ^d ± 0.20
OMZ (40 mg/kg B.Wt.)	2.5 ^c ± 0.36	3.63 ^b ± 0.21	0.062 ^c ± 0.08	3.16 ^c ± 0.20
L.S.D	0.578	0.354	0.022	0.354

*Data are expressed as the mean ±SD (n = 5 per group). Mean values with the different letters in the same column mean significantly different at level p≤0.05.

Effect of LVEE and OMZ on ulcer score, ulcer index, ulceration (%) and preventive index

Table (3) indicated the effect of LVEE and OMZ on ulcer score, ulcer index, ulceration (%) and preventive index. The results showed that the

negative group did not have ulcer score, ulcer index and ulceration (%) because it received saline solution only. However positive group giving ethyl alcohol had the highest ulcer score, ulcer index and ulceration (%) compared to gastric ulcer groups treated with LVEE (50 and 100 mg / kg body weight) and omperazole. Preventive index had opposite trend .The best result was recorded to group 2 (100 mg / kg LVE) compared with rats treated with LVEE (50 mg / kg body weight) and rats treated with omperazole. **Ko and Cho, (2000)** showed that heavy consumption of alcohol can lead to inflammation of the stomach lining (gastritis), as well as stomach ulcers .While, **Speroniet al., (2007)** indicated that verbena can reduce the effect of alcohol due to its content of flavonoids and polyphenols which consider good antiulcer because of their antioxidant effect . Also, **Tajik et al., (2016)** reported that aqueous extract of verbena for unknown reasons can more strongly treat ethanol-induced gastric ulcers in rats compared to ranitidine as an effective ulcer drug .These results had the same trend reported by **Mashayekhi-Sardoet al . , (2020)** showing that treatment with LVEE (200 mg / kg) caused a fundamental decrease in the ulcer index and ulcer score .

Table (3): Effect of LVEE and OMZ on ulcer score, ulcer index, ulceration (%) and preventive index in rats exposed to ethanol

Groups	US	UI	Ulceration(%)	Preventive index
control (-)	-	-	-	-
control (+)	8.5	850	90.3	15.5
LVEE (50 mg/kg B.Wt.)	8	800	70.5	30.6
LVEE (100 mg/kg B.Wt.)	2.5	250	22.4	90.3
OMZ (40 mg/kg B.Wt.)	6.3	630	50.23	77.5

US= ulcer score

UI =ulcer index

Effect of OMZ and LVEE on oxidant and antioxidant status

Data in Table (4) shows the effect of LVEE on MDA, CAT and SOD level of negative control and gastric ulcer groups. Treatment with LVE (50,100 mg kg) and 40 mg/kg omperazole significantly improved the levels of CAT and SOD when compared to positive control rats. However, administration with LVEE led to decrease in MDA level. The highest reduction in MDA was observed in rats treated with LVEE (100 mg/ kg body weight) .

Table (4): Effect of OMZ and LVEE on oxidant and antioxidants status in the stomach of rats exposed to ethanol

Groups	MDA (nmol/g tissue)	CAT (U/g tissue)	SOD (U/mg tissue)
control (-)	57.63 ^c ± 1.61	18.36 ^a ± 0.50	8.46 ^a ± 0.89
control (+)	87.93 ^a ± 1.77	3.13 ^c ± 0.32	1.83 ^c ± 0.30
LVEE (50 mg/kg B.Wt.)	82.56 ^b ± 1.35	7.3 ^d ± 1.13	2.66 ^c ± 0.76
LVEE (100 mg/kg B.Wt.)	62.86 ^d ± 2.00	15.46 ^b ± 0.89	8.16 ^a ± 0.65
OMZ (40 mg/kg B.Wt.)	73.6 ^c ± 1.80	10.8 ^c ± 1.08	5.56 ^b ± 0.60
L.S.D	3.139	1.543	1.224

*Data are expressed as the mean ± SD (n = 5 per group). Mean values with the different letters in the same column mean significantly different at level p ≤ 0.05.

