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Article

Gastroprotective potential and mechanisms of action of *Hedera nepalensis*

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Hedera nepalensis (H. nepalensis), belonging to the family Araliaceae, is a medicinal plant traditionally used to treat stomach problems. The current study investigated the gastroprotective potential and the mechanism of action of *H. nepalensis* in diclofenac-and ethanol-induced ulcer models. Anti-oxidant and lipid peroxidation inhibitory prospects of *H. nepalensis* were checked out by free radical scavenging assay and UV spectrophotometer respectively. Effect of *H. nepalensis* on the pH, gastric total acidity of gastric juice and protective effects of *H. nepalensis* against ulcer models have been examined. Histopathological studies have been carried out. The aqueous methanol extract of *H. nepalensis* (100 µg/mL) showed anti-oxidant (83.55%) and lipid peroxidation inhibitory (70.88%) potential at 1000 µg/mL; the extract had no buffer potential. The extract (400 mg/kg) significantly (81.12% and 63.46%) showed gastroprotective effect in diclofenac and ethanol-induced rat ulcer models respectively. Histopathological studies confirmed the biochemical findings. FTIR analysis showed the presence of carboxylic acid, alkanes, conjugated alkanes, aldehydes and alkyl-aryl ethers. Gallic acid, M-coumaric acid and quercetin were found by HPLC analysis. *H. nepalensis* exhibited significant protection against diclofenac and ethanol induced gastric damage by anti-oxidant and lipid peroxidation suppression effects suggesting potential broad utility in treatment of diseases characterized with gastric damage.

Keywords: Antioxidant. Diclofenac. Ethanol. Gastroprotective. *Hedera nepalensis.*

INTRODUCTION

Gastric ulcer has become the leading source of hospitalization of patients in modern world. Every year, 4 million peoples worldwide suffer from peptic ulcer disease (Chung, Shelat, 2017). Ulcers form when there is an inequity between protective and aggressive factors in the stomach. These factors include acid and mucus secretions, blood flow, prostaglandins, *Helicobacter pylori* infection and cell regeneration in stomach. Some exogenous factors, such as smocking, non-steroidal anti-inflammatory drugs, alcohol and stress may lead to development of gastric ulcer (Klein *et al.,* 2010).

Antacids, H_2 receptor blockers and proton pump inhibitors are being used to treat ulcer. High cost and development of unwanted effects such as impotence, arrhythmia, hypersensitivity and gynecomastia demand more effective and less toxic agents to treat ulcer (Ramis *et al.,* 2012). Nowadays medicinal plants and various phytochemicals derived from plants have been used to treat a number of diseases. Even in the developed countries, most of the population relies on use of traditional medicinal plants to treat their illness (Luna *et al.,* 2005).

Hedera nepalensis belonging to the family Araliaceae, is a medicinal plant found in Japan,

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Afghanistan, West Asia and the Himalayas (Jafri *et al.,* 2017). Traditionally, *H. nepalensis* is used to treat diabetes, ulcer, abscesses, cancer and as a diaphoretic and cathartic agent (Ahmad, Javed, 2007; Qureshi *et al.,* 2007). Flavonoids, tannins, steroids, terpenoids and glycosides have been found in *H. nepalensis* (Kanwal *et al.,* 2011). Decoction from plant is effective against lice. The juice prepared from leaves of this plant is used as a blood purifier and to treat diabetes (Akhtar *et al.,* 2013).

The present study was aimed to investigate the gastro-protective activity of *Hedera nepalensis* in diclofenac-and ethanol-induced gastric ulcer rats. The acidity, pH, ulcer score and histopathological studies have been performed to evaluate the gastroprotective potential of aqueous methanol extract of *H. nepalensis* (AMEHN). The phenolic contents, anti-oxidant and lipid peroxidation inhibitory potential of plant have also been determined. Moreover, FTIR was utilized to assess the functional groups present in the aqueous methanol extract of whole plant.

MATERIAL AND METHODS

Animals

Albino rats of either sex (7-8 weeks old, 150–200 g) were used. Animals were housed in the animal house at the Faculty of Pharmacy, Lahore College of Pharmaceutical Sciences. The temperature was kept at 22±2 ˚C. The animals were exposed to a 12-hour dark/light cycle for one week. Animals were fed chaos and water as needed. The animals were handled in accordance with National Research Council guidelines (UOS/ORIC/1478).

Collection and extraction of *H. nepalensis*

The aerial parts of the plant were collected from Sawat Khyber Pakhtunkhwa (35° 22’ 42” North, 72° 10’ 47” East), Pakistan in August and September 2017. The plant was identified and authenticated by Dr. Zaheer-ud-Din Khan, Department of Botany, Government College University Lahore (Ref. #: GC. Herb. Bot. 3469). The aerial parts of the plant were shade dried and crushed into powder. The aqueous methanol extract was prepared by cold maceration. A dark greenish mass was obtained with percentage yield of 13.48%.

Phytochemical testing

AMEHN was analyzed for the detection of alkaloids, saponins, flavonoids, steroids, tannins, resins, anthraquinones, glycosides and phenols (Odeja *et al.,* 2015).

Buffer potential estimation

Buffer potential of AMEHN was determined to know the change in pH by adding 1 and 2 mL of 0.1 N HCl and NaOH. The pH of 1 mL of extract (100 mg/mL) was determined alone and with addition of 0.1 N HCl/ NaOH (Adefisayo *et al.*, 2017).

