Protective Effects of Aqueous Extract of Indian Gooseberry Fruit Phyllanthus emblica and Neem Leaves Azadirachta indica and Their Mixture on Ulcer Healing and Oxidative Stress in Rats



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Protective Effects of Aqueous Extract of Indian Gooseberry Fruit *Phyllanthus emblica* and Neem Leaves *Azadirachta indica* and Their Mixture on Ulcer Healing and Oxidative Stress in Rats

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Abstract:

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most common cause of peptic ulcers (approximately 25%). Because synthetic drugs frequently have side effects, using natural plant extracts, is now thought to be an alternative method of treating the condition. Since the use of herbal products has been increasing, the study's objectives were to determine the chemical composition and total phenolic compounds of Indian gooseberry fruit and Neem leaves as well as to study the possible protective effects of aqueous extracts from Indian gooseberry and Neem leaves and their mixture on oxidative stress and ulcer healing in rats. After adjusting for a week, thirty female Wister rats weighing 150 ± 10 g were split up into two major groups. As a negative control group, Group (1) was the first to be fed the basal diet (-ve). The second major group divided into 4 groups as follow: Group (2), the Positive Control Group (+ve), was given distilled water orally every day. And 500 mg/kg BW/day of aqueous Indian gooseberry extract was administered orally as a pretreatment to Group (3). Aqueous extract of Neem leaves (350 mg/kg BW/day) was administered orally as a pretreatment to Group (4). 250 mg of Indian gooseberry +250 mg of Neem / kg BW/day were administered orally as a pretreatment to Group (5). All rats, with the exception of those in the negative control group, received a single oral dosage of 500 mg acetyl salicylic acid/ kg BW, after YV days of pretreatment. Rat groups' stomachs and livers were collected for further examination at the completion of the experiment (21 days). Results showed that, ingestion of Aspirin at 500 mg/ kg BW resulted in significant increases of gastric juice volume and in the pH of gastric juice, with significant increment of gastric mucosal injury area when compared with values of the negative control group, on the other hand ingestion of aqueous extract of Indian gooseberry, Neem and their mixture enhanced the gastric protection by increasing the ulcer inhibition percentage, ingestion of Aspirin resulted in a notable and significant increase of gastric and liver MDA, and both reduced glutathione (GSH) and (Catalase) showed significantly lower values as compared with negative control group. In groups of rats treated with gooseberry, Neem and their mixture there were a significant reduction in MDA and significant increments in GSH and

CAT of stomach and liver tissues. From these results it could be concluded that both Indian gooseberry and Neem were effective in preventing gastric ulcer and reducing oxidative stress.

Key Word: Gastric ulcer, oxidative stress, Indian gooseberry, Neem, and protective effect.

التأثير الوقائي للمستخلص المائي لفاكهة عنب الثعلب الهندي وأوراق النيم وخليطهما على التئام القرحة والإجهاد التأكسدي في الجرذان

ملخص البحث:

من الاسباب الشائعة للاصابة بقرحة المعدة هو تناول الادوبة المضادة للالتهابات غير الستيروبدية، (٢٥%)، وحيث ان الادوبة المخلقة صناعيا غالبا ما يصاحبها حدوث اثار جانبية لذا فان تناول النباتات الطبية او مستخلصاتها والمستخدمة تقليديا تعتبر الان اتجاه بديل للعلاج من بعض الأمراض. لذا فان هذه الدراسة تهدف الى تقدير التركيب الكيميائي والفينولات الكلية في فاكهة عنب الثعلب الهندي واوراق النيم، ودراسة الاثار لتناول مستخلص مائي من فاكهة عنب الثعلب الهندي واوراق النيم المجففه وخليطهما للوقاية من قرحة المعدة والمساعدة في سرعة الشفاء واثرهما على الاجهاد التاكسدي في الجرذان. تم استخدام ثلاثون من اناث الجرذان وزن ١٥٠+ ١٠ جرام، تم قسمت الي مجموعتان رئيسيتان: المجموعة الاولي (١) هي المجموعة الضابطة السلبية (عدد ٦) وتغذت على الغذاء القياسي، والمجموعة الرئيسية الثانية قسمت الى اربعة مجموعات: المجموعة الثانية (٢) هي المجموعة الضابطة الموجبة وتم تغذيتها على الغذاء القياسي. بينما تم تغذية المجموعة الثالثة (٣) على الغذاء القياسي بالاضافة الى ٥٠٠ مللجم / كجم من وزن الجسم من مستخلص عنب الثعلب المائي عن طريق الأنبوب المعدى، بينما تم تغذية المجموعة الرابعة(٤) على الغذاء القياسي بالاضافة الي ٣٥٠ مللجم / كجم من وزن الجسم من المستخلص المائي لاوراق النيم عن طريق الأنبوب المعدي، بينما تم تغذية المجموعة الخامسة (٥) على الغذاء القياسي بالاضافة الى خليط من ٢٥٠ مللجم/ كجم من وزن الجسم من مستخلص عنب الثعلب المائي+٢٥٠ مللجم / كجم من وزن الجسم من المستخلص المائي لاوراق النيم عن طريق الأنبوب المعدي، وبعد مرور ٢١ يوما تم اعطاء جرعة من حمض الاسيتيل ساليسيليك •• مللجم/ كجم من وزن الجسم/ الجرذ، لكل المجموعات عدا المجموعة الضابطة السلبية (١) وذلك لاحداث تقرح حاد بالمعدة. اظهرت نتائج الدراسة ان معاملة جرذان المجموعة الضابطة الموجبة بجرعة من حمض الاسيتيل ساليسيليك (٥٠٠ مللجم/كجم من وزن الجسم) قد ادت الى زيادة مساحة تقرح المعدة ودرجة الحموضة مع ارتفاع معنوى في تركيز المالوندايالدهيد بانسجة المعدة والكبد مقارنة بتلك في المجموعة الضابطة السلبية. وبالمقابل فان معاملة الجرذان بالمستخلص المائي لفاكهة عنب الثعلب والمستخلص المائي لاوراق النيم او خليطهما ادت الى تحسن في نسبة وقاية جدار المعدة من التقرح مع انخفاض معنوي في مستوى المالوندايالدهيد وارتقاع معنوي في مستويات الجلوتاثيون والكاتاليز بانسجة كلا من المعدة والكبد. ومن نتائج هذه الدراسة يمكن استنتاج ان المستخلص المائي

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لكلا من فاكهة عنب الثعلب واوراق النيم كانت فعالة في وقاية المعدة من التقرحات وخفضت من الاجهاد التاكسدي وكان خليطهما اكثر فعالية من استخدام كلا منهما منفردا. الكلمات المفتاحية: قرحة المعدة- الاجهاد التاكسدي- فاكهة عنب الثعلب الهندي- اوراق النيم-التاثير الوقائي.

Introduction:

Gastric hyperacidity and gastroduodenal ulcers are health problems among varied population, gastric ulcer arise from the imbalance between acidpepsin secretion and mucosal resistance (**Dorababu** *et al.*, **2006**). It represented as general gastrointestinal diseases and their symptoms include mucosal injury and inflammation. Hyperacidity occurs due to increased secretion of hydrochloric acid from the gastric mucosa (**Chattopadhyay** *et al.*, **2004**). The causes of gastric ulcers, according to **Balaraddiyavar** *et al.*, **(2024)**, include alcohol, smoking, *Helicobacter pylori* infection, damage of mucosal and nonsteroidal anti-inflammatory drugs (NSAID)-induced mucosal damage. These drugs damage the mucosal lining of the stomach by decreasing the formation of prostaglandins (PGs), interfering with platelet aggregation, and impeding ulcer healing. Acute drug-induced ulcers account for around 25% of all peptic ulcers. NSAID-induced damage is probably exacerbated by gastric acid.