Lemon verbena due to the presence of polyphenols like flavones and verbascoside shows great antioxidant possibilities and inhibition of lipoxygenase enzyme (Casamassima *et al.*, 2017) and also, catechol groups have scavenging activity (Obied *et al.*, 2008). Verbascoside extracted from lemon verbena leaves owns anti-inflammatory characteristics because it reduces superoxide radical generation and glutathione peroxidase function, and consequently reduce inducible nitric oxide synthase activity (Speranza *et al.*, 2010). In addition to, Funes *et al.*, (2009) reported that verbascoside as a strong radical scavenger can cause decrement in MDA levels where the Daily use of lemon verbena extract for athletes significantly reduced MDA levels in neutrophils and kept up these cells from oxidative stress compared to the control group (Funes *et al.*, 2011). Mashayekhi-Sardo *et al.*, (2020) supported the previous results, as they indicated that the use of the aqueous and alcoholic extract of *verbena* (100 and 200 mg/kg body weight) leads to a decrease in the level of malonaldehyde in stomach tissues and improves stomach ulcers caused by indomethacin. This possibly by discouragement the lipoxygenase enzyme or inhibiting leukotriene receptors and lipid peroxidation. This result confirms the findings of Rashid *et al.*, (2022), in which they showed that lemon verbena has promising cytotoxic and antioxidant activities.

Sensory properties of bread supplemented with lemon verbena leaves powders

Interpretation of the data confirmed that fortification of bread with verbena powder had a significant positive effect on sensory characteristics. Adding the lemon verbena powder to bread in concentrations (1%- 1.5%) increased the consumer acceptance in terms of flavor, color, texture, appearance, taste and overall acceptability.

This results are agreement with **HematianSourki *et al* ., (2021)**they showed that adding various concentrations of verbena powder and essential oils to cookies improved the taste and aroma properties during the storage period, when evaluated sensory. This finding is also in support of what **Shirzadet *al.*, (2021)** said, as they emphasized that lemon verbena can maintain shelf life, food safety, and retention of biochemical compounds by reducing the decomposition process in strawberries in cold storage. Also, **Li *et al.*, (2021)** reported that the locust bean gum and sodium alginate active coatings compacted with essential oil of lemon verbena improved the quality and protracted the shelf life of yellow croaker during storage.

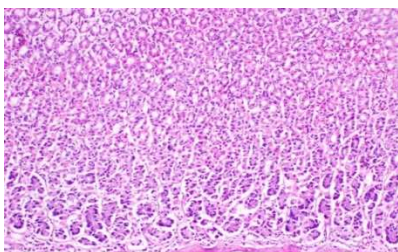
Table (5): Mean scores for the sensory properties of bread supplemented with lemon verbena leaves powders

Variable	Control	1% LV	1.5% LV	LSD
Color	8.06 ^a ± 0.25	8.36 ^{ab} ± 0.15	8.66 ^a ± 0.16	0.382
Flavor	8.16 ^b ± 0.15	8.16 ^b ± 0.05	8.80 ^a ± 0.26	0.358
Taste	8.20 ^b ± 0.26	8.53 ^b ± 0.35	9.13 ^a ± 0.20	0.561
Texture	8.03 ^a ± 0.15	8.5 ^{ab} ± 0.5	9 ^a ± 0.20	0.645
Compressibility	8.63 ^a ± 0.15	8.6 ^a ± 0.1	8.9 ^a ± 0.1	0.240
Apprance	9.23 ^a ± 0.25	8.16 ^b ± 0.28	9.06 ^a ± 0.30	0.565
Over all acceptability	8.23 ^a ± 0.20	7.86 ^a ± 0.15	8.30 ^a ± 0.26	0.426

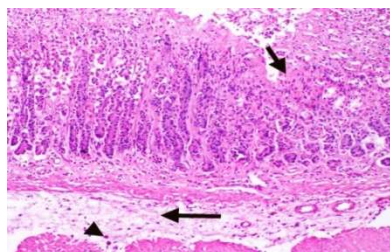
*Values are mean ± SD. Values in the same raw sharing the same superscript letters are not statistically significantly different. LV: lemon verbena.

