



EVALUATION OF ANTI-OXIDANT AND ANTI ULCER ACTIVITIES OF ETHANOLIC EXTRACT OF INDIGOFERA MYSORENSIS BY USING *INVITRO* METHODS

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ABSTRACT:

The present study is evaluated the antioxidant potential and antiulcer effect of AEIM. The results analysed from the present study have indicated that AEIM possesses antioxidant and antiulcer effect on aspirin induced ulcers. The preliminary phytochemical screening of whole plant extracts indicates presence of alkaloids, Carbohydrates, Glycosides, Flavonoids, Tannins, Proteins, Amino Acids. The antiulcer effect is screened in ethanol extract of *Indigofera mysorensis* on NSAID induced anti-ulcer study. The results get from these study have been shown that ethanol extract of *Indigofera mysorensis* produce antiulcer effect. In aspirin induced model, there is reduction in ulcer index, total acidity, total volume of gastric contents, total protein concentration and higher concentration of glutathione content and pH of gastric secretion they compared with control group. Famotidine used as a standard comparison agent.

Keywords: Evaluation, Anti-Oxidant, Anti-Ulcer, Ethanolic Extract, *Indigofera Mysorensis*, *Invitro Methods*

INTRODUCTION

Gastric hyperacidity is a very common global problem affecting millions of people worldwide due to an imbalance between aggressive and protective factors.[1] The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, bacterial products (*Helicobacter pylori*), and drugs. The current treatment of peptic ulcer is mainly done with H₂ receptor antagonists, proton pump inhibitors, and antimuscarinics. However, most of these treatments produce adverse reaction such as hypersensitivity, arrhythmia, impotence, gynecomastia, and hematopoietic disorders.[2]



Antioxidants apparently protect the living system from oxidative insults, which is a hallmark feature of cancer, cardiovascular disease, and diabetes.[3] This oxidative damage is caused by reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl, and nitric oxide (NO) radical.[4] These ROS accumulations lead to damage to crucial biomolecules such as nucleic acids, lipids, proteins, polyunsaturated fatty acids, carbohydrates, and DNA in living system also directly stimulate histamine release from mast cells.[5] Most of the antioxidant presents in vascular plants such as Vitamin C and E, carotenoids, flavonoids, and tannins.[6] The naturally polyphenolic compounds, especially flavonoids have been largely studied for their strong antioxidants capacity.[7]

The genus *Indigofera* belongs to family, Fabaceae which is ranked the third largest family of the blossoming plants after Orchidaceae and Asteraceae with approximately 650 genera and 18000 different species. The family in overall is characterized by the pod (legume) type of fruit developing from a single carpal with marginal placentation. The family Fabaceae is divided in to three sub-families (Caesalpiinoideae, Mimosoideae, Faboideae). In Pakistan, the species of genus *Indigofera* are found in mountainous areas of North West Frontier Province, Azad Jammu and Kashmir, Northern Areas of Dir, from 1500 to 3000 meters [8]. In India, some species are available in Himalayas, Kasi Afghanistan and W. China [9]. Fast-growing when young but slowing with age [10]. Tolerates light shade [11]. A tall shrub, 2.5m, covered bristly white hairs, copiously branched shrub with short imaripinnate, leaflets 9-33; flowers in axillary racemes, in erect often almost stalk less Oleg and Rix, (1985) bright red or rosy or radish purple standard petal sessile, stamens diadelphous; pod cylindrical 10-12 seeded have a vanillas cent [12]; flowers are mostly 6-10 mm; calyx bristal haired, with lobes as tubes; bracts minute. Leaves and leaflets very variable, leaflets elliptic to oblanceolate, mostly 4-12mm, with white hairs. Pod 1.3-2.5cm, straight, hairless [13].