Treatment with traditional medicinal plant extracts is currently regarded as an alternate method of illness control because synthetic medications frequently have some negative side effects. Due to its nutritional and therapeutic properties, Indian gooseberry (Phyllanthus emblica L.) has become extremely important in indigenous traditional medicine. It is particularly utilized to treat numerous conditions like inflammation, gastric ulcers, and hyperacidity (Saini et al., 2022), and because of Neem's antiulcer and anti-inflammatory properties, Neem (Azadirachta indica) considered as an important sources for antioxidants and other compounds (Bhajoni et al., 2016). Neem plays fundamental role in prevention of many ailments (Alzohairy, 2016). In addition, gallic acid, ellagic acid, pectin, quercetin, linoleic, oleic, and myristic acid are among the phytoconstituents of Indian gooseberries that have been shown to be beneficial for lowering inflammation (Krishnaveni et al., 2010, Huang et al., 2021, and Ansari et al., 2024). Neem Azadirachta indica, is used to alleviate many ailments (Subapriva and Nagini 2005 and Atangwho et al., 2012). Different parts of Neem have been shown to decrease inflammation, and that could be attributed to its higher content of phenolic constituents (Chaudhar et al., 2019 and Pingali et al., 2020).

Since the use of herbal products has been growing rapidly, the objectives of the current study were to investigate the potential protective effects of aqueous extracts from Indian gooseberry (*Phyllanthus emblica L.*) fruit and Neem (*Azadirachta indica*) leaves and their mixture on ulcer healing and oxidative stress in rats.

MATERIALS AND METHODS:

Indian gooseberry fruit and Neem leaves was purchased from Agriculture Seeds Spices, and Medical Plant Company (Harraz), Cairo, Egypt. Commercial kits used for determining Malondialdehyde (MDA), reduced glutathione (GSH) concentration and Catalase (CAT) were provided by Bio-diagnostic Co., Egypt. Aspirin (Rivo tablets, 320 mg acetyl salicylic acid) was purchased from local pharmacy.

For preparation of the aqueous extract, the studied plant part was dried and ground. 100 g of the dry powder was mixed with 500 ml of distilled water using an electric mixer and left for 24 hours at room temperature. The mixture was then filtered using cloth filtration to remove suspended particles. It was then placed in a centrifuge at 3000 rpm for 10 minutes. The extract was then filtered using filter paper to obtain a clear solution.

Moisture, protein, fat, fiber and ash estimated in accord to the method of **AOAC** (2019), whereas the percentage carbohydrate was calculated. Carbohydrate = 100 - (moisture + crude fiber + ash + fat+ crude protein). Using the method of **Singleton and Rossi**, (1965) estimations of the total phenolic content of the extracts were completed. Determination of total flavonoids content was in accord with method of **Zhishen** *et al.*, (1999).

Study design: Experimental procedures were applied in agreement with guiding principle for care and use of laboratory animals. The Agricultural Research Center in Giza, Egypt, supplied thirty female Wister rats weighing 150 ± 10 g. For a total of three weeks, the animal groups were housed at 20–25 degrees Celsius. For a week, the rats received a standard diet. In according to Reeves et al., (1993), a standard diet was formulated. Then the rats have been divided up into two primary groups after a week of adaption. As a negative control group (-ve), the first group, Group (1), was given the baseline diet. The remaining 24 rats were split into four separate groups, each consisting of six animals, at random. Group 2 received oral distilled water as the positive control group (+ve). Group 3 ingested 500 mg/kg BW/day of aqueous Indian gooseberry extract orally. According to Gupta (2016), Group 4 administered an oral treatment of Neem aqueous leaf extract (350 mg/kg BW / day). And 250 mg of Indian gooseberry + 250 mg of Neem / kg BW/day were administered orally as a pretreatment to Group 5. After 21 days of pretreatment, and to induce acute mucosal injury, the method of Mahmoud and Abd El-Ghffar (2019) was applied. All rats, except those of the negative control group, were subjected to12 hours fasting, and then a single dose of 500 mg of acetyl salicylic acid per kilogram of body weight was ingested with epigastric tube. By the end of experimental (21 days) all rats were sacrificed, with the removal of their stomachs and livers, which sited in a sterile container for further examination. Rapidly after the stomach was removed, the two openings of the stomach were knotted together and injected with 3 ml of distilled water. The juice of stomach was then collected in sterile container. Procedure mentioned by Bandyopadhyay et al., (2004), ulcer inhibition was measured. For

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measuring of the ulcer area (UA), the total area of each stomach lesions was measured. UA and ulcer inhibition were calculated according to the equation reported by **Robert** *et al.*, (1984). Stomach juice from each rat was centrifuged. The gastric juice volume was measured. Then, by following **Moore's** (1968) method, the pH of the supernatant was measured. In accord to the methods of **Albro** *et al.*, (1986) and Ellman (1959), respectively, the concentrations of reduced glutathione and Malondialdehyde in tissue of the stomach and liver were estimated. The Catalase activity was estimated following the **Aebi** (1984) method.