Histopathological examination of stomach

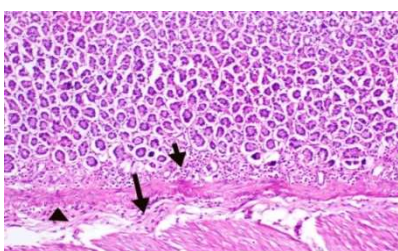
Microscopical examination of stomach of rats from group 1 showed normal histological gastric layers (pic. 1a) . On contrary, stomach of rat from group 2 revealed focal necrosis of gastric mucosa, sub mucosal edema and inflammatory cells infiltration (pic. 1 b). Meanwhile, stomach of rats from group 3 exhibited apparent normal histological gastric layers, whereas, some sections from this group revealed slight sub mucosal edema (pic. 1 c). Furthermore, gastric tissues of rats from group 4 showed no histopathological alterations except slight sub mucosal edema (pic. 1 d) in some section .On contrary, Otherwise, stomach of rats from group 5 described few mucosal and sub mucosal inflammatory cells infiltration as well as slight sub mucosal edema (pic. 1 e). The best result was recorded for group 4.



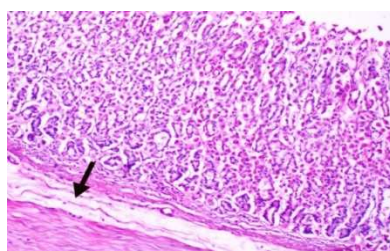
Picture (a)



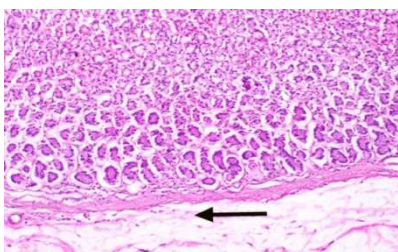
Picture (b)



Picture (c)



Picture (d)



Picture (e)

Picture 1. Effect of OMZ and LVEE on histopathological lesions in gastric mucosa in rats exposed to ethanol (H&E , 100) (n = 5 per group). (a) control group; (b) experimental group; (c) LVE (50 mg/kg) group ; (d)LVE (100mg/kg) group and (e) OMZ group (40mg/kg).The black arrows indicate sub mucosal edema and inflammatory cells infiltration.

Conclusion

In brief , the results of this study showed that oral treatment with LVEE at a dose of 50 and 100 mg/kg body weight has a precautionary influence on the augmentation of stomach ulcers induced by alcohol. Regarding the evidence for the effectiveness of verbena in treating stomach

ulcers and how this occurs, many researches must be conducted to prove the effective role of verbena in reducing stomach ulcers, because there is little research in this scope.

References

- Aebi, H. (1984):** Catalase *in vitro*. In: Methods in Enzymology, Academic Press, New York, 105:121-126.
- AIN.American Institute of Nutrition. (1993) :**Purified diet for laboratory Rodent, Final report. Journal of Nutrition, 123:1939-1951.
- Al-Yahya, A. A. and Asad, M. (2016):** Antiulcer activity of gum Arabic and its interaction with antiulcer effect of ranitidine in rats. Biomed. Research, 27(4): 1102-1106.
- Asnaashari, S.; Dastmalchi, S. and Javadzadeh, Y. (2018):** Gastroprotective effects of herbal medicines (roots). *International Journal of Food Properties*, 21: 901–919.
- Bakr , S.H. (2020) :** Curative effect of quercetin in rats inflicted with aspirin-induced gastric ulcer. Journal of the Faculty of Specific Education , 6(28):211-227.
- Bilia, A. R.; Giomi, M.; Innocenti, M.; Gallori, S. and Vincieri, F. F. (2008):** HPLC–DAD–ESI–MS analysis of the constituents of aqueous preparations of verbena and lemon verbena and evaluation of the antioxidant activity. *Journal of Pharmaceutical and Biomedical Analysis*, 46(3):463-470.
- Carleton, H.(1976):**Carleton"sHistoPathological Technique (4th Ed.) , London, Oxford University Press, New York, Toronto
- Casamassima, D.; Palazzo, M.; Vizzarri, F.; Costagliola, C.; Corino, C. and Di Costanzo, A. (2017) :** Dietary effects of plant extracts, based on verbascoside, lycopene and horseradish on several blood variables and plasma oxidative status in growing rabbits. *Livestock Science* , 206: 148-153.
- Choupani, M.; Delouee, S. A. and Alami , M. (2014) :** Antioxidant properties of various solvent extracts of lemon verbena (*LippiaCitriodora*) leaves . *International Journal of Advanced Biological and Biomedical Research*, 2(42): 494-500.

