



PRELIMINARY PHYTOCHEMICAL STUDY AND PHARMACOLOGICAL SCREENING OF EXTRACTS OF LEONOTIS NEPETIFOLIA FOR HEPATOPROTECTIVE ACTIVITY

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Abstract:

The present study aimed to perform preliminary phytochemical evaluation and screening of various extracts of *Leonotis nepetifolia* as hepatoprotective. Extraction of plant material using different solvents and TLC study using ethylacetate, n-hexane and hydroalcoholic extracts. Screening of various extracts by Paracetamol induced hepatotoxicity on Sprague Dawley rats and extracts were used for the evaluation of hepatoprotective activity. It presents biochemical test including SGOT and SGPT and antimicrobial activity was also evaluated. Phytochemical evaluation showed the presence of alkaloid, flavonoid and glycosides. The examination indicated the antimicrobial activity and hepatoprotective activity more significantly in ethyl acetate extract.

Index terms: Leonotis nepetifolia, Lamiaceae, phytochemical and hepatoprotective.

INTRODUCTION:

Medicinal plants are known to provide a rich source of raw materials for traditional medicine for the treatment of various ailments since time immemorial. According to World Health Organisation, 80% people in developing countries particularly those living in villages still depend on traditional plant-based medicines principally due to their low price. [1] Natural plants contain phytochemicals which are considered to contribute to their bioactivity. Phytochemicals are compounds or secondary metabolites in plants that have beneficial or lethal effects in the body. The phytochemicals are often elaborated for the plant defence against pests and herbivores or to gain an advantage over competing agents. These phytochemicals inadvertently protect humans against pathogens and provide nutrition for normal cell health and repairs. [2] The family Lamiaceae (mint family) is widely distributed in temperate regions, comprising about 3500 species distributed among 200 genera, most of them being herbaceous, less often shrubs, and rarely trees.[3] *Leonotis nepetifolia* is one among the weed plant which has pantropic in its distribution and considered as a weed of waste land and cultivated areas. [4] Jaundice is the commonest ailments affecting the citizens of both developed and poor countries. The local inhabitants rely on medicinal plants for treatment. Jaundice is the yellowish staining of the skin and sclera that is caused by high levels in blood of the chemical bilirubin. When the level of bilirubin level is high they tend to be brown. At the present era, consumption of these herbal medicines is increasing at a high speed due to its less or no side effect and cost effectiveness as compared to synthetic medicines. Polluted and dirty water is the prime reason for the spread of jaundice which has confirmed by the recent test done on the water being supplied to the households. The allopathic systems of medicines which are so far formulated and underuse are not completely safe on the ground and free from toxicity. General management of jaundice includes high water intake, proper nutrition, and low-fat

diet. Further they are very much costly for routine use. Herbal medicines are often retained popularity due to historical and cultural reasons. There is also no scientific evidence based review on the traditional medicinal plants used for treatment of jaundice. Various medicinal plants has been used for the management and treatment of jaundice. The use of herbal medicines to treat liver diseases has increased worldwide, and this is due to belief that herbal medicines are harmless and free from serious adverse reactions. In addition they are available and easily obtained from nature. Moreover, the limited therapeutic choices and sometimes unsatisfactory therapeutic failure of modern medicine including herbal preparations. Worldwide, a great number of remedial plants and their formulations have been claimed to have hepatoprotective activity. Around 160 phytoconstituents from 101 plants have been proved to possess liver-protecting activity. It is known that 80% of the world population are using medicinal plants and 30% are prescribed by physicians. [5] Liver cirrhosis or hepatic cirrhosis is a condition in which the liver does not function properly due to long term damage. The increasing use of herbal medicines reflects their perceived effectiveness in the treatment and prevention of liver disease, and the belief that these treatments are safe as they are natural. Liver cirrhosis is mainly caused due to toxic chemicals, excess consumption of alcohol, infections and autoimmune/ disorder. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Enhanced lipid peroxidation and other oxidative damages in liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis. It has been estimated that about 90% of the acute hepatitis is due to the viruses. The major viral agents involved are Hepatitis A, B, C, D, E & G. Of these, Hepatitis B infection often results in chronic liver diseases and cirrhosis of liver. Primary liver cancer has also been shown to be produced by these viruses. [6]

