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Comparison Effect of an aqueous extract of *Alpinia* and *Fenugreek seeds* on Male Rats with Gastric Ulcer

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ABSTRACT

This study aimed to investigate the effects of aqueous extracts of Fenugreek seeds (FSAE) and Alpinia (AAE) on ethanol-induced stomach ulcers in rats. For five weeks, thirty male rats weighing 150 -170 gram. Then, divided into two major groups. The first one included five rats were fed a basal diet as part of a normal control group (negative group). To induce stomach lesions, twenty-five rats in the second group were fasted overnight and subsequently administered a single oral dose of 5 ml per kg of ethanol. These groups were split into 5 subgroups, as follow: (G 2:positive control group, which was not given any treatment and was given the basal diet, G 3: Rats given the basal diet, besides 250 ml/kg/daily of FSAE orally, G 4: Rats given the basal diet, in addition to 500 ml/kg/daily of FSAE orally, G 5: Rats given the basal diet, in addition to 250 ml/kg/daily of AAE orally: and G 6: Rats given the basal diet, in addition to 500 ml/kg/daily of AAE orally. Histological examinations, chemical assays, and biochemical assessments were conducted on both serum and gastrointestinal mucosa samples. The results revealed that the overall antioxidant capacity of FSAE was notably greater than that of AAE. However, there were no observable differences in the overall levels of flavonoids and phenols between FSAE and AAE.When compared to the positive control group, serum levels of AST, ALT, creatinine, urea, and uric acid were significantly lower in all groups that received support from FSAE (250 and 500 ml/kg) and AAE (250 and 500 ml/kg). For G6 and G4, respectively, the best findings were obtained. The study found that groups with varying percentages of FSAE and AAE had higher serum levels of GSH, GPX, SOD, and PH, but lower levels of MDA and MPO.The G6 achieved the best outcomes. Various FSAE and AAE treatments significantly increased stomach mucosa GSH, GPX, SOD, and PH levels, while MDA and MPO levels decreased in all groups compared to the untreated group.Compared to the positive control group, all groups receiving diets supplemented with different proportions of FSAE and AAE exhibited significant enhancements in stomach histopathology. Among these, G6 demonstrated the most favorable outcomes. Consequently, this study recommends the utilization of Alpinia aqueous extract as a potent agent for gastric protection. It also suggests its therapeutic potential in managing gastrointestinal disorders linked with oxidative stress and inflammation, especially in the prevention and treatment of ethanol-induced gastric ulcers.

Keywords: gastric biochemical parameters -Fenugreek seeds aqueous extract - inflammatory parameters - Alpinia aqueous extract -oxidative stress markers - gastric ulcer.

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INTRODUCTION

Gastric ulcers, a prevalent digestive ailment, can arise from various potential causes. Among these factors, alcohol consumption is a significant contributor **(Aroke *et al*., 2020)**. Alpinia officinarum Hance species, a lesser galangal in the Zingiberaceae family, is used in traditional Chinese medicine for treating stomach pains, renal calculus, diabetes, bronchitis, chronic enteritis, heart and kidney diseases, and has anti-inflammatory, carminative, emmenagogue, aphrodisiac, and abortifacient properties (**Yi Sun *et al*., 2016)**. Ethnopharmacological significance: Alpinia officinarum Hance, a perennial herb, holds historical importance in traditional medicine for its therapeutic applications in addressing various conditions including pain, inflammation, stomachaches, and colds (**Abubakara *et al*., 2018).**

Extensive pharmacological investigations have revealed the robust in vivo and in vitro bioactivities of numerous components found in A. officinarum, encompassing lipid regulation and antioxidant properties. Over ninety phytochemical constituents have been documented and isolated from A. officinarum, with phenolic compounds, particularly diarylheptanoids, constituting the majority of these bioactive elements (**Abubakara *et al*., 2018).**

A. officinarum is used in over thirty-three formulations in various countries for treating gastro oesophageal reflux disease, gastritis, stomachaches, and gastric ulcers, and in capsule forms like Jiangxiang Anwei and Weitong laying (**Ding *et al*., 2015)**.

Herbs are extremely valuable in Indian homes for medicinal purposes. One of the most promising nutritionally valuable medicinal plants is fenugreek (Trigonella foenum-graecum), which is found in Asia, Europe, Africa, and Australia. It is a standard medical procedure for several ailments. Fenugreek has a known medical use after a number of studies on its constituent chemicals. Seeds provide dietary fiber because of their high fiber content. Fenugreek contains gum, fiber, alkaloids, flavonoids, saponins, and volatile substances. Among its many medical applications are as an antioxidant, hypoglycemic, hypocarcinogenic, hypocholesterolemia, antibacterial, and stomach stimulant (**Aher *et al*., 2016)**.

Trigonella foenum graecum (L.), commonly known as fenugreek, belongs to the Fabaceae family. This herbaceous plant has been extensively studied for its antioxidant properties present in both its seeds and leaves. Additionally known as methi, fenugreek is utilized in Ayurvedic medicine to address various health issues, including digestive problems, arthritis, bronchitis, wounds, and abscesses (**Aggarwal and Shishodia, 2006)**.

Stomach ulcers respond well to fenugreek seeds. The aqueous extract and gel fraction from fenugreek seeds showed significant effects on ulcer prevention. The cytoprotective characteristics of the seeds are due to their anti-secretory characteristics and effects on mucosal glycoproteins. Moreover, fenugreek seeds have been observed to inhibit ethanol-induced increases in lipid peroxidation. Other than that, the mechanism is that it raises the antioxidant capacity of the gastric mucosa, which reduces mucosal injury. Several investigations have demonstrated that omeprazole was less effective in preventing the formation of lesions than the soluble gel fraction derived from the seeds. According to (**Platel and Srinivasan, 2000)**, these results show that fenugreek seeds may have antiulcer properties.

Fenugreek seed extract, rich in polyphenols, can protect erythrocytes from oxidative degradation, maintain hemoglobin and PCV levels, and potentially treat indomethacin-induced stomach ulcers (**Singaravelu *et al*., 2018)**.

The application of Trigonella foenum seed produced an antiulcer effect. Omeprazole, a drug that blocks the proton pump and is used to treat digestive disorders like gastritis, gastric ulcers, duodenal ulceration, and gastroesophageal reflux disease, has effects similar to those of fenugreek seeds. In a rat model where ethanol was used to induce stomach ulcers, the gel portion and aqueous extract from fenugreek seeds have effects on mucosal glycoproteins, and ant secretory activity has a protective role against ulcers (**Pandian *et al*., 2002)**.