- Cipriani, T. R.; Mellinger, C. G.; de Souza, L. M.; Baggio, C. H.; Freitas, C. S.; Marques, M. C., ... and Iacomini, M. (2006):**A polysaccharide from a tea (infusion) of *Maytenus I licifolia* leaves with anti-ulcer protective effects. *Journal of Natural Products*, 69(7):1018-1021.
- Dubey, N.K.(2014):** Plants as A Source of Natural Antioxidants. CABI: Varanasi, India, 2014.
- Eno, A. E.; Azah, N.; Edet, E. E. and Itam, E. H. (2004):** Induced secretion of pepsin –rich gastric juice in the rat by the crude extract from elaeophobiadrupifera leaves: Adual pathway mechanism. *Nigerian Journal of Physiological Sciences*, 19(1-2): 53-59.
- Enyew, A.; Asfaw, Z.; Kelbessa, E. and Nagappan, R. (2014):**Ethnobotanical study of traditional medicinal plants in and around Fiche District, Central Ethiopia. *Current Research Journal of Biological Sciences*, 6(4):154-167.
- Farahmandfar, R.; Asnaashari, M.;Pourshayegan, M.;Maghsoudi, S. and Moniri , H. (2018):** Evaluation of antioxidant properties of lemon verbena (*Lippiacitriodora*) essential oil and its capacity in sunflower oil stabilization during storage time. *Food Science & Nutrition*, 6(4): 983–990.
- Funes, L.; Carrera-Quintanar, L.; Cerdán-Calero, M.; Ferrer, M. D.; Drobnic, F.;Pons, A. andMicol, V. (2011):** Effect of lemon verbena supplementation on muscular damage markers, proinflammatory cytokines release and neutrophils’ oxidative stress in chronic exercise. *European journal of applied physiology*, 111(4): 695-705.
- Funes, L.; Fernández-Arroyo, S.; Laporta, O.;Pons, A.; Roche, E.; Segura-Carretero, A., ... and Micol, V. (2009):** Correlation between plasma antioxidant capacity and verbascoside levels in rats after oral administration of lemon verbena extract. *Food Chemistry*, 117(4): 589-598.
- Ghasempour, M.; Omran, S.M.; Moghadamnia, A.A. andShafiee, F. (2016):** Effect of aqueous and ethanolic extracts of *Lippia citriodora* on candida albicans. *Electron. Physician*, 8(8): 2752–2758.
- Guzman-Gomez, O.; Garcia-Rodriguez, R.; Quevedo-Corona, L.; Perez-Pasten-Borja, R.; Rivero-Ramirez, N. and Rios-Castro, E.(2018):** Amelioration of ethanol-induced gastric ulcers in rats