Medicinal plants are known to give rich wellsprings of crude materials for self-medication in the treatment of different diseases since time in remembrance. The misuse of therapeutic plants for recuperating is quite much as old as mankind itself. The early man realized that a couple of plant concentrates could treat different illnesses.[7] For instance, 60000 years before our human advancement, the Neanderthal man had simple information on the utilization of restorative plants developed significantly in the Greek and Roman periods, with famous figures like Hippocrates, Galen, and Dioscorides.[8]Herbal medication is utilized to treat numerous conditions, like asthma, dermatitis, premenstrual disorder, rheumatoid joint inflammation, headache, menopausal indications, persistent weakness, and gut disorder among others. As of late, “The World Health Organization assessed that 80% of individuals overall depend on natural drugs for some piece of their essential medical care.”[9] The genus *Leonotis* has 12 species widely distributed in Pantropics which is represented by one species, *Leonotis nepetifolia* in India.[10]*Leonotis nepetifolia* is an important medicinal plant of reputed Indian traditional systems of medicine such as Ayurveda, Unani, and Siddha. This plant exhibited various biological activities and has been attributed to a variety of physiological effects. Labiatae has been used in the primitive medical treatment of cancer. Thus, every component of this plant has useful medicinal properties. [11]

HEPATOPROTECTIVE ACTIVITY:

The liver is the key organ of metabolism and is continuously and variedly exposed to xenobiotics. Environmental pollutants, and chemotherapeutic agents. If the natural protective mechanisms of the liver are empowered during all such exposures, this will lead to hepatic injury. Liver diseases are a problem worldwide, and the conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Thus efforts have shifted toward medicinal plants as new sources of hepatoprotective agents. A number of plant based traditional medicines or formulations containing herbal extracts are sold for liver disorder. The drug possess various pharmacologic activities including anti-inflammatory, antioxidant, and hepatoprotective. Herbal therapy is a holistic therapy, Medicinal plants are widely used and assumed to be safe. Presently, lot of work is going on the herbal medicine as an hepatoprotective. Moreover even the allopathic medicine has not claim any medication for the treatment of jaundice. Currently, In India generally doctors & peoples prefer the herbal medicine for the treatment of liver cirrhosis and jaundice. So observing the present scenario the screening of herbal medicine for liver cirrhosis and jaundice has become an important aspect and the literature review also reveals that the results are obtaining from the herbal medicines as an hepatoprotective. There are various medicine showing hepatoprotective activity and listed as follows in table no. 1 [12]:-

Table No. 1 showing medicine of hepatoprotective activity

Sr. No.	Name of Plant	Part Used	Active Constituents	Solvent Used
1)	Melastoma malabathricum L.	Leaves	Flavonoids, phenolic components	Methanol
2)	Acacia Catechu	Heart wood	Saponins, flavonoids, alkaloid	70% Methanol
3)	Feijoa Sellowiana	Fruits peel	Polyphenols, carbohydrates, vitamin A	Methanol
4)	Ficus religiosa	Leaves	Flavonoids	Methanol
5)	Fagonia schweinfurthi	Whole plant	Total phenols and flavonoid	Ethanol
6)	Zingiber officinale Roscoe	Rhizome	Flavonoids, polyphenols	Water
7)	Acacia nilotica Linn	Aerial plants	Carbohydrate, cardiac glycoside, saponin, tannins	Methanol
8)	Astragalus Kahiricus	Roots	Triterpenes, flavonoids, phenolic compounds	Ethanol
9)	Podophyllum hexandrum	Rhizome	Tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides	n-hexane
10)	Abelmoschus manihot (L.)	Flowers	Tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides	Isolated
11)	Origanum vulgare	Leaves	Terpenoids, phenolic compounds, flavonoids, tannin, saponins	Aqueous
12)	Ostostegia persica Boiss	Aerial parts (shoot)	Essential oil, flavonoids	Methanol

13)	<i>Daucus Carota</i>	Seeds	Monoterpenoids, Flavonoids, Quercetin, Limolene	Methanol
14)	<i>Pisonia aculeate</i>	Leaves	Alkaloids, phenolic compounds, tannins, saponin & flavonoids	Methanol
15)	<i>Cyathea gigantean</i>	Leaves	phenolic compounds, tannins, flavonoids	Methanol
16)	<i>Garcinia indica</i>	Fruit rind	Xanthones, flavonoids, benzophenones, Lactones & phenolic acids	Aqueous
17)	<i>Premna esculenta</i> Roxb.	Leaves	Polyphenols, flavonoids	Ethanol
18)	<i>Cissus Quadrangularis</i>	Stem	B-Carotene	Methanol
19)	<i>Asparagus racemosus</i>	Root	Alkaloids, tannins, saponins, steroids, and flavonoids	Hydro- alcohol
20)	<i>Feronia limonia</i>	Stem Bark	Flavonoids, tannins, phenols	Methanol
21)	<i>Nerium oleander</i>	Flower	Terpenoids, cardiac glycosides, tannin, flavonoids, saponins	Methanol
22)	<i>Vitis vinifera</i>	Root	Terpenoids, cardiac glycosides, tannin, flavonoids, saponins, polyphenols, phytosterols	Ethanol
23)	<i>Moringa oleifera</i>	Seed Oil	Flavonoids, terpenoids,	Oil isolated
			anthraquinine, tannin, steroids, saponins, alkaloids	