In our study *Indigofera mysorensis plant* extracts its phytochemical investigation will be a useful tool for the identification and authentication of theplant for industrial and further research purpose, which will be related to the antioxidant activity. Antioxidants, which can scavenge free radicals, have an important role in pharmacological systems. Antioxidants are emerging as prophylactic and therapeutic agents. Hence, antioxidant was also evaluated for the potent extract. And now I have under taken the study of evaluation anti-oxidant and antiulcer activity of *Indigofera mysorensis plant* extracts by using *invitro* methods

MATERIALS & METHODS

Collection and Authentication of Plant: The whole plant of *Indigofera mysorensis* collected in the month of June, 2022 from chittur dist. The plant materials were identified and authenticated by Prof. Madhav Shetty, Dept. of botany, Taxonomist, SV University, Tirupati. A voucher was kept in the Department of Pharmacognosy for reference

Aspirin Induced Ulcer

Table No 1: Dose dependent studies: (Animal: rats)

SI NO	DRUG	DOSE	ROUTE OF	NO. OF ANIMALS	PARAMETERS FOR STUDY
1.	Control (water)	-----	Oral administration	6	1.Ulcer index & Ulcer score 2.Total acidity 3.Acid volume 4.pH 5.Glutathione 6.Total protein
2.	Standard (famotidine)	3mg/kg	Oral	6	
3.	AEIM	100mg/kg	Oral	6	
4.	AEIM	200mg/kg	Oral	6	
5	AEIM	400 mg/kg	Oral	6	

RESULTS AND DISCUSSION

Soxhlet Extraction of *INDIGOFERA MYSORENSIS*

The percentage yield of the *Indigofera mysorensis* was found to be 20.88 % w/v.

Table No 2: Extraction of *Indigofera mysorensis*

Plant	Part used	Method of Extraction	Solvents	Percentage Yield (% W/V)
<i>Indigofera mysorensis</i>	Wholeplant	Maceration	Ethanol(95%)	20.88

Table 3: phytochemical evaluation

Parameters	value
1. Alkaloid	+
2. Carbohydrates	+
3. Glycosides	+
4. Flavonoids	+
5. Tannins & Phenolic compounds	+
6. Proteins & Amino acids	+
7. Saponins	-
8. Sterols or Triterpenes	-

In Vitro Antioxidant Activities

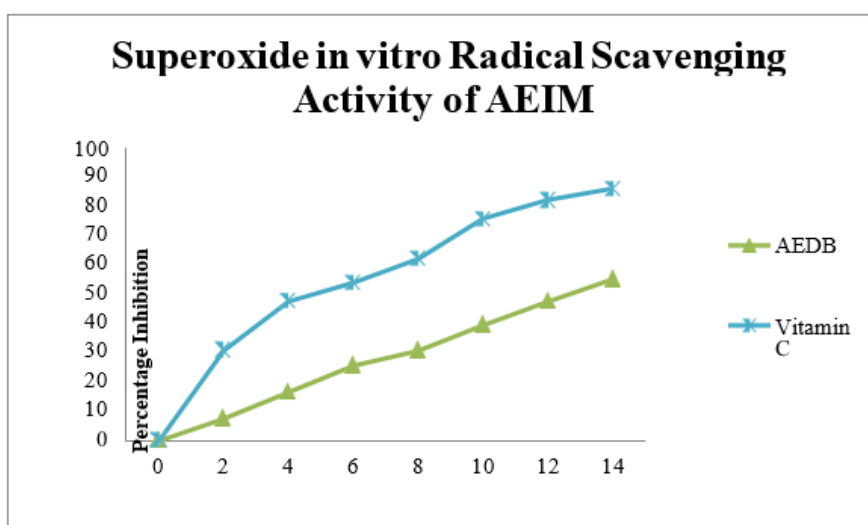
Effect of superoxide radical scavenging activity:

Table No 4: Effect of AEIM on Superoxide *in vitro* Radical Scavenging Activity

Concentration (µg/ml)	Absorbance		Percentage inhibition	
	Ethanol extract	Vitamin C	Ethanol extract	Vitamin C
0	0.78±1.22	0.78±1.5	0±0.00	0±0.00
2	0.72±2.31	0.54±3.5	7.6±1.35	30.76±0.71
4	0.65±3.1	0.41±0.78	16.6±4.71	47.43±1.79
6	0.58±1.27	0.36±1.55	25.64±3.6	53.84±2.53
8	0.54±1.72	0.28±2.3	30.76±1.55	61.94±4.22
10	0.47±5.5	0.19±3.6	39.47±2.44	75.64±1.67
12	0.41±3.7	0.14±2.3	47.43±3.39	82.05±2.36
14	0.35±1.78	0.11±3.2	55.12±0.67	85.89±0.37

