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EVALUATION OF ANTI - OXIDANT AND HEPATOPROTECTIVE ACTIVITY OF *IXORA COCCINEA* LEAF EXTRACTS BY USING *INVITRO* AND *INVIVO* MODELS.

Vinjavarapu. L. Anusha[1], A.Naga vamsi[2], B.Siva sai[2], E.Brahma reddy[2], B.Sindu[2], S.Kiran[2]

[1]Associate. Professor, Department of Pharmacology, SIMS college of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India.

2. Department of Pharmacy, SIMS college of Pharmacy, Mangaldas Nagar, Guntur, Andhra Pradesh, India.

Introduction:

ANATOMY OF LIVER:

•The liver is one of the largest gland in the body and after the dermis .The liver weighs about three and a half pounds it constituents about 2.5% of adults body weight.The different type of cells propagate from the liver lobes are parenchymal and
non-parenchymal type of cells.

•The liver is situated below the diaphragm which occupies the side of the hypochondriac region in the abdominopelvic cavity.

FUNCTIONS OF LIVER:

•The liver has well over 500 functions and is known as the laboratory of the humanbody.
•The liver is tied to almost all the bodily processes as it is responsible for filtration of all incoming foods and fluids.

LIPID METABOLISM:

• Liver stores some triglycerides from fatty acids through acetyl coenzyme A known as beta oxidation.
•It possibly converts excess acetyl coenzyme A to ketone bodies (ketogenesis)
•It synthesises lipoproteins ,the transportation of fatty acids,triglycerides(TG)and cholesterol from the body cells.

PROTEIN METABOLISM:

•Most of the plasma proteins ,such as alpha and beta globulins,glycol proteins (albumin and fibrinogen) are synthesised from liver cells .
•It converts resulting toxic ammonia into much less toxic urea for excretion in urine.

REMOVAL OF DRUGS AND HORMONES:

- Liver can detoxify substances such as alcohol or excrete drugs likepenicillin,erythromycin,and so on into bile.
- It also triggers or chemically alters thyroid hormones and steroid hormones(oestrogen and aldosterone).

AIMS AND OBJECTIVES;

Plants that cure liver diseases so considerable interest has developed in the examination of these numerous plant remedies which are useful in liver diseases. So it is necessary to find new drugs of importance in hepato protective activity with fewer side effects. Moreover it is necessary to produce scientific validation to drugs of herbal origin in common use under Ayurvedic Siddha Unani systems of medicine.

Phytochemical investigation will be a useful tool for the identification and authentication of the plant for industrial and further research purposes. Total phenol content of a tested material is 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, antioxidants were also evaluated for the potent extract.

And now I have undertaken the study of evaluation of antioxidant and hepatoprotective activity of ixora coccinea leaf extracts by various hepatotoxin induced albino rat models.

- To select plants based on their ethno medical uses and preparation of their extracts.
- To screen phytochemical profiles.
- To screen the selected extract for antioxidants using various in vitro methods.
- To screen the potent plant extract for their in vivo hepatoprotective activities.

PLANT PROFILE:

TAXONOMIC CLASSIFICATION OF *IXORA COCCINEA*

Kingdom	Plantae
Sub kingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	*Asteridae*
Order	*Rubiales*
Family	*Rubiaceae*
Genus	*ixora*
Species	coccinea

Ixora coccinea plant

DISTRIBUTION AND HABITAT:

Ixora coccinea is a common leafing shrub native to Southern India and Sri Lanka and widely cultivated in Indonesia, Malaysia, the Philippines, Vietnam, Cambodia, Laos and Thailand. It

has become one of the most popular leafing shrubs in South Florida USA gardens and landscapes. It grows in tropical areas with medium annual rainfall in well drained soils.

DESCRIPTION:

Ixora coccinea is a low-growing tropical shrub notable for its bright coloured leaves which are composed of many small blooms massed together into dense, flat-topped leaf heads. Ixora coccinea is one of the few Ixora species that make good indoor plants along with several kinds developed from it. (It takes up to five years for Ixora coccinea to grow to its maximum height of about 1.2m (4 feet). It is a much-branching shrub, with leathery, shiny, pointed oblong leaves up to 10cm (4 inch) long and 5cm (2 inch) wide arranged in pairs or whorls of three or more in 1-2 cm (0.4-0.8 inch) long stalks, Leaf colour is dark green, often bronzish when the leaves are new. Tubular leaves, which are up to 5 cm (2 inch) long and fiery red, open at the month into four petals arranged in the form of a cross about a centimetre (0.4 inch) wide. The entire leaf head has a diameter of 8-12 cm (3-5 inch). Normal leafing period is summer, but occasional leaves also appear in the autumn. Various kinds of Ixora which have Ixora coccinea as a parent produce differently coloured blooms, chiefly in shade of orange, yellow and pink, as well as red.