24)	<i>Alchornea cordifolia</i>	Leaf	saponins, alkaloids, flavonoids, tannins	Methanol
25)	<i>Hibiscus vitifolius</i> Linn.	Roots	Flavonoids, phenolic compounds	Chloroform, Methanol

Description of *Leonotis nepetefolia*

Scientific classification:

Kingdom: Plantae

Division: Angiosperms

Class: Eudicots

Sub class: Asterids

Order: Lamiales

Family: Lamiaceae

Genus: *Leonotis*

Species: *L. nepetefolia*



Fig1. *Leonotis nepetefolia* plant

The mature plant attains the height up to 2 meter. The orange yellow coronated verticillaster inflorescence and distinct plant odour are amongst the unique characters of this plant. Corolla tubular, blipped, tube with five annular rings within, upper lip hooked, lower lip 3-lobed. Stamens are four, didynamous hooded by the upper lip, exserted. Filaments minutely bearded, disc copular. Ovary bicarpellary, tetra ocular ovule one per locule. Basal is style gynobasic, stigma bifid, capitellate. Nutlets are four which are oblong, trigonous and seeds are also oblong. The drug possess various pharmacologic activities including anti-inflammatory, antioxidant, and hepatoprotective.[13]

MATERIAL AND METHODS:

Collection and Authentication of Plant:

Leonotis neptefolia plant was collected locally from Gondia and authenticated from Botany Department of RTMNU, Nagpur University, Nagpur. The Specimen Voucher no. was deposited at RTMNU university Herbarium, Nagpur.

Extraction of Leaves, roots, stem, seeds, and flower of *Leonitis neptefolia*

The parts of plants were dried in shade, pulverized into coarse powder and about 500 g of powder was extracted with n-hexane, ethyl acetate and hydroalcoholic mixture by Soxhlet extraction method. The resulting filtrate was concentrated to dryness and the percentage yield was calculated and stored in clean container till further use.

Characteristics of extracts

All the extracts were evaluated for their characteristics like colour, appearance, percentage field.

Preliminary phyto-chemical Screening

Preliminary phyto-chemical screening was performed to identify phytochemicals in various solvent extracts of *Leonotis nepetefolia*. The extracts were subjected for phytochemical screening using standard procedures.

Thin Layer chromatography (TLC):

The plant extract was subjected to thin layer chromatographic studies, to find out the probable number of compounds present in it.

Test for Antibacterial activity:

The antimicrobial activities of the extracts were evaluated by Disc diffusion method. The extracts were diluted in dimethylsulphoxide (DMSO) at the different concentration. The diameters of inhibition zone was measured.

Hepatoprotective Activity:

Sprague Dawley rats (180-250g) of either sex were used for the study. All experimental procedures were carried out under compliance with Institutional Animal Ethical Committee (IAEC). Groups were formed according to the protocol. Firstly, saline solution was administered orally to the Group 1 animals in the dose of 10ml/kg. Group 1, group 2, group 3, group 4 and group 5 received orally 500mg/Kg b.w. of paracetamol for inducing hepatotoxicity. And after 48 hours the last three groups received orally 100mg/kg n-hexane ethyl acetate and hydro alcoholic extracts. After 5days blood was withdrawn for the biochemical estimation.

RESULTS AND DISCUSSION

Characteristics of extracts of *L. neptefolia*

All the extracts were evaluated for their characteristics like color, appearance, percentage yield.

Characteristics of extracts are shown in table no 2:-

Table No. 2 Characteristics of Extracts

Sr no.	Extracts	Colour	Appearance	% yield
1.	N-hexane	Brown	Sticky mass	4.06%
2.	Ethyl acetate	Dark Brown	Sticky mass	2.75%
3.	Hydro alcoholic	Brownish Black	Sticky mass	3.21%

Preliminary Phyto-chemical Screening of *Leonotis nepetefolia* extracts

Table No. 03: Phyto-chemical screening of n-hexane, ethyl acetate, hydroalcoholic extract of *Leonotis nepetefolia* plant:

Plant Constituents	Test/reagent	Extracts		
		n-hexane	EA	HA
Test for Alkaloids	Dragendroff's	+	-	+
	Hager's	+	+	+
	Mayer's	+	+	+
	Wagner's reagent	-	+	-
Test for Saponins	Foam Test	-	-	-
Test for Glycosides	Keller-killiani test	+	+	+
	Legal test	-	+	-
	Borntager's Test	+	+	+
	Baljet's Test	+	-	+
Test for Phenols and Tannins	Ferric Chloride	+	+	+
	Lead Acetate	+	-	+
	Acetic Acid	+	+	-
Test for Flavonoids	Sinoda test	+	+	+
	Sulphuric acid test	+	+	+
	Alkali test	+	+	+
Test for Carbohydrates	Molisch test	-	-	-
	Fehlings test	-	-	-
Test for Protein and Amino acids	Millon's Test	-	+	+
	Biuret Test	+	+	+
	Ninhydrin Test	+	+	-
Test for fats or oil	Spot test	-	-	+
Steroid Test	Salkowaski's Test	-	-	-