Histopathological examination: After being dissected, stomach tissue samples were preserved in 10% neutral formalin for 24 hours. And the method of **Banchroft** *et al.*, (1996) was applied to investigate the histopathological changes in tissue of the rat stomach.

Data were collected then the least significant differences (LSD) were calculated by using analysis of variance (ANOVA) and the difference considered significant at P ≤ 0.05 (Snedecor, and Cochran, 1980), the statistical analysis Package SPSS (Version 20) was used for processing of raw data.

RESULTS AND DISCUSSION:

As shown in table (1), the moisture and carbohydrate contents of dried Indian gooseberry was higher (P \leq 0.05) than that of dried Neem leaves. On the other hand the protein, oil, fiber and ash contents were lower (P \leq 0.05) than that in Neem leaves.

Table (1): Chemical composition of Indian gooseberry fruit and Neem leaves (Mean \pm S.D.).

	Indian gooseberry fruit	Neem leaves
Moisture (g %)	13.50 ± 0.27 ^a	2.40 ± 0.05 ^b
Protein (g %)	2.39 ± 0.26^{b}	14.39 ± 0.26^{a}
Oil (g %)	0.44 ± 0.06 ^b	4.79 ± 0.05 ^a
Fiber (g %)	3.13 ± 0.38 ^b	15.47 ± 0.10^{a}
Ash (g %)	2.31 ± 0.28 ^b	9.40 ± 0.07^{a}
Carbohydrates (g %)	78.22 ± 0.01 ^a	53.92 ± 0.01 ^b

Values in the same row ended with dissimilar letter were significantly different at $P \le 0.05$.

From Table (2 and 3) it could be noticed that, the gooseberry fruit has a significantly high total phenolic content (195.73 \pm 3.95 mg GAE/g DW) than that of the Neem leaves (96.00 \pm 0.34 mg GAE/g DW), in addition the total flavonoids content of the gooseberry fruit was significantly higher than that of Neem leaves representing 17.87 \pm 0.75 and 8.00 \pm 0.50 mg RE/ g DW, respectively.

 Table (2): Total phenolic and total flavonoids contents in Indian gooseberry fruit and Neem leaves (Mean + S.D.).

	Indian gooseberry fruit	Neem leaves
Total Phenolic (mg GAE/g DW)	195.73 ± 3.95 ^a	$96.00 \pm 0.34^{\text{ b}}$
Total flavonoids (mg RE/g DW)	17.87 ± 0.75^{a}	$8.00 \pm 0.50^{\text{ b}}$

Values in the same row ended with dissimilar letter were significantly different at $P \le 0.05$. Table (3): A phenolic component in Indian gooseberry fruit and Neem leaves (µg/g DW).

phenolic Compounds	Indian gooseberry fruits	Neem leaves
Gallic acid	8006.54	3710.00
Chlorogenic acid	1264.16	353.08
Catechin	2582.83	289.07
Rutin	0.00	71.071
Quercetin	0.96	302.03
Caffeic acid	399.05	179.03
Catechol	12321.25	2160.00
Ellagic acid	6412.75	910.00
p-Coumaric acid	34.26	163.04
Vanillin	168.79	671.00
Caffeine	101.00	83.00
Kaempferol	1.20	3.35
Salicylic acid	309.77	2130
Cinnamic acid	214.33	680.00

Gomez *et al.*, (2023) reported that, the fruit of the Indian gooseberry is extremely astringent due to its abundance of phenolic chemicals. The main phenolic components found in Indian gooseberry fruits are tannins. According to **Zhao** *et al.*, (2015), several tannins have been detected in Indian gooseberry fruits, including gallic acid, ellagic acid, corilagin, pyrogallol, and chebulagic acid. The fruit of the Indian gooseberry, has a variety of bioactive phytochemicals, the vast majority of which are polyphenols (galic acid, quercetin, corilagin, chebulinic acid, chebulagic acid, apeigenin, leutolin, etc.), as reported by **Gupta** *et al.*, (2021).

