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# EVALUTION OF HEPATOPROTECTIVE ACTIVITY OF COMBINATION DOSE OF ECLIPTA ALBA AND BETEL LEAF EXTRACT AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS AND COMPARISON OF RESULTS AGAINST STANDARD DOSE OF SILYMARIN

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**ABSTRACT**

1) ***ECLIPTA ALBA***
- ***Background***:-*The plant eclipta alba commonly known as 'Bhringraj',is used as a hepatoprotective agent. In Ayurveda Bhringraj is considered as a powerful liver tonic.It is a weed which grows in tropical and subtropical regions all over the world. It is widely dispersed throughout India,Brazil,Thiland and China.The plant is known to have some important pharmacological activities such as antimicrobial, analgesics, anti-inflammatory ,anti-viral, hepato-protective, etc*

2) ***PIPER BETEL***
- ***Background***:- *Piper betel linn is commonly known as Paan leaf or betel leaf is belonging to family piperaceae.It have various pharmacological activities like antioxidant, anti-inflammatory and antimicrobial properties. It commonly used as a hepato-protective tonic for liver.*
- ***Objective***:-*The main objective of the study is to evaluate the hepatoprotective activity of combination dose of Eclipta alba and Piper betel against paracetamol or acetaminophene induced hepatotoxicity in albino wistar rats and comparison of results against standard dose of silymarin that is the khown hepatoprotective agent.*
- ***Keywords***:- *Eclipta alba, Liver injury,Hepatoprotective activity,Silymarin, betel leaves, Albino rats.*

## ❖ INTRODUCTION

The liver plays central role in metabolism and detoxification. It is essential organ responsible for numerous essential metabolic functions. Several drugs such as Xenobiotics and environmental toxins can damage the liver. Paracetamol is commonly used as analgesics and antipyretic drug but at high doses it can damage the liver. Hepatotoxicity is one of the most common adverse effect with paracetamol or acetaminophene. Exessive paracetamol intake generates the toxic metabolite N-acetyl-p-benzoquinone{NAPQI},through cytochrome p450 metabolism. This NAPQI deplets hepatic glutathione and leads to oxidative stress cellular necrosis and hepatic dysfunction.

Synthetic Hepato-protective drugs such as silymarin and N-acetyl cysteine are commonly used as Hepato-protective agents . However they have limited efficacy and potential side effects. Medicinal plants are rich in phytochemicals such as flavonoids, alkaloids, tannins and phenolic compounds that exhibit potent antioxidant and membrane stabilizing properties that can mitigate oxidative stress induced hepatocellular injury. Among various medicinal plants, Eclipta alba and betel leaf has been reported for its hepato-protective, antioxidant and anti-inflammatory activities.

### 1) ECLIPTA ALBA

Eclipta alba is popularly known as ''king of hairs''used in indigenous system of medicine as a hepato-protective drug. Seedling leaves of plant are ovate to egg shaped with short toothed margins. Stems are reddish purple with short flat up turned hairs. Roots are cylindrical and greyish in colour. The flowers of eclipta alba are green early and then turns white with short rays.
In ayurvedic medicine the leaf extract is considered ''Rasayana'' for longevity and rejuvenation. Eclipta alba has traditional external uses, like athlet foot, eczema and dermatitis. It is also used as anti- venom against snakebite in china and brazil.
This plant is known to have various pharmacological properties and it is traditionally used in treatment of eclipsy.

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It contains active constituents such as wedelolactone ,eclalbasaponins, and flavonoids. Wedelolactonone and demethylwedelolactone have hepato-protective action. It enhances hepatic regeneration and restores normal liver enzyme levels. Many hydrocarbons like ecliptal,alpha-formylterthienyl are present in extract. The whole plant of eclipta alba contains many triterpenene like saponin,eclabatin along with alpha-amyrin and b-amyrin, ursolin acid, oleanolic acid and triterpine glycosides eclalba saponins 1-6.

- **PHYTOCHEMISTRY OF ECLIPTA ALBA**

Eclipta alba(L) contains various phytoconstituents which includes coumestans, alkaloids,flavonoids, glycosides, triterpenoids. The leaves contain stigmasrerol, wedelolactone, dimethylwedelolactone. The roots contain hentriacontanol and heptacosanol. The aerial part contains phytosterol and beta amyrin.

**1) Alkaloids**

The main alkaloid isolated from Eclipta alba is ecliptine. These compound is mainly responsible for its hepato-protective, antimicrobial and antioxidant activities.

**2) Coumestans**

Wedelolactone, demethylwedelolactone and nor- wedelolactone are the most important coumestans found in the plant. These compounds are primarily responsible for liver protecting anti inflammatory and antioxidant effects.

**3) Flavonoids**

Major flavonoids identified includes luteolin ,apigenin and quercetin.Flavonoids act as a strong free radical scavenger and contribute to the anti- inflammatory anti-cancer and antioxidant properties of the plant.