According to (**Negm and Aboraya, 2023)**, the composition of A. officinarum powder (per 100 grams dried) consists of 2.23 grams of fat, 5.56 grams of protein, 3.24 grams of ash, 59.94 grams of carbohydrates, 11.76 grams of moisture, and 17.27 grams of fiber. Fenugreek seeds contain approximately 25% fiber, 23–26% proteins, 0.9% lipids, and 58% carbohydrates (**Wani and Kumar, 2018)**.

Aim of the study

Therefore, the aim of this study was to assess the impact of an aqueous extract derived from Trigonella Fenugreek seeds and Alpinia officinarum Hance on male rats afflicted with gastric ulcers.

MATERIALS & METHODS

Materials:

- Dried Fenugreek seeds (Trigonella) and Alpinia (Alpinia officinarum) were obtained from the National Research Centre in Dokki, Cairo, Egypt.

- El-Gomhoreya Company, located in Cairo, Egypt, supplied the minerals, vitamins, choline chloride, casein, cellulose, and ethanol (96.75%) used in the study.

- Starch and oil were bought from a neighbourhood market in Cairo, Egypt.

- Thirty albino male rats (Sprague Dawley Strain), weighing between 150 and 170 grams, were obtained from the Food Technology Research Institute in Giza.

Methods:

Chemical analysis

Quantifying the aggregate quantities of flavonoids, phenols, and antioxidants

The total flavonoids and phenols in the aqueous extracts of alpinia and fenugreek seeds were quantified following the methodologies described by (**Ivanov et al., 2015) & (Liu et al., 2009).**

Preparation of Alpinia &Fenugreek seeds aqueous extracts

100 grams of dried alpinia and fenugreek seeds were boiled in distilled water for 15 minutes, then chilled and filtered in a sterile (**vial.Nwabufo and Olusanya, 2017).**

Biological Experiment

Feed cups were utilized for diet provision to reduce food wastage, while a glass tube that was placed through the cage's wire provided the rats with water. following the method outlined by Reeves et al., 1993. The components for the basal diet, totaling one hundred grams, were prepared. Animal investigations were conducted with the consent of the UResearch Animal Facility-Institutional Animal Care and Use Committee, Cairo University, under approval number URAF-E-4-23.

The components of the basal diet were formulated according to the (**AIN technique, 1993)**, comprising choline chloride (0.20%), cellulose (5%), a vitamin mixture (1%), maize oil (10%), casein (12%), mineral mixture (4%), and starch (remaining quantity). After period of adaptation , the animals were divided into two main groups. The first one, termed the normal control , consisted of five rats fed the standard diet for five weeks. The second group of 25 rats received a single oral dose of 5 milliliters per kilogram of ethanol after an overnight fast to induce stomach lesions, as per the protocol outlined by **(Tolulope et al., 2019).** Subsequently, this group was further divided into five subgroups, each comprising five rats. Group 2: Positive control group, receiving only the basal diet without any additional treatment. Group 3: Rats fed the basal diet, supplemented with an oral dose of 250 ml/kg of Fenugreek seeds aqueous extract. Group 4: Rats fed the basal diet, supplemented with an oral dose of 500 ml/kg of Fenugreek seeds aqueous extract. Group 5: Rats fed the basal diet, supplemented with an oral dose of 250 ml/kg of Alpinia aqueous extract. Group 6: Rats fed the basal diet, supplemented with an oral dose of 500 ml/kg of Alpinia aqueous extract.

Every week for the five weeks of the experiment, food consumption was noted and each rat's weight was recorded. Using the following formula, the body weight gain and food efficiency ratio (FER) were calculated in accordance with (**Chapman *et al*., 1959)**:

$$(\text{BWG}) \quad = \frac{\text{Final Weight\_ Initial Weight}}{\text{Initial Weight}}$$

$$(\text{FER}) \quad = \frac{\text{Daily body Weight gain(g)}}{\text{Food intake (g/d)}} * 100$$

Rats were fasted for an entire night at the conclusion of the experiment before being slaughtered. After blood collection, the blood was centrifuged. For biochemical analysis of inflammatory measures and oxidative damage markers, serum was separated and kept at -20°c. For instance, ELISA kits (catalog NO.MBS2636626), (catalog NO.MBS744364), (catalog No.CSB-E12144r), (catalog NO.CSB-E08555r), (catalog NO.CSM-E08722r) were used to analyze malondialdehyde (MDA), glutathione peroxidase (GPX), glutathione (GSH), superoxide dismutase (SOD), myeloperoxidase (MPO) and PH, respectively. (**Reitman & Frankel's, 1957)** analysis of aspartate amino transferase (AST) and alanine amino transferase (ALT) was followed. Serum uric acid was measured using the methods described by (**Fossati *et al*., 1980)**, creatinine by (**Bartels & Bohmer, 1971)** and urea by (**Marsh *et al*., 1965).**

Additionally, for the purpose of biochemical analysis (gastric biochemical parameters), the stomach mucosa was removed and kept at -20 celsius degree. This allowed for the analysis of the following: glutathione peroxidase (GPX) (catalog NO.MBS744364), myeloperoxidase (MPO) (catalog NO.CSM-E08722r) &PH, superoxide dismutase (SOD) (catalog NO.CSB-E08555r), aglutathione (GSH) (catalog No.CSB-E12144r).

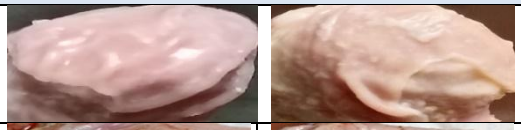
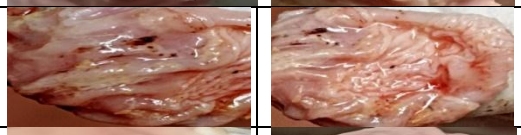
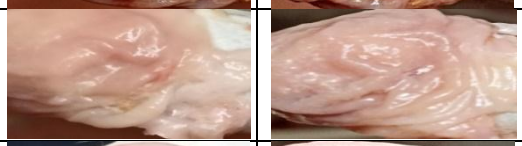
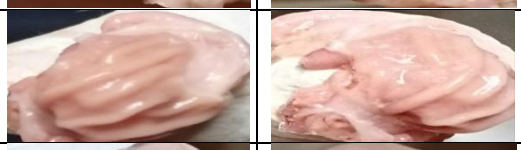
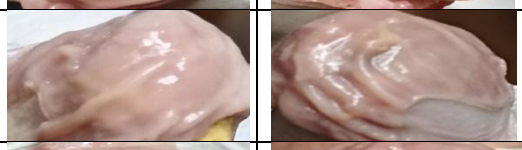
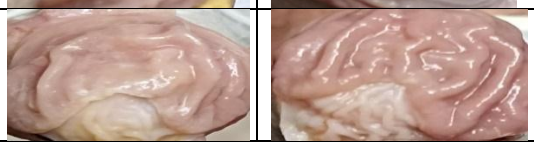
Histopathological Examination

Each rat's stomach was removed, photographed (Table 1), and then subjected to a histopathological examination in accordance with (**Bancroft *et al*., 2012).**

Statistical analysis

The findings are presented as mean ± SD. According to (**McClave & Benson, 1991)**, one-way analysis of variance, or "ANOVA," was used to statistically analyze the data.