Results are mean ± SD of three individual experiments

Fig. No 1: Effect of AEIM on Superoxide *in vitro* Radical Scavenging Activity



Effect of AEIM on DPPH radical reducing activity

Table No 5: study of *in vitro* DPPH Radical Scavenging Activity

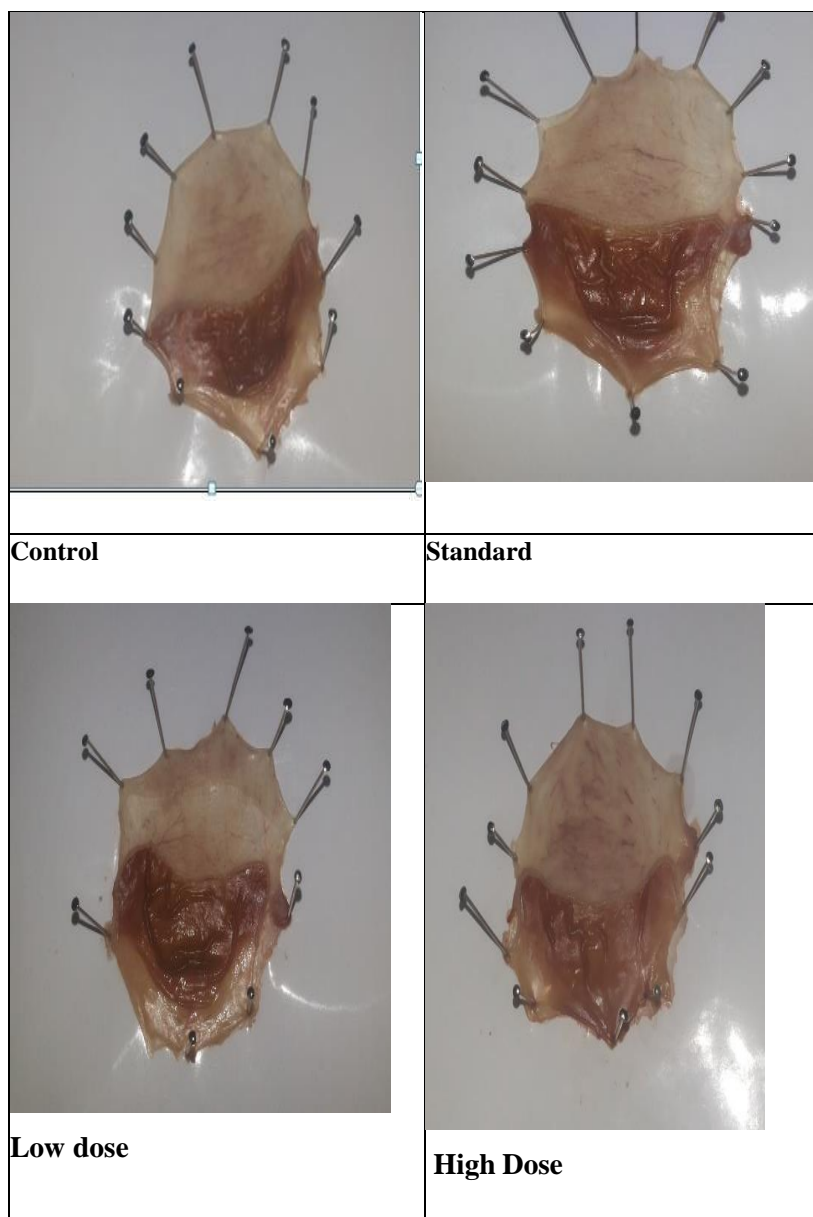
Concentration (µL/ml)	Absorbance		Percentage inhibition	
	Ethanol extract	VitaminC	Ethanol extract	VitaminC
1	0.694±0.21	0.58±1.23	0.5±0.71	15.8±2.3
10	0.676±1.31	0.487±3.5	4.35±.56	29.9±2.9
20	0.640±3.22	0.361±1.23	9.03±0.78	49±5.6
30	0.60±1.52	0.121±1.5	14.2±1.3	67.8±4.9
40	0.566±4.35	0.101±3.2	19.2±1.27	87.3±4.3