Antioxidant activity

Ascorbic acid, quercetin and AMEHN (10 mg each) were weighed and dissolved separately in methanol (10 mL). The resultant solution (1000 µg/mL) was obtained. The above stock solutions (1 mg/mL) were undergone serial dilution so that lower concentrations viz. 20, 40, 60, 80, 100 µg/mL were obtained. By using DPPH free radical, quenching effect of plant extract and standards was determined. 2.22 mg of DPPH was dissolved in methanol (100 mL) to prepare fresh 0.1 mM solution of DPPH (Awan *et al.*, 2020). 3 mL of extract and standard solutions at different concentrations were mixed in 1 mL of methanol solution of DPPH. The solution was kept at 25° C for 30 minutes and analyzed on UV spectrophotometer at □=517 nm (Harlalka *et al.*, 2007). The standards used were quercetin and ascorbic acid. Radical scavenging activity was evaluated by following expression:

$$\% \text{ Oxidant inhibition} = \left(\frac{Ac - As}{Ac}\right) \times 100$$

Whereas,
Ac denotes absorbance of DPPH without AMEHN i.e., control and
As denotes absorbance of DPPH with AMEHN i.e., sample

Lipid peroxidation inhibition evaluation of AMEHN

TBARS assay was performed to assess lipid peroxidation inhibition activity of the extract. Egg homogenate (lipid rich medium) was used in this analysis. AMEHN (0.1 mL) and quercetin (2 mg/mL), as well as 0.5 mL of 10% v/v egg homogenate, were placed in a test tube and diluted to a final volume of 1 mL with water. To induce lipid peroxidation in each solution, 0.5 mL of 0.07 M ferrous sulphate solution was added and incubated for 30 minutes. The following chemicals were dissolved in 1.5 mL of 20% ascorbic acid, whose pH was adjusted to 3.5 with NaOH, 1.5 mL of 0.8 percent w/v TBA in 1.1 percent SDS, and 0.5 mL of 20% TCA. The resulting mixture was vortexed and warmed for 60 minutes at 95 ˚C. Entire methodology is recapped in the same way but without incubation of TBA for each sample. After cooling the solution, butanol (5 mL) was included in it and then centrifuged for 10 minutes (395x g). Absorbance was measured at 532 nm of organic layer (Jahantighi *et al*., 2016). Lipid peroxidation percentage was calculated as follows;

$$\% \ anti - lipid \ peroxidation = \left[1 - \left(\frac{E}{C}\right)\right] \times 100$$

Whereas,
C denotes absorbance of control
E denotes absorbance of sample (Abs 532_{+TBA} – Abs 532_{-TBA})

Study design for diclofenac induced gastric ulcer

The rats were divided into 5 groups (n=5). Group I (Normal control) was administered vehicle; 1% carboxymethyl cellulose (CMC) (10 mL/kg) orally. Group II (Disease control) was treated with single dose of diclofenac sodium (100 mg/kg) dissolved in 1% CMC. Group III (Standard drug) received three doses at 12hr interval of omeprazole (20 mg/kg) dissolved in 1% CMC. Group IV and V (AMEHN treated) received three doses of AMEHN (200 and 400 mg/kg respectively) at 12 hours interval. Diclofenac sodium was administered after one hour of last dose of AMEHN and omeprazole (Kang *et al*., 2014).

Study design for ethanol induced gastric ulcer

Same protocols (as for diclofenac) were followed for ethanol induced gastric ulcer model. However, dose for 80% ethanol was 5 mL/kg. Rats were anesthetized with chloroform after 12hrs interval of last dose (Liu *et al*., 2001).

The rats were provided with regular diet and monitored to find the signs of ulcer. Their stomachs were dissected out. Gastric contents after dissection were collected and the stomachs were rinsed with normal saline, afterwards macroscopic examination i.e., ulcer score, ulcer index, percent protection, pH determination, total acidity estimation and histopathological examination were carried out.

Ulcer Scoring, ulcer Index and percentage protection

To determine the induction of ulcers, the stomach was isolated, washed, and inspected with a magnifying glass. Normal colored stomach (0), red coloration (0.5), spot ulcer (1), hemorrhagic streak (1.5), deep ulcers (2), perforation (3).

The Ulcer index (UI) was computed using the following formula:

$$UI = (Un + Us + Up) \times 10^{-1}$$

Whereas; Un = average number of ulcers per animal, Us = average number of severity of scores, Up = percentage of animals with ulcers.

And percentage protection has been calculated by;

$$\%Protection = (C-T/C) \ X \ 100$$

Whereas; C = ulcer index in control group, T = ulcer index in treated group (Sattar *et al*., 2019).

Determination of gastric total acidity and pH

The gastric juice was obtained from stomach of rat; pH of gastric juice was then checked with the help of pH litmus paper. For the determination of gastric total acidity, gastric juice was diluted with 1 ml of distilled water, 2-3 drops of phenolphthalein were added in the mixture as

an indicator and titrated against 0.01N NaOH until pink colour. The total acidity (meq/L/100 g) was calculated by given formula (Lee, Kallal, Feldman, 1996; Abebaw, Mishra, Gelayee, 2017);

$$Total\ acidity = \frac{V\ (NaOH) \times N \times 100mEq/L}{0.1}$$

Whereas;
V; volume of NaOH used
N; normality of NaOH

Histopathological Studies

For histopathological studies, little partitions of stomach from each exploratory gathering were fixed in 10% formalin and submerged in paraffin. Areas of 5 µ were taken with a standard microtome and stained with hematoxylin and eosin. The areas were inspected for presence of degenerative and necrotic elements (Sattar *et al*., 2019).