Table (1): image of the stomach

Organs / Groups	the stomach image
Group(1)	
Group(2)	
Group(3)	
Group(4)	
Group(5)	
Group(6)	

Group (1): (-ve) control, **Group(2): (+ve)** control, **Group(3):** 250 ml/kg of Fenugreek seeds aqueous extract**, Group(4):** 500 ml/kg of Fenugreek seeds aqueous extract**, Group(5):** 250 ml/kg of Alpinia aqueous extract**, Group(6):** 500 ml/kg of Alpinia aqueous extract**.**

RESULTS AND DISCUSSION

Table (2): Trigonella foenum graecum and Alpinia officinarum Hance's aqueous extract's active ingredient.

Content / Samples	Antioxidants (%)	Total phenols (mg/ml)	Total flavonoids (mg/ml)
Fenugreek seeds aqueous extract	**89.54 ^{a} ± 0.18**	**0.64 ^{a} ± 0.14**	**0.28 ^{a} ± 0.08**
Alpinia aqueous extract	**74.67 ^{b} ± 0.12**	**0.71 ^{a} ± 0.21**	**0.35 ^{a} ± 0.05**
LSD 0.05	**0.35**	**0.40**	**0.15**

Mean values in the same row with distinct superscript letters indicate a significant difference $P \leq 0.05$.

The aqueous extract from fenugreek seeds exhibits notably higher total antioxidant activity compared to that from alpinia officinarum, as demonstrated in Table 2 illustrates. Conversely, phenols and flavonoids in total were not significantly present between fenugreek seeds or alpinia

aqueous extract. According to (**Negm and Aboraya, 2023),** the composition of alpinia officinarum aqueous extract includes 46.51± 0.40 GAE/g total phenols, 30.97± 1.85 QE/g total flavonoids, and 3.05± 0.26 DPPH% antioxidant activity. Conversely, fenugreek seed extract exhibits substantial antioxidant potential with total phenolic contents of 454.93 ± 3.57 mg GAE/g, total flavonoid contents (TFC) of 135.04 ± 2.12 µg/CE, and total antioxidant capacity (TAC) of 162.51 ± 3.81 per gram of dry plant extract, as highlighted by (**Fatima *et al*., 2022)**.

Table (3): The impact of administering aqueous extracts of Alpinia officinarum Hance and Trigonella foenum graecum on nutritional parameters in rats with gastric ulcer

groups / Parameter	Negative Group(1)	Positive Group(2)	Fenugreek seeds aqueous extract		Alpinia aqueous extract		LSD 0.05
			250 ml/kg Group(3)	500 ml/kg Group(4)	250 ml/kg Group(5)	500 ml/kg Group(6)	
FI (g/d)	19.53 ^{a} ± 0.16	19.63 ^{a} ± 0.21	19.3 ^{a} ± 0.66	19.15 ^{a} ± 0.35	19.05 ^{a} ± 0.19	19.05 ^{a} ± 0.31	0.63
BWG (g)	0.14 ^{a} ± 0.09	0.46 ^{a} ± 0.36	0.35 ^{a} ± 0.18	0.20 ^{a} ± 0.14	0.25 ^{a} ± 0.12	0.28 ^{a} ± 0.18	0.35
FER (%)	4.05 ^{a} ± 2.48	12.81 ^{a} ± 9.75	9.89 ^{a} ± 5.37	5.81 ^{a} ± 4.13	7.14 ^{a} ± 3.14	8.05 ^{a} ± 4.99	9.79

(FI) feed intake, (BWG) body weight gain and (FER) feed efficiency ratio

Mean values in the same row with distinct superscript letters indicate a significant difference $P \leq 0.05$.

When examining the body weight gain, feed efficiency ratio, and feed intake of the control group (G) in comparison to normal rats, the data presented in Table (3) indicated a non- significant differences. Moreover, when comparing both control rats ("G"1 and 2) to the treatment groups (3, 4, 5, and 6), there was no significant difference observed in body weight gain, feed efficiency ratio, or feed intake.The results were validated by (**Teixidor-Toneu *et al*., 2016)**. They found A. officinarum is combined with other herbs in a msahan mixture, which is used as traditional medicine in Morocco. To cure conditions like musculoskeletal diseases, gynecological conditions, and general health, the plant materials are pulverized, dried, and combined with meals. Similar to this, the Marrakech region of Morocco uses the roots to heal colds and promote weight gain. (**Javaid *et al*., 2021)** found that chronic administration of Alpinia led to reduction in weight gain and Lee index of rats compared to the obesogenic diet-fed rats**. (Zhang *et al*., 2020)** reported food intake bettween the groups was not significantly different .

Table (4): The impact of administering the aqueous extract of Alpinia officinarum Hance and Trigonella foenum graecum on liver and kidney function in rats with gastric ulcers

groups / Parameter	Negative Group(1)	Positive Group(2)	Fenugreek seeds aqueous extract		Alpinia aqueous extract		(LSD) 0.05
			250 ml/kg Group(3)	500 ml/kg Group(4)	250 ml/kg Group(5)	500 ml/kg Group(6)	
Liver function							
ALT (U-L)	58.28 ^{d} ± 11.47	160.61 ^{a} ± 10.63	102.36 ^{bc} ± 10.20	64.62 ^{d} ± 8.75	120.29 ^{b} ± 17.82	85.93 ^{bc} ± 8.45	20.72
AST (U-L)	93.98 ^{c} ± 12.25	235.04 ^{a} ± 6.58	166.83 ^{b} ± 14.78	108.34 ^{c} ± 14.61	149.25 ^{b} ± 14.12	94.09 ^{c} ± 9.01	21.86
Kidney function							
Creatinine (mg-dl)	0.64 ^{c} ± 0.08	1.22 ^{a} ± 0.04	0.87 ^{b} ± 0.10	0.74 ^{bc} ± 0.06	0.86 ^{b} ± 0.07	0.59 ^{c} ± 0.12	0.14
Urea (mgl-dl)	20.2 ^{d} ± 1.17	57.19 ^{a} ± 3.08	43.06 ^{b} ± 4.34	29.98 ^{c} ± 2.25	41.49 ^{b} ± 3.59	26.26 ^{c} ± 3.91	5.76
Uric acid (mg-dl)	2.99 ^{d} ± 0.13	5.98 ^{a} ± 0.14	5.05 ^{b} ± 0.18	4.20 ^{c} ± 0.30	3.80 ^{c} ± 0.38	3.10 ^{d} ± 0.23	0.43
Alanine amino transferase (ALT) & Aspartate amino transferase (AST)							

Mean values in the same row with distinct superscript letters indicate a significant difference $P \leq 0.05$.