PROPAGATION:

Propagate Ixora coccinea from stem cuttings 5-8 cm (2-3 inch) long taken in spring. Trim each cutting immediately below a leaf, remove that leaf and dip the cut end in hormone rooting powder. Plant the cutting in a 5-8 cm (2-3 inch) pot containing a moistened equal-parts mixture of peat moss and coarse sand or perlite. Enclose the whole in a plastic bag or propagating case and stand it in bright filtered light at a temperature of 21-27°C (70-81°F). When the cutting has rooted probably in four to six weeks - uncover it gradually over a two or three week's period in order to acclimatise the new plant to the less humid atmosphere of the room. When the new plant is fully uncovered, begin to water moderately (allowing a couple of centimetres (0.4-0.8 inch) or so of the potting mixture to dry out between watering again) and apply standard liquid fertiliser once every two weeks. About three months after the start of the propagation move the new plant into a slightly bigger pot of the recommended potting mixture for adult plants and treat it as mature.

USES:

(It is primarily an ornamental plant. Cut specimens are long lasting and are often used in floral arrangements. It is sometimes used as a hedge. In tropical Asia the leaves, bark and leaves of this plant are used in traditional medicine.

MATERIALS AND METHODS:

COLLECTION AND AUTHENTICATION OF PLANT:

Ixora coccinea leaf was procured from the botany central council for research in ayurvedic and siddha govt of india .The dried leafs are authenticated by Chelladurai.V research officer botany central council for research in ayurveda and siddha govt of india.

PHYTOCHEMICAL SCREENING:

The plant may be containing the following compounds such as carbohydrates,proteins,and lipids.That is utilised as food by man.It also contains the compounds like tannins,glycosides,alkaloids,volatile oils. The compound that is responsible for lots of medicinal properties.

CLASSIFICATION OF TESTS: TEST FOR CARBOHYDRATES

- Molisch test
- Fehling test
- Benedict's test

TEST FOR ALKALOIDS

- Mayer's reagent
- Dragendorff's reagentsTEST FOR FLAVONOIDS

TEST FOR STEROIDS

- Salkowski test
- Liebermann burchard test

TEST FOR TANNINS

- Millions reagents
- Ninhydrin test
- Keller -killani test
- Foam test

TEST FOR TERPENOIDS.

IN *VITRO* ANTIOXIDANT ACTIVITIES

Superoxide radical scavenging activity

PRINCIPLE:

The superoxide anion radical scavenging activity was determined by nitro blue tetrazolium (NBT) reduction method of McCord and Fridovich (1969). The assay is based on the ability of drugs to inhibit the reduction of nitro blue tetrazolium (NBT) by Superoxide, which is generated by the reaction of photo reduction of riboflavin within the system. The superoxide radical thus generated reduces the NBT to a blue coloured complex.

I. REAGENTS:

- Nitro blue tetrazolium (NBT) - 1.5nm (12.3mg/10ml)
- Riboflavin - 0.12µm (4.5mg/100ml)
- .NaCN/EDTA -0.0015% NaCN in 0.1M EDTA
- Phosphate buffer - 0.06M (pH 7.8)

II.PROCEDURE:

The reaction mixture contained EDTA (0.1 M), 0.3mM NaCN, Riboflavin (0.12mM), NBT (1.5 n moles), Phosphate buffer (67mM, pH 7.8) and various concentrations of the seed oil extract in a final volume of 3ml. The tubes were illuminated under an incandescent lamp for 15 min. The optical density at 560 nm was measured before and after illumination. The inhibition of superoxide radical generation was determined by comparing the absorbance values of the control with that of seed oil extract and fraction-IV. Vitamin C was used as positive control. The concentration of fraction-IV required to scavenge 50% superoxide anion (IC50 value) was then calculated.

DPPH radical reducing activity

I.PRINCIPLE:

It is a rapid and simple method to measure antioxidant capacity. It involves the use of free radicals, DPPH (2, 2-Diphenyl-1-picryl hydrazyl) (Aquino et al, 2001). The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reducedDPPH-H. The resulting decolourisation is stoichiometric with respect to the number of electrons captured.

I. REAGENT:

- DPPH 3 mg in 25 ml methanol (stored in dark bottle)
- Methanol

II.PROCEDURE:

Freshly prepared DPPH (187 µl) was taken in different test tubes protected from sunlight. Tothis solution added different concentrations (0, 25, 50, 75,100,150,200µg/ml) of seed oil extract and fraction-IV. The volume was made up to 1 ml with methanol. Keep the tubes in dark and after 20 min absorbance was measured at 515 nm. Methanol was used as blank and vitamin C was used as positive control. The concentration of test materials to scavenge 50% DPPH radical (IC50 value) was calculated from the graph plotted with % inhibition against Concentration.