It is worth to mention that, the total phenolic and total flavonoids content can vary according to many factors such as the plant's growing conditions, drying method, extraction process and the part of the plant used. **Tewari** *et al.*, (2023) emphasized that, the proximate composition, ascorbic acid content, and polyphenol content were all significantly impacted by cultivar variances. Neem have been shown to decrease inflammation, and that could be attributed to its higher content of phenolic constituents (**Chaudhar** *et al.*, 2019 and Pingali *et al.*, 2020). Neem oil is rich sources of varied phenolic compounds (Gosse *et al.*, 2005). And these compounds contribute to the antioxidant qualities of Neem extracts (Al-Hashemi and Hossain, 2016).

Table (4): Effect of Indian gooseberry fruit and Neem leaves extracts on gastric juicevolume, pH of gastric juice, gastric mucosal injury area and gastricprotection in Aspirin induced gastric ulcer rats (Mean+ S.E.).

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			Gastric Mucosal Injury		
	Gastric juice Volume (mL)	pH of gastric juice	Gastric Mucosal Injury	Ulcer Inhibition	
Groups			Area (mm ²)	(%)	
Control (-ve)	0.28 ± 0.03 ^d	$5.20 \pm 0.20^{\text{ b}}$	$0.00\pm 0.00^{\rm d}$		
Control (+ve)	1.15 ± 0.01 ^a	5.74 ± 0.56^{a}	8.96± 0.39 ^a		
Group 3 (500 mg	0.50 ± 0.02 ^c	$5.20\pm0.30^{\text{ b}}$	1.35 ± 0.17 ^b	84.94	
IGE)					
Group 4 (350 mg	0.39 ± 0.06 ^c	$5.50 \pm 0.10^{\text{ b}}$	0.97 ± 0.49 ^b	89.12	
NE)					
Group5 (250 mg	0.29 ± 0.05 ^d	$5.20\pm0.20^{\text{ b}}$	0.73 ± 0.36 ^c	91.91	
IGE+ 250 mg NE)					

Values in the same column ended with dissimilar letter were significantly different at $P \leq 0.05$.

From table (4) it could be noticed that, ingestion of Aspirin at 500 mg/ kg BW resulted in a significant increases of gastric juice volume $(1.15\pm0.01 \text{ ml})$ and in the pH of gastric juice (5.74 ± 0.56) , with significant increment of gastric mucosal injury area $(8.96\pm0.39 \text{ mm}^2)$ when compared with values of remaining groups. Ingestion of aqueous extract of Indian gooseberry, Neem and their mixture enhanced the gastric protection by increasing the ulcer inhibition percentage as shown in Figure (1).



Fig., (1): Effect of aqueous extract of Indian gooseberry, Neem and their mixture on gastric protection percentage in aspirin induced gastric ulcer rats.

The Indian gooseberry and Neem gastroprotective properties have been illustrated in varied models of animal studies. Al-Rehaily et al., (2002); Chatterjee et al., (2011) and Gupta et al., (2021) illustrated that, Emblica officinalis extract shows significant antiulcer. antisecretory, and gastroprotective effects in animal models. Fruit extract protects against damage of the gastric mucosal, reduces secretion of gastric acid, and increases antioxidant activity. Furthermore, Chatterjee et al., (2011) stated that, the officinalis extract enhanced anti-inflammatory Emblica responses bv diminishing (TNF- α , IL-1 β) the pro inflammatory cytokines and elevating (IL-10) the anti-inflammatory cytokines. On the other hand, studies on Neem extracts indicated that, Neem extracts' has an anti-inflammatory, antioxidant, and wound-healing properties, which make it considered as an efficient in

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preventing gastric ulcers (Govindachari *et al.*, 1998; Biswas *et al.*, 2002 and Osunwoke *et al.*, 2013). In addition, Chattopadhyay *et al.* (2004) confirmed that, Neem leaf extract hinder secretion of gastric acid by diminishing activities of H+-K+-ATPase, decrease oxidative stress's damage, and DNA damage in gastric mucosa. A study by Bandyopadhyay *et al.*, (2004) on human, confirmed significant reductions in gastric acid secretion and supported its antiulcer property.