Data presented in **Tab. 4** revealed a notable elevation in serum levels of AST, ALT, creatinine (Cr), urea (UN), and uric acid (UA) among positive rats in comparison to normal control rats. Conversely, rats administered aqueous extracts containing 250 and 500 ml of fenugreek seeds, as well as 250 and 500 ml of Alpinia per kg, exhibited significantly reduced serum levels of AST, ALT, UN, UA, and Cr when contrasted with the positive control group. Our results agreed with (**Kaviarasan and Anuradha, 2007)** stated that It was discovered that fenugreek seed possesses hepatoprotective qualities. Fenugreek seed polyphenolic extract functions as a shield against ethanol-induced hepatic abnormalities.Additionally, (**Kaviarasan and Anuradha, 2007)** reported that fenugreek powder's ethanolic extract inhibited ethanol-induced toxicity in Chang liver cells, and that its protective activity is equivalent to that of the common hepatoprotective drug sylimarin. It was discovered that fenugreek polyphenolic extract prevented ethanol-induced liver damage by regulating hepatic injury indicators (ALT, AST, ALP, LDH, and GGT), boosting hepatocyte viability, and lowering apoptotic nuclei. Creatinine levels significantly decreased when fenugreek was taken orally. prior studies demonstrating enhanced kidney functioning following fenugreek supplementation and has a favorable impact on the elderly's hepatic enzyme regulation (**Marine *et al.*, 2020).**In another study, confirmed that Alpinia officinarum improving liver function parameters, and suppressing oxidative stress, as well as modifying antioxidant defense mechanisms (**Niazvand *et al.*, 2023)**. Additionally, (**Rajesh *et al.*, 2013)** revealed that, decrease AST, ALT levels when compared to model control group indicating that Alpinia Officinarum is effective in reducing abnormal AST, ALT levels. (**Lin *et al.*, 2018)** found Rhizoma Alpiniae Officinarum extract, which has high contents of total phenolics and flavonoids, showed significant hypouricemic and renal protective effects on hyperuricemic rats, and reducing urea nitrogen and creatinine levels. (**Palanirajan *et al.*, 2022)** indicated a reduction in urea, uric acid, and creatinine levels upon administration of Alpinia purpurata extract. Additionally, it was observed that serum marker enzymes including Protein, AST, ALT, ALP, and LDH were significantly elevated ($p<0.05$) in the prostate cancer-induced group. However, oral administration

of Alpinia purpurata for 16 weeks to both induced and simultaneously treated groups markedly normalized these values.

Table (5): The impact of administering aqueous extracts of Alpinia officinarum Hance and Trigonella foenum graecum on gastric biochemical measures

groups / Parameter	Negative Group(1)	Positive Group(2)	Fenugreek seeds aqueous extract		Alpinia aqueous extract		(LSD) 0.05
			250 ml/kg Group(3)	500 ml/kg Group(4)	250 ml/kg Group(5)	500 ml/kg Group(6)	
GSH (ng-mg)	223.63 ^{a} ± 22.34	39.9 ^{d} ± 5.05	95.01 ^{c} ± 15.42	139.11 ^{b} ± 10.33	86.52 ^{c} ± 16.05	160.03 ^{b} ± 8.50	25.14
GPX (U-mg)	200.73 ^{a} ± 17.37	43.32 ^{d} ± 5.68	98.22 ^{c} ± 16.25	145.85 ^{b} ± 16.50	92.24 ^{c} ± 10.60	148.13 ^{b} ± 12.41	24.48
SOD (U-mg)	215.33 ^{a} ± 7.16	50.11 ^{d} ± 8.89	85.19 ^{c} ± 11.32	138.50 ^{b} ± 7.80	107.14 ^{c} ± 19.05	155.37 ^{b} ± 16.88	22.59
MDA (nmol-mg)	0.63 ^{d} ± 0.14	9.23 ^{a} ± 0.34	6.64 ^{b} ± 0.57	2.24 ^{c} ± 0.67	5.83 ^{b} ± 0.56	1.77 ^{c} ± 0.35	0.84
MPO (ng-mg)	0.50 ^{d} ± 0.11	4.82 ^{a} ± 0.18	2.90 ^{b} ± 0.44	1.45 ^{c} ± 0.39	3.15 ^{b} ± 0.40	1.52 ^{c} ± 0.44	0.63
PH	7.58 ^{a} ± 0.28	4.52^{d} ± 0.52	5.94 ^{c} ± 0.17	6.85 ^{b} ± 0.16	6.03 ^{c} ± 0.29	6.94 ^{b} ± 0.27	0.54

Glutathione (GSH), Glutathione peroxidase (GPX), Superoxide dismutase (SOD), Malondialdehyde (MDA), Myeloperoxidase (MPO) & Potential hydrogen (PH)

Mean values in the same row with distinct superscript letters indicate a significant difference $P \leq 0.05$.

The study assessed the impact of an aqueous extract from fenugreek seeds and an aqueous extract from alpinia on oxidative stress and gastric protection. Gastric biochemical markers, including GSH, GPX, SOD, MDA, MPO, and PH, were utilized to measure stomach acidity and identify oxidative stress. According to Table (5), Rats induced with ethanol displayed swift damage to their stomach mucosa and substantial changes in oxidative stress biochemical markers compared with control group. Damage to the stomach mucosa resulted in a notable decrease in GSH, GPX, SOD, and PH levels in the positive control group compared to the negative control group. However, rats administered aqueous extracts of fenugreek seeds and Alpinia exhibited a significant increase in PH, SOD, GPX, and GSH levels. Among these, the most promising outcomes were observed for G6 and G4, respectively. (**Johnley *et al.*, 2020)** reported similar outcomes and noted that treatment with alpinia rhizomes extract showed a significant increase in SOD, CAT and GSH compared to the positive control group. Also (**Singaravelu *et al.*, 2018)** found comparable outcomes and noted that, Compared to control positive group, fenugreek-treated group exhibited a substantial decrease in ulcer index, gastric juice volume, and acidity. An analysis of oxidative enzymes revealed a noteworthy rise in Catalase, SOD, and GSH levels in the treated group in contrast to the positive control group.