- pretreated with phycobiliproteins of *Arthrospira* (*Spirulina*) *Maxima*. *Nutrients*, 10 (6) : 763.
- Hano, J.; Bogajske, J.; Danek, L. and Wantuch, C. (1976):** Effect of neuroleptic on the development of gastric injury related to oxidation, stress, and lipid peroxidation rats Laboratory investigation. *Polish Journal of Pharmacology*, 80 (8): 161-169.
- HematianSourki, A.; Ghani, A.; Kiani, F. and Alipour, A. (2021):** Phytochemical profiles of lemon verbena (*Lippiacitriodora* HBK) and its potential application to cookie enrichment. *Food Science and Nutrition* , 9(6): 3100-3113.
- Huang, C.; Chen , Y.; Chuan Wang, D.; Chiu, C.; Lin, W.; Huang, C. and Hsu, M. (2014):** Cytoprotective Effect of American Ginseng in a Rat Ethanol Gastric Ulcer Model. *Molecules*, 19: 316-326.
- Ismail, H. I., Chan, K. W., Mariod, A. A. and Ismail, M. (2010):** Phenolic content and antioxidant activity of cantaloupe (*Cucumismelo*) methanolic extracts. *Food chemistry*, 119(2): 643- 647.
- Kadasah, S.; Al Eid, A.S.; Alawad, S.S.; Al Shahrani, A.S.; Alruwaih, A.S.; Elfaki, I. and Arshaduddin, M. (2021):** Gastro protecting influence of Topiramate in ethanol produced gastric ulcers in rats. *Toxicology reports* , 8 : 1031–1039.
- Ko, J. K. and Cho, C. H. (2000):** Alcohol drinking and cigarette smoking: a " partner" for gastric ulceration. *Zhonghua yi xue za zhi= Chinese medical journal; Free China ed*, 63(12): 845-854.
- Kubica, P.; Szopa, A.; Dominiak, J.; Luczkiewicz, M. and Ekiert, H. (2020):** *Verbena officinalis* (Common Vervain)—a review on the investigations of this medicinally important plant species. *Plantamedica* , 86 (17): 1241-1257.
- Li, B.; Wang, X.; Gao, X.; Ma, X.; Zhang, L.; Mei, J. and Xie, J. (2021) :** Shelf-Life Extension of Large Yellow Croaker (*Larimichthyscrocea*) Using Active Coatings Containing Lemon Verbena (*Lippacitriodora*Kunth.) Essential Oil . *Frontiers in nutrition* , 8 : 1-14
- Mashayekhi-Sardoo, H.; Razavi, B. M.; Ekhtiari, M; Kheradmand, N. and Imenshahidi, M. (2020) :** Gastroprotective effects of both aqueous and ethanolic extracts of Lemon verbena leaves against indomethacin-induced gastric ulcer in rats. *Iranian Journal of Basic Medical Sciences* , 23(12) : 1639-1646.

- Menichini, F.; Tundis, R.; Bonesi, M.; Loizzo, M. R.; Conforti, F.; Statti, G. and Menichini, F. (2009):** The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinense* Jacq. cv Habanero. *Food Chemistry*, 114(2): 553–560.
- Mohtashami, S.; Babalar, M. and Mirjalili, M. H. (2013):** Phenological Variation in Medicinal Traits of *Dracocephalum moldavica* L. (Lamiaceae) under Different Growing Conditions. *Journal of herbs, spices & medicinal plants*, 19(4): 377-390.
- Moradi, M.; Mirzaei, A.; Naseri, R. and Vafa, P. (2014):** Effects of drought stress on qualitative and quantitative traits of mungbean. *International Journal of Agricultural and Biosystems Engineering*, 8(2): 148-152.
- Motawi, T. K.; Hamed, M. A.; Hashem, R. M.; Shabana, M. H. and Ahmed, Y. R. (2012):** Protective and therapeutic effects of *Argyrea speciosa* against ethanol-induced gastric ulcer in rats. *Zeitschrift für naturforschung c*, 67(1-2): 47-57.
- Naser Aldeen M. G.; Mansoor R. and AlJoubbeh M. (2015) :** Fluctuations of phenols and flavonoids in infusion of lemon verbena (*Lippia citriodora*) dried leaves during growth stages. *Nutrition and Food Science*, 45(5): 766–773.
- Nishikimi, M.; Appaji, N. and Yagi, K. (1972):** The occurrence of superoxide anion in the reaction of reduced phenazmethanesulfate and molecular oxygen. *Biochemical and biophysical research communications*, 46(2): 849-854.
- Obied, H. K.; Prenzler, P. D. and Robards, K. (2008):** Potent antioxidant biophenols from olive mill waste. *Food Chemistry*, 111(1): 171-178.
- Ohkawa, H.; Ohishi, W. and Yagi, K. (1979):** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, 95: 351–358.
- Oke, F.; Aslim, B.; Ozturk, S. and Altundag, S. (2009):** Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. *Food Chemistry*, 112(4): 874–879.
- Park, H.; Cho, D.; Huang, E.; Seo, J.Y.; Kim, W.G.; Todorov, S.D. (2020):** Amelioration of alcohol induced gastric ulcers through the administration of *Lactobacillus plantarum* APSulloc 331261 isolated from green tea. *Frontiers in microbiology*, 11: 420.
- Popova, M.; Bankova, V.; Butovska, D.; Petkov, V.; Nikolova-Damyanova, B.; Sabatini, A. G. and Bogdanov, S. (2004) :** Validated