Fourier-transform infrared spectroscopy (FTIR) analysis

AMEHN was analyzed spectroscopically for the determination of functional groups. 1 mg sample along with 2.5 mg KBr was grounded. Grounded powder was filled in micro cup of 2 mm internal diameter and loaded onto FTIR set at temperature 26° C ± 1° C. The sample was scanned in infrared range of 4000-400 cm. The spectrum obtained was compared with reference chart for identification of functional groups (Chandra, 2019).

High performance liquid chromatography (HPLC) analysis

50 mg of AMEHN, 16 mL of distilled water, 24 mL methanol were mixed. 10 mL of 6 M HCl was added in above solution. Solution was kept in oven for 2 hours at 95°C to obtain aglycons of flavanol glycosides. Samples were then centrifuged for ten minutes (395x g). Upper layers of samples were sonicated and filtered S 0.45 µm sized filter of cellulose acetate. Then the samples were injected into HPLC as described previously (Tokuşoğlu, Ünal, Yildirimk, 2003).

RESULTS

Phytochemical Screening

Phytochemical screening of aqueous methanol extract of *H. nepalensis* showed the presence of alkaloids, flavonoids, phenols, resins, saponins and tannins as shown in Table I.

TABLE I - Phytochemical screening of aqueous methanol extract of *H. nepalensis*

Phytochemicals	Test	Observations	Inferences
Alkaloids	Mayer's test	Formation of ppt.	+
Anthraquinones	Ammonia test	No pink, violet or red color in ammonical phase	-
Flavonoids	Magnesium test	A red or intense red coloration	+
Glycosides	Fehling test	No red precipitates	-
Phenols	Ferric chloride test	Green/dirty green color	+
Resins	Acetone test	Green color solution	+
Saponins	Foam test	Foam observed	+
Steroids	Salkowshi test	No reddish-brown cooler observed	-

TABLE I - Phytochemical screening of aqueous methanol extract of *H. nepalensis*

Phytochemicals	Test	Observations	Inferences
Tannins	$FeCl_3$ test	Blue black/blue green ppt.	+

(+) = Present, (-) = Absent

Buffer potential

AMEHN did not show any variation in pH by addition of 0.1N NaOH and 0.1N HCl solutions. Thus, it might be assumed that AMEHN did not have buffer capacity (as shown in Table II).

TABLE II - Buffer potential of aqueous methanol extract of *H. nepalensis*

Solution concentration	pH
1 mL extract	5.51±0.34
1 mL 0.1 N HCl	1.58±0.21
1 mL extract + 1 mL HCl	2.49±0.11
1 mL extract + 2 mL HCl	1.97±0.18
1 mL 0.1 N NaOH	12.70±0.92
1 mL extract + 1 mL NaOH	11.02±0.17
1 mL extract + 1 mL NaOH	12.03±0.51

Values are expressed as mean (n = 3).

Antioxidant activity

AMEHN exhibited antioxidant activity that was concentration dependent. The extract at concentration 100 µg/mL had remarkable free radical scavenging ability (83.55%). Quercetin and ascorbic acid free radical scavenging activity was (95.59%) and (98.23%) respectively as shown in Table III.

TABLE III - Antioxidant potential of *H. nepalensis*

Solution concentration (µg/mL)	Antioxidant potential		
	Quercetin	Ascorbic acid	AMEHN
20	81.71±0.77	94.15±0.17	31.07±0.536
40	84.28±0.50	96.04±0.36	65.76±0.183
60	88.81±0.41	96.35±0.31	80.07±0.30
80	92.62±0.59	96.73±0.33	82.34±0.43
100	95.59±0.42	98.23±0.27	83.55±0.28

Values are taken as Mean±SEM, (n=3), AMEHN=Aqueous methanol extract of *H. nepalensis.*

Lipid peroxidation inhibition

Lipid peroxidation inhibitory action of AMEHN was analyzed and compared to reference inhibitors quercetin (Table IV). Inhibitory action of AMEHN, was decreased to 0.00 at lower concentration (62.5 µg/mL) however, quercetin showed 63.99 ± 0.05% inhibition at lowest concentration of 62.5 µg/mL. Notwithstanding, AMEHN exhibited better anti-lipid peroxidation activity at higher conc. as shown in Table IV.

TABLE IV - Inhibition of lipid peroxidation by AMEHN

Concentrations (µg/mL)	% Inhibition of lipid peroxidation	
	Quercetin	AMEHN
62.5	63.99±0.05	0.00
125	68.56±0.28	37.77±0.44
250	70.80±0.16	61.81±0.09
500	73.93±0.07	68.07±0.31
1000	74.38±0.19	70.88±0.45

Values are taken as Mean±SEM, (n=3), AMEHN=Aqueous methanol extract of *H. nepalensis.*

Effects of AMEHN on gastric ulcer induced by diclofenac

Mucosal damage by diclofenac represented as severity score and ulcerative lesions were decreased to a large extent by AMEHN. The Disease control group had significantly increased gastric ulcer score (11.40±1.12) compared to control group. The number of lesions decreased (0.60±0.03) at 200 mg/kg dose likewise number of ulcerative lesions decreased (0.40±0.05) at 400 mg/kg dose. Severity score was decreased to (1.40±0.40, 0.60±0.10) at 200 and 400 mg/kg doses respectively in comparison to disease control (11.40±1.12). Ulcer index was decreased to a remarkable extent at 200 mg/kg (2.2) and 400 mg/kg (2.16). The ulcer protection was observed to be 80.77% and 81.12% at respective doses in comparison to disease control and standard drug treatment group (82.17%) (Table V).