Al-Rehaily *et al.*, (2002) stated that, the progress of gastric lesions was significantly reduced by gooseberry extract, when administrated at doses of 250-500 mg/kg body weight. The results indicate that, the gooseberry extract has cytoprotective, antisecretory, and antiulcer characteristics. In addition, Chatterjee *et al.*, (2011) illustrated that, extract of Indian gooseberry was effective in healing of gastric ulcer.

Gupta *et al.*, (2021) declared that, Indian gooseberry extract has been reported to exhibit gastroprotective effect, the gastro-protective and healing effects of the gooseberry fruit extract were supported by the histopathological results (Al-Rehaily *et al.*, 2002; and Chatterjee *et al.*, 2011). Recently Balaraddiyavar *et al.*, (2024) illustrated that, gooseberry fruit's antisecretory and antioxidant properties considered as the main cause of its stomach protective effect. Rats ingested with butanol extract of the fruit orally showed increased stomach mucus (Bandyopadhyay *et al.*, 2000). An aqueous extract of Indian gooseberry fruit (100 mg/kg body weight orally for 7 days) had a healing effect on rats' stomach ulcers (Pakrashi *et al.*, 2003). It has been hypothesized that the ability of Indian gooseberry extract to activate prostaglandins (PGs) in the gastric mucosa is accountable for the mucosal regenerative (Konturek *et al.*, 1987 and Alvarez *et al.*, 1999).

Neem has a complex of several antioxidants, such as limonoids, nimbin, nimbidin, and nimbolide, as reported by Govindachari et al., (1998), which resulted in its anti-inflammatory, antigastric ulcer, and antitumor properties (Biswas et al., 2002). Furthermore, Osunwoke et al., (2013) revealed that, Neem leaf extracts enhance wound healing activity by promoting neovascularization and enhanced anti-inflammatory response. Recent study of Sandu and Pandy (2024) confirmed that, Neem's anti-inflammatory, woundhealing, and antioxidant qualities are linked to its use in the treatment of stomach ulcers. The administration of lyophilized extract powder for 10 days at a dose of 30 mg twice daily demonstrated a significant (77%) decline in gastric acid secretion in human (Bandyopadhyay, et al., 2004). a study by Pillai and Santhakumari (1984), nimbidin considerably accelerated the healing of chronic stomach ulcers in albino rats and dogs. Neem leaf extract inhibits gastric acid secretion, and reduces hydroxyl radical (*OH)-induced oxidative membrane damage. By inhibiting H+-K+-ATPase and lowering oxidative membrane damage, Neem leaf extract has antiulcer properties (Chattopadhyay et al., 2004).

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The results illustrated in table (5) and Figures (2, 3 and 4) showed that, in rats of positive control group, ingestion of Aspirin resulted in a notable and significant increase of gastric and liver MDA, indicating higher oxidative stress in tissues of stomach and liver when compared with negative control group value. Furthermore, rats of positive control group had significantly lower gastric and liver GSH and Catalase levels when compared with negative control group. In groups of rats ingested with gooseberry, Neem and their mixture there were a significant reduction in MDA and significant increments in GSH and CAT of stomach and liver tissues as compared to that of positive control group.