50	0.530±2.33	0.046±4.2	23.4±1.32	97.3±4.2
60	0.461±3.5	0.06±4.9	29.2±.79	96.1±3.2
70	0.459±3.6	0.05±4.1	36.3±0.96	96.2±4.56
80	0.40±4.6	0.05±0.22	41.9±0.95	96.2±4.32
90	0.36±2.5	0.04±0.3	48.13±1.32	97.5±3.78
100	0.327±3.72	0.03±.52	54.23±1.56	98.3±3.96

Results are mean ± SD of three individual experiments.

Pharmacological Study

Fig. No 3: Photographs Showing Aspirin Induced Gastric Ulcers



Effect of AEIM on Ulcer Index on NSAID induced Ulcer model

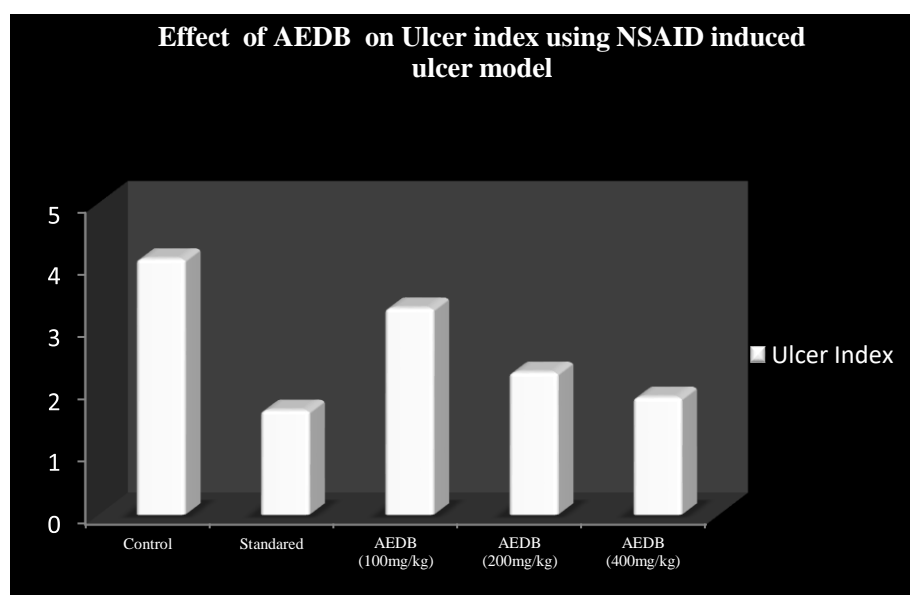
Table No 6: Effect of AEIM on Ulcer Index on NSAID inducedUlcer model

S.No	Treatment	Dose	Ulcer Index
1	Control(water)	-	4.11±0.25
2	Famotidine	3mg/kg	1.68±0.42**
3	AEIM	100mg/Kg	3.32±0.28
4	AEIM	200mg/Kg	2.29±0.21*
5	AEIM	400mg/Kg	1.89±0.11**

AEIM - Alcoholic Extract of *Indigofera mysorensis*

**P<0.001, *P<0.05, compared with control

Fig. No 4 : Effect of AEIM on Ulcer Index on NSAID induced Ulcermodel



Effect of AEIM on Total Acidity on NSAID induced Ulcer model

Table No 7: Effect of AEIM on Total Acidity on NSAID induced Ulcermodel

S.No	Treatment	Dose	Total Acidity(mEq/L)
1	Control(water)	-	73.14±1.13
2	Famotidine	3mg/kg	28.1±0.84**
3	AEIM	100mg/Kg	75.6±0.22*
4	AEIM	200mg/Kg	67.06±0.516*
5	AEIM	400mg/Kg	40.20±0.21**