TABLE V - Effects of AMEHN on gastric ulcer induced by diclofenac

Treatment	Severity score	No. of lesions	Ulcer index	Percentage protection (%)
Normal control	0	0	0	-
Disease control	11.40±1.12	3.00±0.32	11.44	-
Standard drug (20 mg/kg)	0.40±0.15***	0.20±0.02***	2.04***	82.17***
AMEHN (200 mg/kg)	1.40±0.40***	0.60±0.03***	2.20***	80.77***
AMEHN (400 mg/kg)	0.60±0.10***	0.40±0.05***	2.16***	81.12***

Values are expressed in Mean±SEM (n=5), Where, ***=(P<0.001 vs. disease control)

Effect of AMEHN on pH and total acidity of gastric juice

The Disease control group had significantly increased gastric total acidity (73.42±0.81) compared to control group (24.01±0.23). AMEHN (400 mg/kg) increased the pH of gastric juice (5.36±0.07) as compared to Disease control (1.54±0.11). The gastric total acidity was decreased (34.54±1.80) by AMEHN (400 mg/kg) as compared to standard drug treatment (35.39±0.96) (Table VI).

TABLE VI - Effect of AMEHN on pH and total acidity in diclofenac induced ulcer model

Treatment groups	pH	Total Acidity (mEq/L/100 g)
Normal control	5.38±0.12	24.01±0.23
Disease control	1.54±0.11	73.42±0.81
Standard drug (20 mg/kg)	5.52±0.09***	35.39±0.96***
AMEHN (200 mg/kg)	4.78±0.06***	46.20±1.10***
AMEHN (400 mg/kg)	5.36±0.07***	34.54±1.80***

Values are expressed in Mean±SEM (n=5). Where; *** = (P<0.001 vs. disease control)

Histopathological studies

Histopathological studies have been carried out of gastric damage induced by diclofenac. It has been found that the gastric mucosa and superficial epithelium in disease group was completely eroded. In standard drug treated group, superficial epithelium remained intact. AMEHN (400 mg/kg) showed gastric protection similar to standard drug treated group of rats however, mild damage in gastric mucosa and superficial epithelium was observed in AMEHN (200 mg/kg) pretreated rats as shown in Figure 1.