- methods for the quantification of biologically active constituents of poplar-type propolis. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, 15(4): 235–240.
- Radwan, A. G.; Abdel Halem, A. T.; AbouSaif, A. M. and Mabrouk, M. (2003):** Protective effect of thymus extract against stress induced gastric ulcer in rats. *AL-Azhar Journal*, 3(4): 553-562
- Raeesi, M.; Eskandari-Roozbahani, N. and Shomali, T. (2019):** Gastro-protective effect of Biebersteiniamultifida root hydro-methanolic extract in rats with ethanol-induced peptic ulcer. *Avicenna journal of phytomedicine* . 9(5): 410–418.
- Rashid, H. M.; Mahmood, A. I.; Afifi, F. U. and Talib, W. H. (2022):** Antioxidant and Antiproliferation Activities of Lemon Verbena (*Lemon verbena citrodora*): An In Vitro and In Vivo Study. *Plants*, 11(6): 785.
- Rashid, H. M.; Mahmood, A. I.; Afifi, F. U. and Talib, W. H. (2022):** Antioxidant and Antiproliferation Activities of Lemon Verbena (*Lemon verbena citrodora*): An In Vitro and In Vivo Study. *Plants*, 11(6): 785.
- Razavi, B.M.; Zargarani, N. and Hosseinzadeh, H. (2017):** Anti-anxiety and hypnotic effects of ethanolic and aqueous extracts of Lippiacitriodora leaves and verbascoside in mice. *Avicenna journal of phytomedicine* 7(4):353–365
- Reeves, P.; Nielsen, F. and Fahmy, G. (1993):** Reported of the American Institute of Nutrition; committee on the reformulation of the AIN76, A rodent diet. *Journal of Nutrition*, 123:1939-51.
- Romano, B.; Pagano, E.; Montanaro, V.; Fortunato, A. L.; Milic, N. and Borrelli, F. (2013) :** Novel insights into the pharmacology of flavonoids. *Phytotherapy research* , 27(11): 1588-1596.
- Robert, A.; Nezamis, J. E. and Phillips, J. P. (1968):** Effect of prostaglandin E1 on gastric secretion and ulcer formation in the rat. *Gastroenterology*, 55(4): 481-487.
- Sharoba, A. M. A.; El-Desouky, A. I.; Mahmoud, M. H. M., and Youssef, K. M. (2009):** "Quality attributes of some breads made from wheat flour substituted by different levels of whole amaranth meal." *Journal of Food and Dairy Sciences*, 34 (6) : 6413-6429.

- Shin, J.K.; Park, J.H.; Kim, K.S.; Kang, T.H. and Kim, H.S. (2020):** Antiulcer activity of steamed ginger extract against ethanol/HCl-induced gastric mucosal injury in rats. *Molecules* . 25(20): 4663.
- Shirzad, H.; Alirezalu, A.; Alirezalu, K.; Yaghoubi, M.; Ghorbani, B.; Pateiro, M. and Lorenzo, J. M. (2021):** Effect of Lemon verbena *citrodora* essential oil on biochemicals, antioxidant characteristics, and shelf life of strawberry fruit during storage. *Metabolites*, 11(5): 256-53
- Speranza, L.; Franceschelli, S.; Pesce, M.; Reale, M.; Menghini, L.; Vinciguerra, I. ... and Grilli, A. (2010):** Antiinflammatory effects in THP-1 cells treated with verbascoside. *Phytotherapy Research*, 24(9): 1398-1404.
- Speroni, E.; Cervellati, R.; Costa, S.; Guerra, M. C.; Utan, A.; Govoni, P. ... and Stuppner, H. (2007):** Effects of differential extraction of *Verbena officinalis* on rat models of inflammation, cicatrization and gastric damage. *Plantamedica*, 73(03) : 227-235.
- Steel, R. G. and Torrie, J. H. (1980):** Principles and Procedures of Statistics McGraw-Hill Book. Co. Inc., New York, 481
- Tajik, J.; Kheirandish, R.; Azizi, S.; Amanollahi, R. and Mahmoudi, T. (2016):** the effects of aqueous extracts of *Lippiacitrodora*, *Trachyspermumcopticum*, *Dracocephalumpolychaetum* on repair of ethanol induced gastric ulcer in rats. *Comparative Clinical Pathology*, 25 (1): 119-123.
- Tureyen, A. and Ince, S. (2021):** Bupropion inhibits oxidant status and inflammation in ethanol-induced chronic gastritis in rats. *International Journal of Pharmacology*. 17(5): 281–291.
- Watts, B. M.; Ylimaki, G. L.; Jeffery, L. E. and Elias, L. G. (1989):** Basic sensory methods for food evaluation. IDRC, Ottawa, Canada. p.159.
- Wojdyło, A.; Oszmiański, J. and Czemerys, R. (2007) :** Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105 (3): 940–949.
- Zhou, D.; Yang, Q.; Tian, T.; Chang, Y.; Li, Y. and Duan, L.-R. (2020):** Gastroprotective effect of gallic acid against ethanol-induced gastric ulcer in rats: Involvement of the Nrf2/HO-1 signaling and anti-apoptosis role. *Biomedicine and Pharmacotherapy*, 126: 110075.