(Mea	an <u>+</u> S.E.).				
	Control (-ve)	Control (+ve)	Group (3) IGE 500 mg	Group (4) NE 350 mg	Group (5) 250 mg IGE + 250 mg NE
Gastric MDA (µ mol/g. tissue)	190.7±0.94 °	260.5±1.27 ^a	176.8±4.20 ^d	212±3.76 ^b	162±4.86 ^{de}
Gastric GSH (µ mol/g. tissue)	24.3±0.89 ^a	13.6±0.89 °	14.8±0.73 ^{bc}	14.2±0.45 °	16.5±0.61 ^b
Gastric CAT $s^{-1}g^{-1}$	5.5±0.57 ^a	3.3±0.04 ^d	4.7±0.04 ^b	3.9±0.08 °	4.8±0.28 ^b
Liver MDA (µ mol/g. tissue)	370.5±7.31 °	606.2±2.29 ^a	357.3±1.27 ^d	399.4±22.90 ^b	327.6±12.08 e
Liver GSH (µ mol/g. tissue)	190.5±11.64 ^a	82.8±1.47 ^d	152.3±2.41 ^b	121.6±4.41 ^c	141.4±14.61 ^b
$\begin{array}{c} \text{Liver CAT} \\ \text{s}^{-1}\text{g}^{-1} \end{array}$	25.7±0.45 ^a	14.5±0.57 ^d	20.2±0.81 ^b	18.8±0.49 ^c	19.05±0.77

Table (5): Impact of Indian Gooseberry and Neem leaves extracts on the levels of MDA,GSH and Catalase in gastric and liver of aspirin induced gastric ulcer rats(Mean± S.E.).

Values in the same row ended with dissimilar letter were significantly different at $P \le 0.05$.







Fig., (3): Impact of Indian Gooseberry and Neem leaves extracts on GSH in the gastric and liver of Aspirin induced gastric ulcer rats.



Fig., (4): Impact of Indian Gooseberry and Neem leaves extracts on Catalase in the gastric and liver of Aspirin induced gastric ulcer rats.

Indian gooseberry has been known to be effective in decreasing oxidative stress; this may be due to its antioxidants, such as gallic acid (Li *et al.*, 2004). In regard to these effects, Indian gooseberry effectively lowers oxidative stress in stomach and liver tissues (Thilakchand *et al.*, 2013; Packirisamy *et al.*, 2017 and Muthu *et al.*, 2018). And due to the capacity of Neem to decrease the biomarkers of oxidative stress (MDA), and increasing the GSH level and the activity of Catalase in stomach, Neem was considered as an important plant for stomach healing. Results of Garg *et al.*, (1993) further indicated that, Neem leaves prevent cells' apoptosis in gastric mucosa by reducing oxidative stress, which helps protect the stomach mucosal coating from damage caused by stress, ethanol, or NSAIDs. The gastric protective and antioxidant effects of Neem may be attributed to its phytochemicals, in addition, Asenuga *et al.*, (2022) stated that, Neem is a rich source of nimbin and azadirachtin which perform as an antioxidant and anti-inflammatory and their effects include the reduction of oxidative stress and elevating the level and activity of antioxidant enzymes.



(e) mixture of IGE250mg+ NE250mg group

Fig., (5): Effects of aqueous extracts of Indian gooseberry (IGE) and Neem leaves (NE) and their mixture on gastric histopathology of Aspirin induced gastric ulcer rats.

As it could be noticed from Fig., (5), stomach of rats from negative control group showed the normal histopathological of gastric layers (Figs. 5 a). On the other hand, stomach of positive control group showed histopathological changes demonstrated by necrosis of gastric mucosa coupled with inflammatory cells infiltration (Fig., 5 b), furthermore, rats treated with aqueous extract of Indian gooseberry exhibited infiltration of the stomach mucosa with a few number of inflammatory cells associated with sub mucosal edema (Fig., 5 c). Rats treated with aqueous extract of Neem leaves showed slight sub mucosal edema accompanied with few sub mucosal inflammatory cell infiltrations and necrosis of gastric mucosa with sub mucosal inflammatory cell infiltrations (Fig., 5 d). Rats group treated with mixture of aqueous extract of Indian gooseberry and Neem showed neglected histopathological changes (Fig., 5 e). The aforementioned results were in line with results of other studies who reported that, the histopathological study confirmed that Indian gooseberry has healing effects on gastric ulcers, with mechanisms involving prostaglandin mobilization and antioxidant properties (Konturek et al., 1987 and Alvarez et al., 1999).

In conclusion, aqueous extract of Gooseberry fruit and leaves of Neem, both of which separately showed antiulcer properties through acid suppression by Neem, and antioxidant mucosal protection by gooseberry, their mixture have

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a synergistic protective effect against gastric ulcer, it is recommended to include Indian gooseberry and Neem tea in the diet of those at risk of gastric ulcer.

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