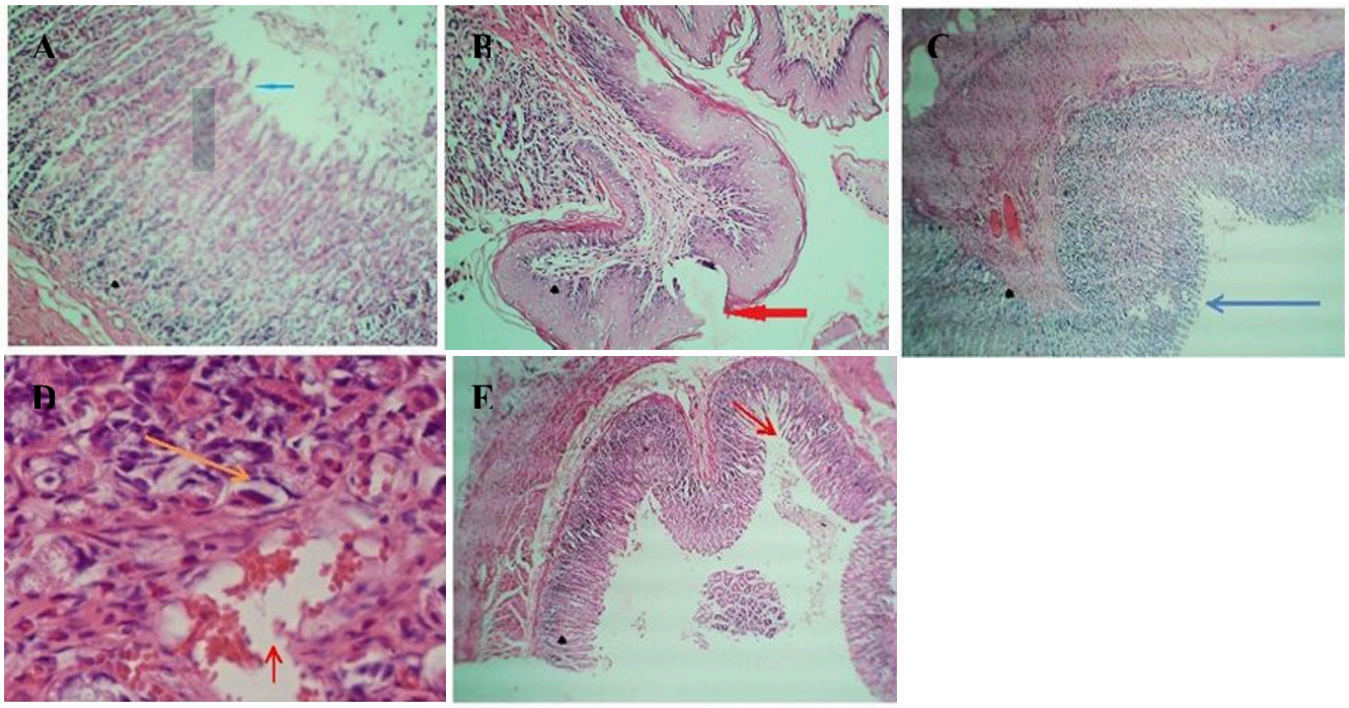

FIGURE 1 - Histopathological studies of stomachs from normal control (A), disease control (B), standard drug treated (C), aqueous methanol extract of *H. nepalensis* (200 and 400 mg/kg) treated group of rats (D) and (E) respectively in diclofenac induced gastric ulcer model. (A) arrow show intact epithelium, (B) arrow showing superficial epithelium was sloughed, necrosis of gastric glands had occurred, (C) mild erosion (arrow), restoration of epithelium by standard drug, (D) less necrosis in gastric glands and restoration of their normal shape (arrow) (E) arrow indicating very less erosion of superficial epithelium by *H. nepalensis* (400 mg/kg). (Light microscope magnification X100; hematoxylin and eosin staining) 215x279mm (300 x 300 DPI).

Effects of AMEHN on gastric ulcer induced by ethanol

Mucosal damage by ethanol represented as severity score and ulcerative lesions are decreased to a large extent by AMEHN. The Disease control group had significantly increased gastric ulcer score (12.80±1.63) compared to control group. The number of lesions decreased (0.60±0.20) at 200 mg/kg dose likewise number of ulcerative lesions decreased (0.80±0.17) at 400 mg/kg dose. Severity score decreased up to (2.00±0.38, 1.20±0.58) at 200 and 400 mg/kg doses respectively in comparison to disease control (12.80±1.63). Ulcer index was decreased to a remarkable extent at 200 mg/kg (4.26) and 400 mg/kg (6.20) doses. Percentage ulcer protection was observed to be 63.46% and 46.83% at respective doses in comparison to disease control and standard drug treatment group (63.64%) (Table VII).

TABLE VII - Effect of AMEHN on gastric mucosal damage induced by ethanol

Treatments	Severity score	No. of lesions	Ulcer index	Percentage protection (%)
Normal control	0	0	0	-
Disease control	12.80±1.63	3.80±0.38	11.66	-
Standard drug treatment	1.80±0.50***	0.60±0.20***	4.24	63.64

TABLE VII - Effect of AMEHN on gastric mucosal damage induced by ethanol

Treatments	Severity score	No. of lesions	Ulcer index	Percentage protection (%)
AMEHN (200 mg/kg)	2.00±0.38***	0.60±0.20***	4.26***	63.46***
AMEHN (400 mg/kg)	1.20±0.58***	0.80±0.17***	6.20***	46.83***

The values were expressed in Mean±SEM, (n=5). Where; ***=(P<0.001 vs. disease control)

Effects of AMEHN on pH and total acidity of gastric juice in ethanol induced ulcer

The Disease control group had significantly increased gastric total acidity (74.65±1.15) compared to control group (24.01±0.23). AMEHN (400 mg/kg) increased the pH of gastric juice (5.52±0.07) as compared to disease control (1.42±0.11). The gastric total acidity was decreased (35.76±1.37) by AMEHN (400 mg/kg) as compared to standard drug treatment omeprazole (34.92±0.80) (Table VIII).

TABLE VIII - Effects of AMEHN on pH and total acidity in ethanol induced ulcer

Treatment groups	pH	Total acidity (mEq/L/100 g)
Normal control	5.38±0.12	24.01±0.23
Disease control	1.42±0.11	74.65±1.15
Standard drug	5.32±0.10***	34.92±0.80***
AMEHN (200 mg/kg)	4.80±0.08**	46.49±0.76***
AMEHN (400 mg/kg)	5.52±0.07	35.76±1.37***

Values are expressed in Mean±SEM (n=5), Where; ***=(P<0.001 vs.disease control), **= (P<0.002 vs. disease control)

Histopathological assessment

Histopathological results showed that gastric mucosa and superficial epithelium were damaged by ethanol. In case of omeprazole pretreated rats, superficial epithelium and gastric mucosa observed to be intact. AMEHN (400 mg/kg) showed similar protection as was seen in omeprazole pretreated group (Figure 2).