Conversely, positive Control exhibited significantly higher MDA activity and MPO levels compared to negative Control. Conversely, rats administered Fenugreek seeds aqueous extract and Alpinia aqueous extract showed a notable reduction in MDA activity and MPO levels. The most notable outcomes were observed for G6.The findings of (**Lin *et al*., 2021)** provide support for these conclusions as they show that by enhancing SOD activity, an extract from the rhizomes of Alpinia officinarum can efficiently scavenge for oxygen free radicals created by ethanol. This, in turn, can increase hydrogen peroxide breakdown, inhibit lipid peroxidation to protect the stomach from oxidation, and avoid injury to the gastric mucosa. Also these conclusions are corroborated by the outcomes of (**Elsayed and Hussein, 2019)** who demonstrated that the fenugreek aqueous extract demonstrated a protective effect, which was demonstrated by a decrease in inflammation. After

comparing the outcomes of several therapies, it was shown that fenugreek extract had the strongest inhibitory effect on MPO.

Table (6): The impact of administering an aqueous extract comprising Alpinia officinarum Hance and Trigonella foenum graecum on inflammatory indicators and oxidative stress markers in serum

groups / Parameter	Negative Group(1)	Positive Group(2)	Fenugreek seeds aqueous extract		Alpinia aqueous extract		(LSD) 0.05
			250 ml/kg Group(3)	500 ml/kg Group(4)	250 ml/kg Group(5)	500 ml/kg Group(6)	
GSH (ng-mg)	186.25 ^{a} ± 18.07	35.53 ^{d} ± 5.62	75.81 ^{c} ± 7.67	149.04 ^{b} ± 16.16	93.39 ^{c} ± 11.69	148.43 ^{b} ± 14.96	23.40
GPX (U-mg)	189.98 ^{a} ± 13.12	30.67 ^{e} ± 8.70	74.97 ^{d} ± 9.63	134.29 ^{b} ± 14.88	100.81 ^{c} ± 17.12	147.35 ^{b} ± 14.16	23.59
SOD (U-mg)	194.49 ^{a} ± 12.03	34.47 ^{e} ± 2.21	73.70 ^{d} ± 6.89	122.48 ^{c} ± 19.21	104.18 ^{c} ± 13.20	145.58 ^{b} ± 8.78	20.76
MDA (nmol-mg)	0.64 ^{d} ± 0.15	8.51 ^{a} ± 0.62	5.25 ^{b} ± 0.31	2.01 ^{c} ± 0.75	2.36 ^{c} ± 0.75	1.22 ^{cd} ± 0.27	0.95
MPO (ng-mg)	0.79^{e} ± 0.16	7.00 ^{a} ± 0.31	4.59 ^{b} ± 0.47	2.71 ^{c} ± 0.42	4.15 ^{b} ± 0.34	1.76 ^{d} ± 0.32	0.62
PH	7.45 ^{a} ± 0.22	4.91 ^{d} ± 0.1	5.82 ^{c} ± 0.37	6.63 ^{b} ± 0.60	6.74 ^{ab} ± 0.07	7.52 ^{a} ± 0.48	0.64

Glutathione (GSH), Glutathione peroxidase (GPX), Superoxide dismutase (SOD), Malondialdehyde (MDA), Myeloperoxidase (MPO) & Potential hydrogen (PH)

Mean values in the same row with distinct superscript letters indicate a significant difference $P \leq 0.05$.

The effects of Fenugreek seeds aqueous extract and Alpinia aqueous extract on inflammatory parameters and oxidative stress markers in serum were evaluated. Utilizing biochemical markers including GSH, GPX, SOD, MDA, MPO, and PH, we were able to measure blood acidity and identify oxidative stress. Table (6) displays these findings. In comparison to the negative control group, the ethanol treatment had lowerd the serum concentrations GSH, GPX, SOD& PH. However, rats fed aqueous extracts of fenugreek seeds and alpinia revealed a notable increase in GSH, GPX, SOD, and PH.The best results were for G6 and G4, respectively. Contrary, to the negative control group, the ethanol treatment raised MDA and MPO serum levels. Simultaneously, rats administered aqueous extracts of fenugreek seeds and Alpinia showed a considerable decrease in MDA and MPO when compared to G2, with G6 and G4 showing the best outcomes, respectively. These results are in agreement with (**Darwish *et al.*, 2020)** that mention adding fenugreek seed powder to the diet causes rats' oxidative damage indicators to decrease. Also these results are in agreement with (**Jin *et al.*, 2019)** who demonstrated that Alpinia conchigera significantly reduced ulcer index, total acidity and pH. furthermore, the extract improved of CAT and SOD relative to rats in respective control groups. These results may be due to Total flavonoids content of Alpinia Officinarum whereas (**Lin *et al.*, 2023)** observed that Alpinia officinarum Hance's total flavonoid successfully reversed the ethanol-induced cell apoptosis (from 23± 1.3 to 8.11 ± 0.93%) and decreased the area of the stomach ulcer in rats (from 11.2 ± 1.89 to 2.19 ± 0.95). Additionally, it improved oxidative stress.

Histopathological Examinations

Examination of stomach Histopathologically:

Histopathological examination of stomach of rats from group 1 (normal control) exhibited the normal histoarchitecture of gastric layers (Figs. 1). In adverse, The rats' stomachs of group 2 (untreated group) revealed histopathological damage characterized by gastric mucosal focal necrosis accompanied with edema of submucosa (Figs. 2). On the other hand, stomach of rats from group 3 (250 ml/kg of Fenugreek seeds aqueous extract) showed slight submucosal edema and focal shortening of the stomach mucosa (Fig. 3).Otherwise, stomach of group 4 rats (500 ml/kg of Fenugreek seeds aqueous extract) showed slightly submucosal edema (Fig. 4). Likewise sections from group 5 (250 ml/kg of Alpinia aqueous extract) demonstrated submucosal edema (Fig. 5). Furthermore, marked improved picture was noticed in stomach of rats from group 6 (500 ml/kg of Alpinia aqueous extract), examined sections exhibited no histopathological lesions (Figs. 6). The results are in agreement with (**Qu, *et al*., 2021)** who reported that Alpinia officinarum has been shown in vivo studies to be able to prevent ethanol-induced damage to the gastric mucosa of mice by reducing inflammation and reducing pain.Additionally, (**Brinal Figer *et al*., 2017)** revealed that, a positive connection was found between the extract dosages and the protective response, meaning that an increase in dose was accompanied by an increase in protection. It was discovered that the highest concentration of extract—1000 mg/kg—completely prevented ulcer ulcers. (**Helmy., 2011)** who found that a popular spice and condiment in Indian homes is fenugreek the high concentration of flavonoids, phenol, saponins, and amino acids in the plant seeds has been shown to have substantial medicinal values. Animal models have been used to demonstrate the antiulcer efficacy of aqueous and gel. (**Jelodar *et al.*, 2005)** reported that Fenugreek has two active alkaloids: lysine and L-tryptophan. It has galactomannans and mucilage, which help to preserve the stomach mucosa. The stomach's parietal cells' ability to secrete hydrochloric acid is inhibited by fenugreek seeds. It lessens lipid peroxidation, which is a primary factor in stomach mucosal inflammation. Additionally, (**Khan *et al.*, 2018)** study reported that primary active component of Alpinia officinarum is the flavonoid galangin. According to earlier research, the antiulcer effects of polysaccharides were primarily attained through one or more of the following pathways: binding to the mucosa surface to provide a protective coating, lowering the secretions of gastric acid and pepsin, preserving the mucus barrier, or lowering oxidative stress or inflammatory response of the gastric mucosa.

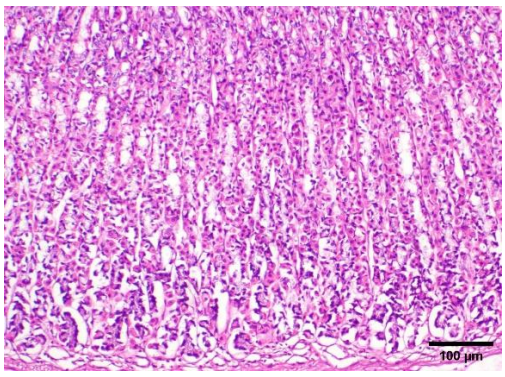


Figure. 1: A microscopy image of rat's stomach from G1 showing the normal histoarchitecture of gastric layers (Hematoxylin & Eosin stain, X 100, scale bar 100µm).

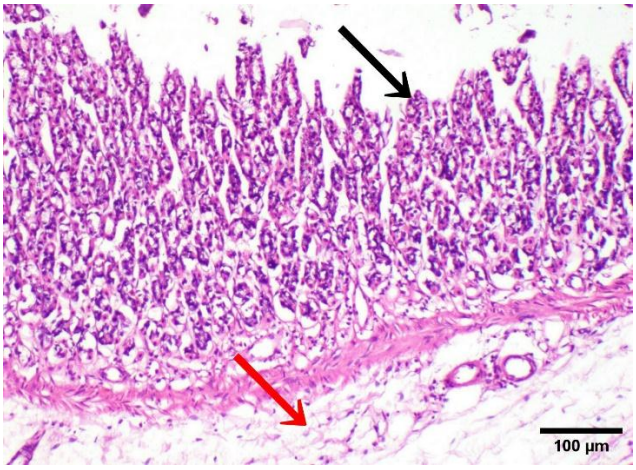


Figure. 2: A microscopy image of rat's stomach from G 2 showing focal necrosis of the stomach mucosa accompanied with edema of the submucosa (Hematoxylin & Eosin stain, X 100, scale bar 100µm).

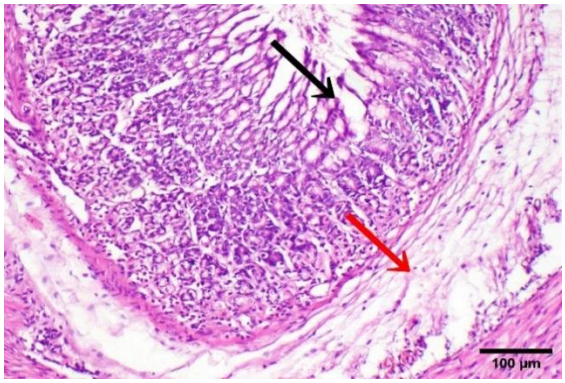


Figure. 3: A microscopy image of rat's stomach from G 3 showing focal shortening of gastric mucosa and slight edema of the submucosa (Hematoxylin & Eosin stain, X 100, scale bar 100µm).

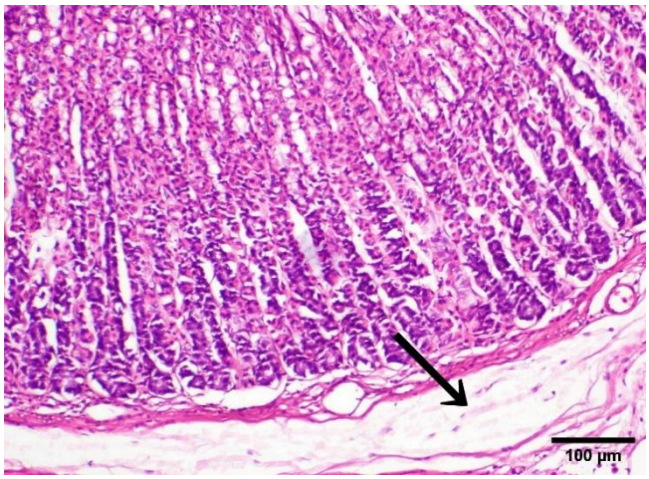


Figure. 4: A microscopy image of rat's stomach from G 4 showing slight edema of the submucosa (Hematoxylin & Eosin stain, X 100, scale bar 100µm).

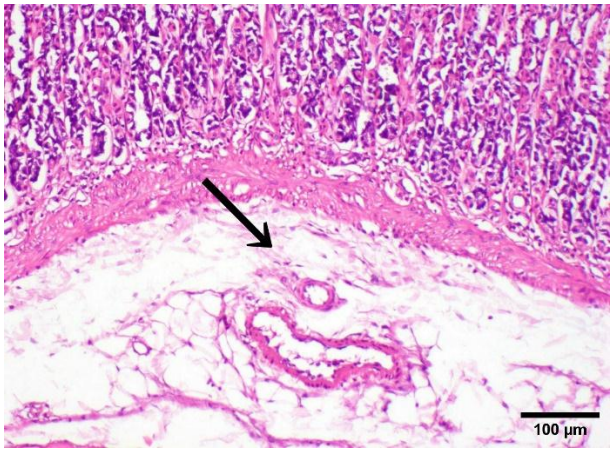


Figure. 5: A microscopy image of rat's stomach from G 5 showing edema of the submucosa (Hematoxylin & Eosin stain, X 100, scale bar 100µm).