**4) Triterpenoids and saponins:-**

The plant eclipta alba contains a series of oleanane type triterpenoid saponins khown as eclalbasaponins(1-6) .These saponins exhibit hepato-protective,immunomodulatory and anti- inflammatory actions.

**5) Sterols**

Stigmasterol and beta-sisosterol are the main sterols present in the plant eclipta alba. They have anti- inflammatory, cholesterol lowering and cyto-protective effects.

**6) Other Compounds**

Essential oils ,thiophenes, polyacetylenes and tannins are also present in the plant. The presence of these compounds enhances the antimicrobial and antioxidant potential of the plant.

- **PHARMACOLOGICAL ACTIVITIES OF ECLIPTA ALBA**

The extract of eclipta alba shows following pharmacological activities .

1) Analgesic and anti-inflammatory activity
2) Antimicrobial effect (Antibacterial, Antifungal, Anti-malarial)
3) Anti-hyperglycemic effect
4) Antioxidant properties
5) Hepato-protective effect
6) Neuropharmacological effect
7) Hypolipedimic effect
8) Anti-cancer activity
9) Hair growth and alopecia
10) Immunomodulatory activity

**2) BETEL LEAF**

Similar to eclipta alba , piper betel linn (Betel leaf) is traditionally used in ayurveda for its antioxidant, antimicrobial ,and detoxifying effects. Betel leaf , scientifically known as piper betle linn, is a perrinnial ,evergreen ,and aromatic climber is belonging to family piperaceae. It is widely cultivated in tropical and subtropical regions of asia,india,shrilanka,Bangladesh,Malaysia and Indonesia. The plant have heart shaped leaves which are used in traditional medicine,cultural rituals. The betel leaf produces distinct aromatic flavor and contains different bioactive compounds such as alkaloids, phenols, terpenes, flavonoids and tannins. It is used in Ayurveda, Unani and folk medicine for its carminative ,antioxidant, hepato-protective properties. The phyto-constituents of betel leaf includes, eugenol,chavicol, hydroxychavicol and phenolic compounds which protects the hepatocytes from oxidative damage.

Beyond its medicinal uses, betel leaf also holds socio-cultural andreligious significance in many Asian communities. It beings an essential component of traditional ceremonies hospitality and festivals.

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- **PHYTOCHEMISTRY OF BETEL LEAF**

Betel leaf is rich in wide range of bioactive phytochemicals.These are,

1) Phenolic compounds (Eugenol,chavibetol,hydroxychavicol,catechol)
2) Alkaloids (Arecoline, Piperine)
3) Terpenes and terpenoids (Caryophyllene,Cardinene,Safrole, Chavicol)
4) Flavonoids (Apigenin, luteolin,quercetin)
5) Tannins ( Gallotannins)
6) Essential oils (Chavibetol,eugenol,and safrole

- **PHARMACOLOGICAL ACTIVITIES OF BETEL LEAF**

The extract of betel leaf possesses following pharmacological activities,These are

1) Hepato-protective Activity
2) Antioxidant Activity
3) Antimicrobial Activity
4) Antifungal Activity
5) Anti-inflammatory Activity
6) Anticancer Activity
7) Anti-diabetic Activity
8) Wound healing Activity
9) Cardio-protective and anti-hyperlipedimic Activity
10) Anthelminitic and Antiseptic Activity
11) CNS stimulant and mood enhancing Activity

Although, individual extracts of eclipta alba and piper betel have shown hepato-protective potential. The combined effects of these 2 plant extracts has not studied yet. Therefore, the present study was designed to evaluate the hepato-protective activity of combination dose of eclipta alba and betel leaf etanolic extract against paracetamol induced hepatotoxicity in rats and compare its effectiveness with the standard hepato-protective agent silymarin .

## ❖ MATERIALS AND METHODS USED

**1) Plant material collection ,preparation and authentication**

- **Source** - Fresh leaves of eclipta alba and betel leaves are collected from healthy plants in india or other location. After collection of leaves or plant verify it from botanist or expert authenticate to ensure that the plant is correct.

After collection ,deposit a voucher specimen in a herbarium for future refrence.

- **Cleaning** - Wash the leaves of eclipta alba and piper betel thoroughly with water to remove the durt dust or other impurities.
- **Drying –** 2 methods are used for drying the leaves.

1) Shade Drying- Dry the leaves in the shade at room tempreture to prevent the degradation of active components.
2) Oven Drying- Another way of drying is dry the leaves in an oven at low tempreture (40 degree) for several days.

- **Grinding –**Once the leaves of eclipta alba and piper betel are completely dry, grind them in a fine powder by using an electric grinder or blender. For this purpose laboratory homogenizer is also used. Sieve the powder to obtain to obtain uniform particle size.
- **Extraction –**Dried powder of each plant (500gm) was extracted separately by maceration or by using soxhlet extraction. Dried powerder of each plant i.e eclipta alba and piper betel (500 gm ) was extracted separately with 70% ethanol( 1:10 W/V) for 48 hrs with intermittent shaking. Filtrate is collected by whatman filter paper and residue reextracted twice. Combined filtrate were concentrated under reduced pressure on rotary evaporator at < 40-45 degree celcius and freeze dried to give concentrated extract.