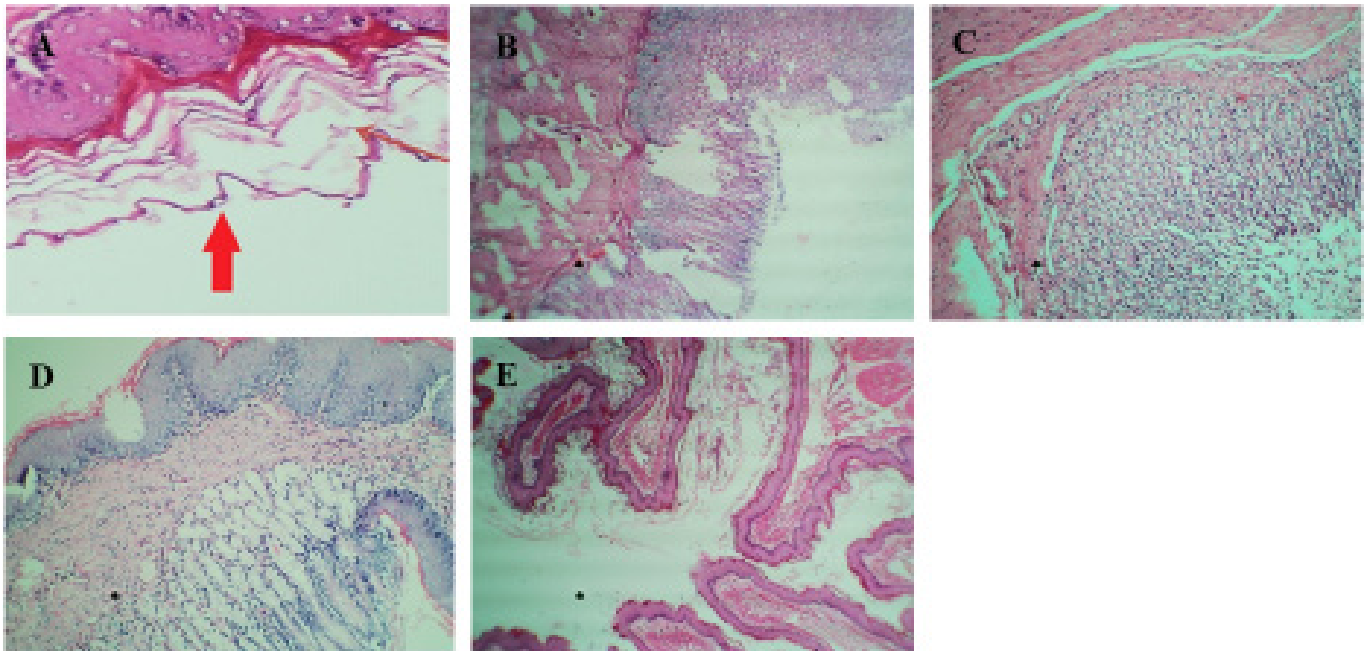

FIGURE 2 - Histopathological studies of stomachs from normal control (A), disease control (B), standard drug treated (C), aqueous methanol extract of *H. nepalensis* (200 and 400 mg/kg) treated group of rats (D) and (E) respectively in ethanol induced gastric ulcer model. (A) arrow shows intact epithelium, (B) damage to superficial epithelium, hemorrhage in lamina propria, (C) area of restoration of epithelium by standard drug, (D) less necrosis (arrow) in gastric glands (E) intact epithelium with no necrotic gastric glands by *H. nepalensis* (400 mg/kg). (Light microscope magnification X100; hematoxylin and eosin staining) 215x279 mm (300 x 300 DPI).

FTIR analysis of *H. nepalensis*

AMEHN was analyzed spectroscopically for determination of functional groups. Aqueous methanol extract of *H. nepalensis* FTIR spectra was compared with standard chart. It was found that functional groups like carboxylic acid, alkanes, conjugated alkenes, aldehydes and alkyl-aryl ethers were present in AMEHN (Table IX; Figure 3).