RESULTS AND CONCLUSION:

Soxhlet extraction of ixora coccinea leaf extraction (ICLE)

- The percentage yield of the ICLE in ethanol by cold maceration was found to be2.93%W/W.

Extraction of *ixora coccinea* leaf extract (ICLE)

PLANT	PART USED	METHOD OF EXTRACTION	SOLVENTS	PERCENTAGE YIELD(%W/V)
Ixora coccinea	leaf	Maceration	Ethanol (95%)	2.93

Preliminary phyto chemical screening .

Ixora coccinea leaf extracts (ICLE) were subjected to various chemical tests as per the standard methods for the identification of the various constituents if this phyto chemical analysis is listed below.

Test for carbohydrates

1.Molisch test

Test for alkaloids

1.Mayer's reagent

Test for flavonoids

1.Millions reagents2.Ninhydrin test

Test for glycosides

1.Keller-killani test

Test for saponins

1.Foam test

QUALITATIVE PHYTOCHEMICAL SCREENING OF *IXORA COCCINEA* LEAFEXTRACT(ICLE)

PLANT CONSTITUENTS	INTERFERENCE Ethanol Extract
Carbohydrates	-
Alkaloids	-
Flavanoids	+
Proteins and amino acids	+
Glycosides	-
Fixed oil	-
Terpenoids	+
Volatile oil	-
Tannins	-

EFFECT OF ICLE ON SGPT CONCENTRATION IN PARACETMOL

TREATMENT	DOSE mg/kg	SGPT(IU/L)
Control	-	93.220±1.22
Toxic control	2mg/kg	157.22±0.16
Standard	100mg/kg	110.22±0.19
Ethanol extract	200mg/kg	127.22±0.21
Ethanol extract	400mg/kg	113.11±1.85

DISCUSSION:

The plant ixora coccinea is widely distributed in south asia .The flower part of plant have been studied its antibacterial activity but the hepatoprotective effect have been never studied, Hence the objective of the study is determining this effect from the dried leaf extract of ixora coccinea
The preliminary phytochemical screening of whole plant extracts indicate the presence of flavonoid, terpenoids, protein and amino acid. may account for antioxidant and hepatoprotective activity.

The antioxidant screening shows that it showed reduced power to DPPH radicals. But the efficiency showed that far below from Vitamin C. The concentration of the ICLE needed to scavenge 50% superoxide anion (IC50) equal to that of standard, hence the leaf juice has significant antioxidant activity.

Liver is one of the important organs of the body hence damage to liver leads to severe pathological problems or death. Liver diseases are mainly caused by toxic chemicals, excessive intake of alcohol, infections and autoimmune disorders. Liver injury caused by hepatotoxins such as carbon tetrachloride, ethanol and acetaminophen is characterised by varying degrees of hepatocyte degeneration and cell death by either apoptosis or by necrosis. SGOT, SGPT, ALP, Total Protein and Total Bilirubin levels are largely used as most common biochemical
markers to evaluate liver injury, Paracetamol (Acetaminophen), a widely used analgesic and antipyretic drug that produces acute liver damage in high doses Paracetamol induced hepatotoxicity is thought to be caused by N-acetyl-p-benzoquinoneimine (NAPQI), a cytochrome P-450 mediated intermediate metabolite. NAPQI can react with sulfhydryl groups such as glutathione and protein thiols. The covalent binding of NAPQI to cell proteinsis considered the initial step in a chain.

While silymarin, an extract of ixora coccinea. Significantly restored total protein activities to normal level. Moreover, histopathological analysis showed that normal liver architecture was disturbed by paracetamol-treated rats; oral feeding with extracts shows normal architecture with mild hepatocyte degeneration compared with normal control groups.
The preliminary phytochemical screening of whole plant extracts indicate presence of flavonoid, terpenoids, protein and amino acid. The antioxidant studies particularly showed that ICLE have slight antioxidant potential but that not inferior than standard vitamin Hepatoprotective study results show that the levels of SGOT, SGPT, ALP and Total Bilirubin were significantly improved may account for hepatoprotective activity All these observations imply that the ICLE could be regarded as a favourable antioxidant and hepatoprotective agent.
As the results indicated that the extract possesses significant hepatoprotective activity, after carrying out a thorough study of clinical trials, the plant can be considered as a low cost, potent, herbal medicine for liver disorder.
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