التأثيرات الوقائية المحتملة لأوراق لوزية الليمون ضد قرحة المعدة فى الفئران : دراسات

بيولوجية وكيموحيوية وتكنولوجيا

الملخص

لوزية الليمون (اللوزية) هي عشبة تتميز باحتوائها على كمية كبيرة من الفينولات مثل الفلافونويدات و فينائل بروبانويدات و التي وجد أنها تلعب دور فعال في السيطرة على اضطرابات الجهاز الهضمي. لذلك سعت هذه الدراسة إلى تقييم التأثيرات الوقائية المعوية المحتملة للوزية ضد قرحة المعدة التي يسببها الإيثانول فى الفئران. بطريقة عشوائية تم تقسيم ثلاثين من ذكور فئران الالبينو إلى خمس مجموعات (٦ / لكل مجموعة). تم حقن الفئران بالإيثانول لتحفيز القرحة المعدية. تم تقدير النشاط المضاد للقرحة للمستخلص الكحولى لأوراق لوزية الليمون بجرعات مختلفة (٥٠ و ١٠٠ مجم / كجم من وزن جسم) ومقارنتها مع أوميبرازول المستخدم كدواء قياسي بجرعة (٤٠ مجم / كجم من وزن الجسم). لمعرفة الآليات الممكنة للعمل ، قدرت بيانات الدراسة الحالية مستويات عوامل الإجهاد التأكسدي. تم إجراء قياسات حجم العصارة المعدية ودرجة الحموضة وغيرها من فحوصات قرحة المعدة وكذلك الفحص النسيجي لقرحة المعدة. أظهرت الفئران التي عوملت بالإيثانول فقط (المجموعة الإيجابية) زيادة معنوية في درجة القرحة ومؤشر القرحة ونقص في إنزيمات مضادات الأكسدة. علاوة على ذلك ، زادت مستويات المألونالدهيد بشكل ملحوظ في المجموعة الإيجابية مقارنة بالمجموعة السلبية. لم تؤدي المعالجة المسبقة للفئران باستخدام المستخلص الكحولى لأوراق اللوزية بمستويات جرعات مختلفة إلى تحسين فحوصات قرحة المعدة وأنشطة مضادات الأكسدة فحسب ، بل أدت أيضاً إلى خفض مستويات المألونالدهيد بشكل ملحوظ في أنسجة المعدة. تشير هذه النتائج إلى أن المستخلص الكحولى لأوراق اللوزية يحتمل أن يمارس آثاره المعوية عن طريق تحفيز الإنزيمات المضادة للأكسدة. أخيراً ، تم استخدام مسحوق أوراق لوزية الليمون في دقيق الخبز بهدف إنتاج خبز مدعم يمكن التوصية به لمرضى قرحة المعدة.

الكلمات المفتاحية : قرحة المعدة ، اللوزية ، نشاط مضاد للأكسدة ، خبز ، مؤشر القرحة ، أوميبرازول