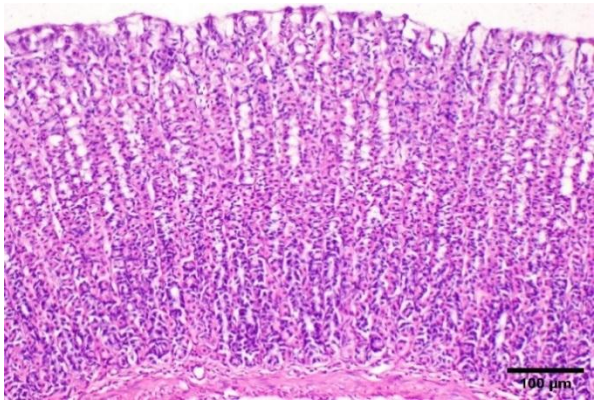


Figure. 6: A microscopy image of rat's stomach from G6 showing histological normal gastric (Hematoxylin & Eosin stain, X 100, scale bar 100µm).

CONCLUSION

Both fenugreek seeds aqueous extract and Alpinia aqueous extract demonstrated notable antiulcer activity against ethanol-induced gastric ulcers, likely attributed to their antioxidant properties. Additional investigations is needed precise mechanism implicit the antiulcer effects for these extracts.

REFERENCES

Abubakara,I.B.; Ibrahim Malamib .;Yakubu Yahayac and Sule,S.M.(2018). A review on the ethnomedicinal uses, phytochemistry and pharmacology of Alpinia officinarum Hance. Journal of Ethnopharmacology .224 , 45–62.

Aggarwal ,B.B and Shishodia, S .(2006). Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 71: 1397-1421.

Aher, R.R.;Belge, S.A.;Kadam, S.R.; Kharade, S.S and Misal ,A.V .(2016). Therapeutic Importance of Fenugreek (Trigonella foenum-graecum L.): A Review. 02 J Plant Sci Res.;3(1): 152.

AIN (American Institute of Nutrition). (1993). Purified diet for laboratory Rodent; Final report. J. Nutrition. 123:1939-195.Compacted Berth Journal of Essential Oil Research. 8(6): 657-664.

Aroke, E. N .; Powell-Roach ,K. L.; Jaime-Lara, R. B.; Tesfaye,M.; Roy,A.;Jackson, P and Joseph, P. V.(2020). Taste the Pain: The Role of TRP Channels in Pain and Taste Perception', Int. J. Mol. Sci., 21, 5929.

Bancroft ,J.D.; Suvarna,K & Layton,C.(2012). Bancroft's theory and practice of histological techniques. 7th ed. E book ISBN. 978-0-7020- 5032–5039.

Bartels, H. & Bohmer, M. (1971). Creatinine standard and measurement of serum creatinine with picric acid. Clin. Chem.; Acta, 32:81.

Brinal Figer.; Pissurlenkar ,R.; Premlata Ambre.;Samidha Kalekar .;Renuka Munshi Fenugreek Seed Extract; In Vitro, In Vivo and In Silico Approaches. Indian J Pharm Sci;79(5):724-730 .

Chapman, D.G.; Castilla,R. & Champell, J.A.(1959).Evaluation of protein efficiency ratio , Canadian Journal of Biochemistry and Physiology,37:679-686.

Darwish, M. M., Shaalan, S., Amer, M. A., & Hamad, S. R. (2020). Ameliorative effects of dried and germinated fenugreek seeds on kidney\failure induced by gentamicin in male mice. *Am. J. Biomed. Sci. & Res*, *9*(6), 459-466.-

Ding, P.;Liu, Y.; Wang, Z and Zhang, J.(2015). Alpinia officinarum Hance (Gaoliangjiang, galangal). In: (Eds.), Dietary Chinese Herbs Chemistry, Pharmacology and Clinical Evidence. Springer, US, New York, NY, pp. 61–67.

Elsayed, M., & Hussein, R. (2019). A comparative study of anti inflammatory and antioxidant activities of fenugreek (Trigonella foenum-graecum) and caraway (Carum carvi) aqueous and oil seed extracts in experimental model of ulcerative colitis. World J Pharm Res, 8, 251-266.

Fatima, H.; Shahid, M.; Pruitt, C.; Pung, M.A.; Mills, P.J.; Riaz, M.; Ashraf, R.(2022). Chemical Fingerprinting, Antioxidant, and Anti-Inflammatory Potential of Hydroethanolic Extract of Trigonella foenum- graecum. Antioxidants. 11, 364.

Fossati, P., Prencipe, L., & Berti, G. (1980). Use of 3, 5-dichloro-2- hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clinical chemistry, 26(2), 227-231.

Helmy, H.M.(2011). Study the effect of fenugreek seeds on gastric ulcer in experimental rats. World J Dairy Food Sci;6:152-8.

Ivanov, I., Petkova, N., Denev, P., & Pavlov, A. (2015). Polyphenols content and antioxidant activities in infusion and decoction extracts obtained from Fragaria vesca L. leaves. Scientific Bulletin:Series F.Biotechnologies,XIX. 145-148.

Javaid, F., Mehmood, M. H., & Shaukat, B. (2021). Hydroethanolic extract of A. Officinarum hance ameliorates hypertension and causes diuresis in obesogenic feed-fed rat model. Frontiers in Pharmacology, *12*, 670433.

Jelodar, G.; Maleki ,M.; Motadayen ,M and Sirus, S.(2005). "Effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats". Indian Journal of Medical Sciences.;59(2):64-9.

Jin, X., Liu, Q. L., Dang, M., Zhang, W., & Li, X. (2019). Gastroprotective and ulcer healing properties of ethanol extract ofAlpinia conchigera rhizome in experimentally induced gastric ulcer in rats. *Tropical Journal of Pharmaceutical Research*, *18*(9), 1873-1879.

Johnley, I. I. R., Somasundaram, G., Salwe, K. J., & Manimekalai, K.(2020). Anti ulcerogenic and anti-oxidant activity of Alpinia galanga rhizomes aqueous extract in indomethacin induced gastric mucosal damage in wistar albino rats. *Biomedical and Pharmacology Journal*, *13*(1), 55-60.