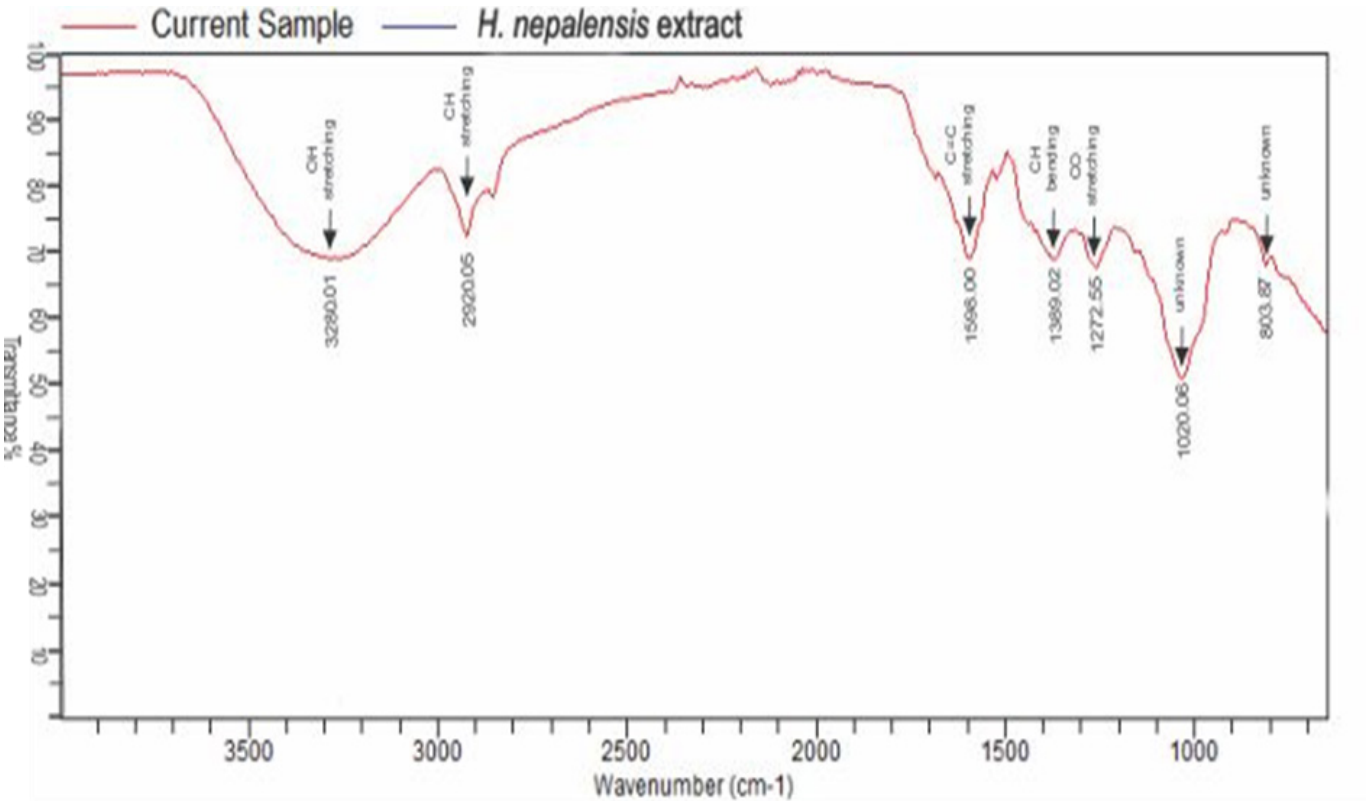

FIGURE 3 - FTIR spectra of aqueous methanol extract of *Hedera nepalensis.*

TABLE IX - FTIR analysis of AMEHN

Sr. no.	Peak value	Transmittance	Bond	Functional group
1	803.87	77.51	Unknown	-
2	1020.06	51.81	Unknown	-
3	1272.55	67.32	C-O stretching	Alkyl aryl ether
4	1389.02	69.55	C-H bending	Aldehyde
5	1598.00	69.75	C=C stretching	Conjugated alkenes
6	2920.05	73.82	C-H stretching	Alkanes
7	3280.01	69.04	OH stretching	Carboxylic acid

FTIR peak values and functional groups of AMEHN

Phenolic content estimation

The extract was analyzed with HPLC which confirmed the presence of phenols like quercetin, gallic acid and M-coumaric acid in 0.78 ppm, 5.24 ppm 3.52 ppm concentrations respectively (Table X; Figure 4).

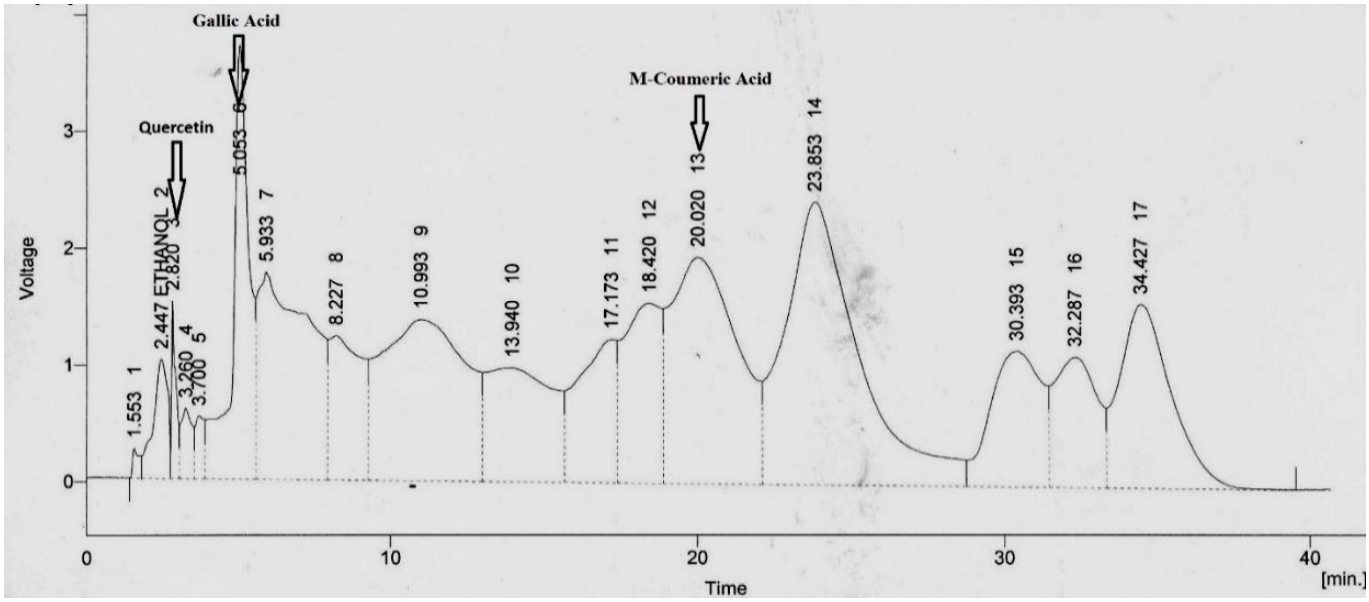

FIGURE 4 - HPLC chromatogram of aqueous methanol extract of *Hedera nepalensis.*

TABLE X - HPLC analysis of AMEHN

Peak no.	Retention time	Compound name	Quantity (ppm)
1	1.55	Unknown	-
2	2.44	Ethanol	-
3	2.82	Quercetin	0.78 ppm
4	3.26	Unknown	-
5	3.70	Unknown	-
6	5.05	Gallic acid	5.24 ppm
7	5.93	Unknown	-
8	8.23	Unknown	-
9	10.99	Unknown	-
10	13.94	Unknown	-
11	17.17	Unknown	-
12	18.42	Unknown	-
13	20.02	M-cumeric acid	5.52 ppm
14	23.85	Unknown	-
15	30.39	Unknown	-
16	32.29	Unknown	
17	34.43	Unknown	-

List of identified compounds by HPLC analysis in aqueous methanol extract of *Hedera nepalensis* (AMEHN)

DISCUSSION

Plants have become an important part of alternative medicines worldwide. A number of traditional medicinal plants have been used to protect gastric mucosa from ulcer causing agents. In this study, *H. nepalensis* was evaluated for its gastroprotective activity against diclofenac and ethanol induced ulcer. The phenolic contents, anti-oxidant and lipid peroxidation inhibitory potential of plant have been determined. FTIR and HPLC analysis has also been carried out.