- **Storage**
  Store the dried extract in a food grade container at 4 degree celcius until use.
- **Combination formulation**
  For combination dosing extracts of eclipta alba and piper betel will mix in a 1:1 W/W ratio and suspended in 0.5 percent CMC i.e carboxymethylcellulose immediately before administration.

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- **Standardization ( Marker quantification)**
  Wedelolactone in eclipta alba is responsible for hepatoprotective action. Similarly , eugenol hydroxychavicol and allylpyrocatechol(APC) in betel leaf extract are responsible for hepatoprotective action.
  They are quantified by HPLC/HPTLC.

**2) Selection of Animals**

- **Species and strain -** 30 adult wistar rats of 150-200 gm weight or swiss albino mice of 25 -35 gram weight of either sex are used for the study. Animals are housed under standard conditions i.e
- Tempreture – 22 to 25 degree celcius
- Relative humidity- 60-70 %
  12 hours light/dark cycle is maintained with free access to standard pellet diet and water. The animals are kept in polypropylene cages. Before experiment the animals are fasted overnight but drinking water is given add libitum.

**3) Experimental design and dosing/Experimental protocol : -**

- **Group distribution-** 5 groups of animals are made ,each containing 6 animals in it.

a) Normal control:- vehicle(0.5% CMC)
b) Paracetamal control:- Paracetamol only + vehicle
c) Standard:- Silymarin 50mg/kg + paracetamol
d) Combination low dose:- E.alba + P.betle extract (100+100 mg/kg BW, P.O)
e) Combination high dose:- E.alba +P. betle extract(200+200mg/kg +paracetamol BW,P.O

- **Dose**
  1) The dose of paracetamol is given is 500mg/kg to induce hepatotoxicity. It can vary also. During experiment the required quantity of paracetamol solution is prepared in normal saline containing 20% propylene glycol
  2) The dose of E.alba and P. betle extract is 100mg/kg p.o ( low dose) High dose is 200mg/kg p.o of each extract. The dose can vary depending on the weight of animals. The extract is suspended in distilled water with 2-3 drops of polysorbate 80( Twinn 80)
  3) Standard dose of silymarin given to animals is 50mg/kg p.o

- **Dosing schedule**- Test extracts and silymarin are given by oral route once daily for 7 days. On day 7(1 hour after last treatment) hepatotoxicity is induced by a single oral dose of paracetamol( 2gm/kg p.o) suspended in warm saline or vehicle. Animals are fasted over night before paracetamol administration but will have free access to water.Animals are sacrifice 24 hours after paracetamol dosing for biochemical and histological analysis.

**4) Biochemical Assay**

**A) Sample Collection**

**a) Blood collection** – At the end of drug treatment, all the animals will anasthetized by application of light chloroform or other anesthetic and blood sample is collected by cardiac puncture into plain tubes. Blood is allowed to clot at room tempreture and centrifuged at 3000 rpm for 10 min to separate plasma/serum. Seprate blood sample are collected from another group of anesthetized animals in glass test tubes and allowed to coagulate for 30 minutes. Serum is store at -20 degree celcius until assays or biochemical estimation.

**b) Liver tissues**- Animals are sacrificed by euthanasia. Liver tissue will excised, rinsed in ice cold saline, blotted and weighed. A portion of liver is fixed in 10% neutral buffered formalin for histopathology ,remaining tissue is snap frozen in liquid nitrogen and store in at -80 degree celcius for another biochemical estimation.

**B) Biochemical estimation( Serum)**

**a) Hepatic marker enzymes-** Serum alanine amino transferase (ALT,SGPT) ,Aspartate amino transferase9AST,SGOT),Alkaline phosphatase(ALP) and total bilirubin is estimated by using commercial diagnostic kits ( crest bio system) according to the manufactures instructions. Total protein and albumin may also be estimated to access synthetic function .

**b) Antioxidant assay- ( Liver Homogenate)**

- **Preparation of homogenate-** Approximately 10% (w/v) liver homogenate will prepared in ice cold phosphate buffer solution by using tissue homogenizer. Homogenate will centrifuged (10,000 x 9 for 15 min at 4 degree celcius) supernatant is collected for assay.

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Following assay are performed for biochemical estimation.

1) Superoxide dismutase(SOD) Assay-
2) Catalase Assay-
3) Reduced glutathione(GSH) Assay-
4) Lipid peroxidation Assay-

**C) Histopathology**

Fixed liver tissues is processed embedded in paraffin and section of tissue are made (4-5 micro meter) and stain it with hematoxylin and eosin. Sections are examine under light microscope for necrosis, fatty change ,inflammation, sinusoidal congestion and score it semi-quantitatively. For example- 0 = normal, 1=mild , 2= moderate, 3=severe by a blinded pathologist.

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