AEIM - Alcoholic Extract of *Indigofera mysorensis*

**P<0.001, *P<0.05, compared with control

Effect of AEIM on Acid Volume on NSAID induced Ulcer model

Table No 8: Effect of AEIM on Acid Volume on NSAID induced Ulcermodel

S.No	Treatment	Dose	Acid Volume (ml)
1	Control(water)	-	5.30±0.21
2	Famotidine	3mg/kg	3.93±0.33**
3	AEIM	100mg/Kg	5.10±0.11*
4	AEIM	200mg/Kg	4.32±0.09*
5	AEIM	400mg/Kg	4.04±0.04**

AEIM - Alcoholic Extract of *Indigofera mysorensis*

**P<0.001, *P<0.05, compared with control

Effect of AEIM on pH in NSAID induced Ulcer model

Table No 8: Effect of AEIM on pH in NSAID induced Ulcer model

S.No	Treatment	Dose	PH
1	Control(water)	-	2.15±0.10
2	Famotidine	3mg/kg	4.88±0.16**
3	AEIM	100mg/Kg	3.49±3.05
4	AEIM	200mg/Kg	3.82±4.77**
5	AEIM	400mg/Kg	4.02±3.33**

AEIM - Alcoholic Extract of *Indigofera mysorensis*

**P<0.001, *P<0.05, compared with control

Effect of AEIM on Glutathione in NSAID induced Ulcer model

Table No 9: Effect of AEIM on Glutathione in NSAID induced Ulcermodel

S.No	Treatment	Dose	Glutathione(mcg/gm)
1	Control(water)	-	0.93±0.012
2	Famotidine	3mg/kg	1.14±0.19*
3	AEIM	100mg/kg	0.88±0.065
4	AEIM	200mg/kg	1.22±0.094
5	AEIM	400mg/kg	1.41±0.024*

AEIM - Alcoholic Extract of *Indigofera mysorensis*

**P<0.001, *P<0.05, compared with control

Effect of AEIM on Total Protein in NSAID induced Ulcer

Table No 10: Effect of AEIM on Total Protein in NSAID induced Ulcer

S.NO	Treatment	Dose	Total ptoeingm/dl
1	Control(water)	-	0.842±0.02
2	Famotidine	3mg/kg	0.720±0.03*
3	AEIM	100mg/kg	0.740±0.09
4	AEIM	200mg/kg	0.672±0.05*
5	AEIM	400mg/kg	0.650±0.02*

AEIM - *Alcoholic Extract of Indigofera maysorensis*

**P<0.001, *P<0.05, compared with control

Fig. No 6: Effect of AEIM on Total Protein in NSAID inducedUlcer Model

CONCLUSION

The present study is evaluated the antioxidant potential and antiulcer effect of AEIM. The results analysed from the present study have indicatethat AEIM possesses antioxidant and antiulcer effect on aspirin induced ulcers.

The preliminary phytochemical screening of whole plant extracts indicates in presence of alkaloids, Carbohydrates, Glycosides, Flavonoids, Tannins, Proteins, Amino Acids. In gastric ulcer tissues, Glutathione (g-glutamylcysteinylglycine, GSH) levels were found to be decreased, aspirin-induced genesis of free radical concentration reduces the cysteine concentration which mediated for GSH released. Values from this study responsible for depletion of gastric GSH is related with induction of gastric lesion in the rats. GSH is a tripeptide and having a superoxide radical scavenger and it protect thiol protein contents essential for release the integrity of tissue against oxidation reaction. In my present study, AEIM treatment showed increase in the glutathione content.

All these data indicate that the AEIM could be regarded as a favourable antioxidant and anti-ulcer effect. Mechanism of action and therapeutic need to establish in future research.

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