Phytochemical evaluation of *H. nepalensis* showed the presence of alkaloids, flavonoids, gums, saponins, phenols, tannins while anthraquinones, glycosides and steroids were absent as shown in Table I. Phytomedicines comprising of anti-oxidants such as flavonoids, tannins and polyphenols have better ability to decrease disease hazards and have healing effects (Jennings *et al*., 2012; Muthusamy *et al*., 2008). Phenols show anti-oxidant ability and scavenge ROS and free radicals from the body showing cytoprotective effect in physiological systems (Scalbert *et al*., 2005). Flavonoids, due to hydroxyl group in main skeleton, also present free radical scavenging potential (Heinonen, Lehtonen, Hopia, 1998).

The aqueous methanol extract of *H. nepalensis* (AMEHN) was studied for its buffer potential by addition of NaOH (0.1N) and HCl (0.1N). AMEHN did not show any buffer capacity and thus having no ability to neutralize acidity of stomach as shown in Table II.

AMEHN was studied for its lipid peroxidation inhibitory and antioxidant potentials. TBARS test was used to evaluate AMEHN *in vitro* anti-lipid peroxidation potential. MDA reacts with TBA to give a red chromogen. It was further analyzed spectrophotometrically (Tiong *et al*., 2013). AMEHN inhibited the formation of MDA. In vitro examination for anti-oxidant activity of test extract was performed by DPPH assay. Antioxidant activity was exhibited by the plant extract (Table III and IV) as reported earlier (Jafri *et al.,* 2017; Kanwal *et al.,* 2011; Hashmi *et al*., 2018). Results showed that AMEHN has significant antioxidant and anti-lipid peroxidation effects. Medicinal plants showing gastro protective effect showed lipid peroxidation inhibitory potential previously (Ismail *et al*., 2012).

The gastroprotective activity of AMEHN was assessed by using diclofenac and ethanol induced ulcer model in rats. NSAIDs are hazard factors of gastric ulcer. Cyclooxygenase inhibitors decrease the mucus secretion, bicarbonate discharge, stifles the combination of prostaglandins, initiation of neutrophils, decrease mucosal blood flow, disturb the mucosal boundary, lipid per oxidation with subsequent development of ulcer (Wallace, 2008). Ethanol is a well-known necrotic agent and induces ulcer by disrupting the mucosal membrane. The parameters like number of lesions, severity score and ulcer index have been increased by oral administration of diclofenac sodium and ethanol in diseased control groups (El-Hady, El Awdan, Ibrahim, 2013).

In present study, diclofenac sodium and ethanol considerably decreased pH (from 5.38 to 1.54 and 1.42 respectively) as shown in Table VI and VIII. Diclofenac and ethanol have significantly enhanced the total acidity of gastric contents in disease control groups (Table VI and VIII). Increase in volume of gastric contents is due to over production of HCl (Pratt, 1992). AMEHN produced gastroprotective effects in pretreated rats against diclofenac and ethanol induced ulcer as shown by decrease in number of lesions, severity score and ulcer index (Table V and VII). Findings of present work are in line with previous studies (De Barros *et al*., 2008; Kim *et al*., 2006; Sumbul *et al*., 2011). Ethanol causes necrotic lesions of the gastric mucosa by lipid peroxidation, free radical production and increased intracellular oxidative stress (Sannomiya *et al*., 2005). Thus, it can be considered that AMEHN has phytochemicals which show anti-oxidant potential to modify the deleterious effects of ethanol (Table VI and VIII). However, further studies on molecular level are required to confirm this mechanism.

Gastric mucosa is protected by prostaglandins synthesized from arachidonic acid whose production is interrupted by ulcerative agents (Yamamoto *et al*., 1992). NSAIDs reduce prostaglandins synthesis from phospholipids leading to gastric mucosal damage. The protective effect of AMEHN in diclofenac induced gastric ulcer model might show its involvement in prostaglandins synthesis. Histopathological studies of also confirmed that AMEHN has gastroprotective effects similar as of omeprazole treated group of rats as shown in Figure 1 and 2.

FTIR analysis of the extract has shown that it contains a variety of natural chemicals like alkyl aryl ether, aldehyde, conjugated alkenes, alkanes, carboxylic acid and others. HPLC analysis also showed presence of phenols like gallic acid, M-coumaric acid and quercetin. M- coumaric and quercetin have good ability to inhibit H+/K+-ATPase pump (De Barros *et al*., 2008). Gallic acid also inhibits vasoconstriction and protein precipitation which lead to ulcer (Sumbul *et al*., 2011). Quercetin is a natural flavonoid which has anti-ulcer activity. It has anti- lipid per oxidant and antioxidant properties. Its antiulcer activity is due to inhibition of ROS, reduced acid production and down regulation of H+/K+ ATPase pump. Quercetin also secures mucosa by elevated mucosal prostaglandin contents (Martin *et al*., 1998).

CONCLUSION

The findings of this study suggested that *Hedera nepalensis* has gastroprotective activity in diclofenac and ethanol induced gastric ulcer, which might be attributed to anti-oxidant and lipid peroxidation inhibitory effect of plant. Such potential might be due to presence of anti-oxidant and phenolic contents in the extract. Thus, *Hedera nepalensis* might be useful to prevent gastritis and gastric ulcer.

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