Kaviarasan ,S& Anuradha ,C.V (2007). Fenugreek (Trigonella foenum graecum) see polyphenols protect liver from alcohol toxicity: a role on hepatic detoxification system and apoptosis. Pharmazie 62(4): 299-304.

Khan,A. M. S.; Khumdmiri, S. U. K.; Khudmiri, S. R.; Al-Sanea, M. M and Ling,P. M.(2018). Fruit-derived polysaccharides and terpenoids:Recent update on the gastroprotective effects and mechanisms. Frontiers in Pharmacology, 9, 569.

Lin, K., Deng, T., Qu, H., Ou, H., Huang, Q., Gao, B& Wei, N. (2023). Gastric protective effect of Alpinia officinarum flavonoids: mediating TLR4/NF-κB and TRPV1 signalling pathways and gastric mucosal healing. *Pharmaceutical Biology*, *61*(1), 50-60.

Lin, K., Qu, H., Tan, Y., Deng, T., Gao, B., & Wei, N. (2021). Effects of the diphenylheptane extract of Alpinia officinarum rhizomes on ethanol- induced gastric ulcers in mice. *Iranian journal of basic medical sciences*, *24*(5), 657.

Lin, L.; Liu, X and Zhao, M. (2018). Screening of xanthine oxidase inhibitor from selected edible plants and hypouricemic effect of Rhizoma Alpiniae Officinarum extract on hyperuricemic rats. Journal of Functional Foods, 50, 26-36.

Liu, S. C., Lin, J. T., Wang, C. K., Chen, H. Y., & Yang, D. J. (2009). Antioxidant properties of various solvent extracts from lychee (Litchi chinenesis Sonn.) flowers. Food Chemistry, 114(2), 577-581.

Marine E. Baset, Tasneem I. Ali1, Hanan Elshamy, Ahmed M. El Sadek , Diana G. Sami , Marwa T. Badawy , Sara S. Abou-Zekry , Hana H. Heiba , Mona K. Saadeldin and Ahmed Abdellatif. (2020). Anti-\ diabetic effects of fenugreek (Trigonella foenum-graecum) comparison between oral and intraperitoneal administration - an animal study International Journal of Functional Nutrition .1: 2.1-9.

Marsh, W. H., Fingerhut, B., & Miller, H. (1965). Automated and manual direct methods for the determination of blood urea. Clinical chemistry11(6), 624-627.

McClave, J. T., & Benson, P. G. (1991). Statistical for business an economics. Maxwell Macm Publishing Co. The USA, 272-295.

Negm, S and Aboraya, A. (2023). Effect of Alpinia officinarum Aqueous Extract on Lipopolysaccharide-Induced Acute Lung Injury in \ Rats. Journal of Specific Education Research,*2023*(78).

Niazvand, F.; Ashtari, A.; Chamkouri, N and Azari, M. (2023). Hepatoprotective effects of Alpinia officinarum rhizome extract on cisplatin-induced hepatotoxicity in rats: A biochemical, histopathological and immunohistochemical study. Journal of Trac Elements in Medicine and Biology, 80, 127306.

Nwabufo, C. K., & Olusanya, O. (2017). Biochemical effect of hibiscut sabdariffa calyx extracts on the reproductive hormones of male wistarrat. Advances in Applied Science Research, 8(2), 38-41.

Palanirajan, A.; Kannappan, P and Kanniappan, G. V. (2022). Anticancer activity of Alpinia purpurata (Vieill) K. Schum. Against MNU and testosterone induced prostate cancer in male Wistar albino rats. Pharmacological Research-Modern Chinese Medicine, 3, 100105.

Pandian, R. S.; Anuradha, C. V and Viswanathan, P.(2002). Gastroprotective Effect of Fenugreek Seeds (Trigonella Foenum Graecum) on Experimental Gastric Ulcer in Rats. J. Ethnopharmacol., 81, 393–397.

Platel ,K and Srinivasan ,K .(2000). Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. Nahrung 44: 42-46.

Qu, H.J .;Lin,K.W.;Li,X.L.;Ou,H.Y.;Tan,Y.F.;Mei Wang and Na Wei. (2021). Chemical Constituents and Anti-Gastric Ulcer Activity of Essential Oils of Alpinia officinarum (Zingiberaceae), Cyperus rotundus (Cyperaceae), and Their Herbal Pair. Chem. Biodiversity, 18, e2100214.

Rajesh, I.;Singh, M.; Maheshwara, A. J. V. U and Prathyusha, M. (2013). Hepatoprotective effect of Alpinia officinarum on hepatic ischemia/reperfusion-induced injury in rats. Am J PharmTech Res, *3*, 2249-4.

Reeves, P. G.; Nielsen, F. H. & Fahmy, G. C. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN- 76A rodent diet. Journal of Nutrition; 123(11):1939-1951.

Reitman, S. & Frankel, S. (1957). Determination of glutamate pyruvate transferase. American Journal of Clinical Pathology, 28:56.

Singaravelu,S.K.; Jaikumar Sankarapillai .; Chandrakumari,A.S and Pammy Sinha. (2018). Effect of Trigonella foenum gracecum (Fenugreek) Seed Extract in Experimentally Induced Gastric Ulcer in Wistar Rats. Pharmacogn J. 10(6): 1169-1173.

Teixidor-Toneu, I.; Martin, G.J.; Ouhammou, A.; Puri, R.K and Hawkins, J.A(2016). An ethnomedicinal survey of a Tashelhit-speaking community in the High Atlas, Morocco. J. Ethnopharmacol. 188, 96–110.

Tolulope, O. A., David, O. A., Sylvester, E. S., & Ugochukwu, U. (2019). The histochemical and biochemical effects of coconutmilk in wistar rats with ethanol-induced gastric ulcer. Anatomy Journal of Africa, 8(2), 1558-1569.

Wani, S. A.and Kumar, P.(2018). Fenugreek: A Review on Its Nutraceutical Properties and Utilization in Various Food Products. J. Saudi Society Agri. Sci., 17, 97–106.

Yi Sun.; Masahiko Kurokawa.; Motofumi Miura.; Tomohito Kakegawa.; Shigeyasu Motohashi and Ken Yasukawa.(2016). Bioactivity and Synthesis of Diarylheptanoids From \ Alpinia officinarum. Studies in Natural Products Chemistry, 49,:157-.

Zhang, T.; Qiu, J.;Wu, X.;Huang, S.;Yuan, H and Park, S. (2020). Schizonepeta Tenuifolia with Alpinia Oxyphylla Alleviates Atopic Dermatitis and Improves the Gut Microbiome in Nc/Nga Mice. Pharmaceutics, 12(8), 722.