-ve : Indicates absence; **+ve** : Indicates presence

Thin Layer chromatography (TLC):

The plant extract was subjected to thin layer chromatographic studies, to find out the probable number of compounds present in it.

Table No.4 Result of TLC of n-hexane, Ethyl acetate, Hydro alcoholic extract of Leonotis nepetefolia.

Sr. No.	Solvent System	Ratio	Spraying Reagent	Rf value
N-hexane Extract				
1.	Toluene: chloroform: Methanol	6:3:1	Iodine chamber & 50% H ₂ SO ₄	0.67
2.	Benzene: chloroform: Ethyl acetate : methanol	4:3:2:1	Iodine chamber & 50% H ₂ SO ₄	0.65
3.	Toluene: ethyl acetate: diethyl amine	7:2:1	Iodine chamber	0.59
Ethyl acetate Extract				
4.	Chloroform: Diethyl amine	9:1	Iodine chamber	0.86
5.	Chloroform: methanol: Ammonia	8:4:1	Iodine chamber	0.83
6.	Methanol: Water	8:2	Iodine chamber	0.88
Hydro alcoholic Extract				
7.	Cyclohexane: ethanol: Diethyl amine	5:4:1	Iodine chamber & 50% H ₂ SO ₄	0.46
8.	Acetone: Diethyl amine	3:7	Iodine chamber & 50% H ₂ SO ₄	0.34
9.	Ethanol: water: Benzene	2:7:1	Iodine chamber & 50% H ₂ SO ₄	0.42

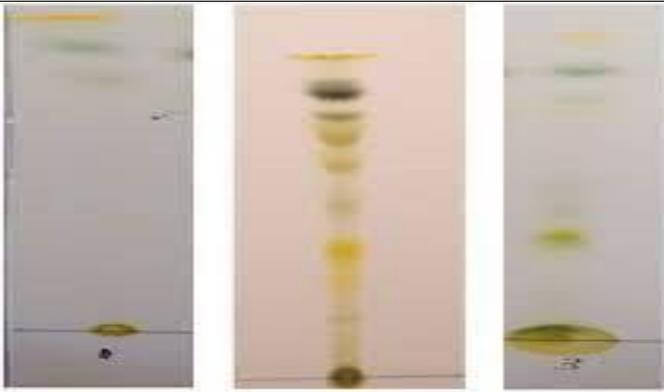
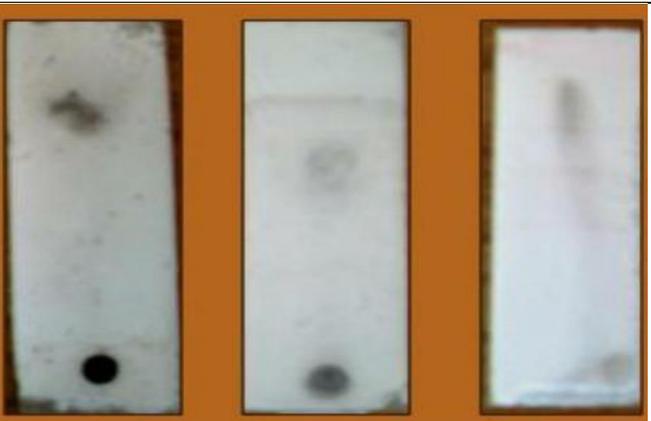
Test Sample	TLC plate
Ethyl Acetate	
N-hexane	
Hydro alcohol	



Table no 5 antibacterial activity

Sr. No.	Extract	Concentration	Disc Diffusion Method			
			Test Organisms			
			S. aureus	E. coli	B. Subtilis	K. Pneumonia
1.	N-Hexane	500ug/ml	4mm	5mm	4mm	3.5mm
		700ug/ml	3	4	5	4
2.	Ethyl Acetate	500ug/ml	8	7	7	6
		700ug/ml	8	8	7	7
3.	Hydro Alcoholic	500ug/ml	3.5	5	5	5
		700ug/ml	4	3	3	4
4.	Amoxicillin	500ug/ml	8	6	8	7
		700ug/ml	6	6	8	7

Ethyl acetate extract of *Leonotis nepetefolia* showed significant activity against all the selected test cultures.

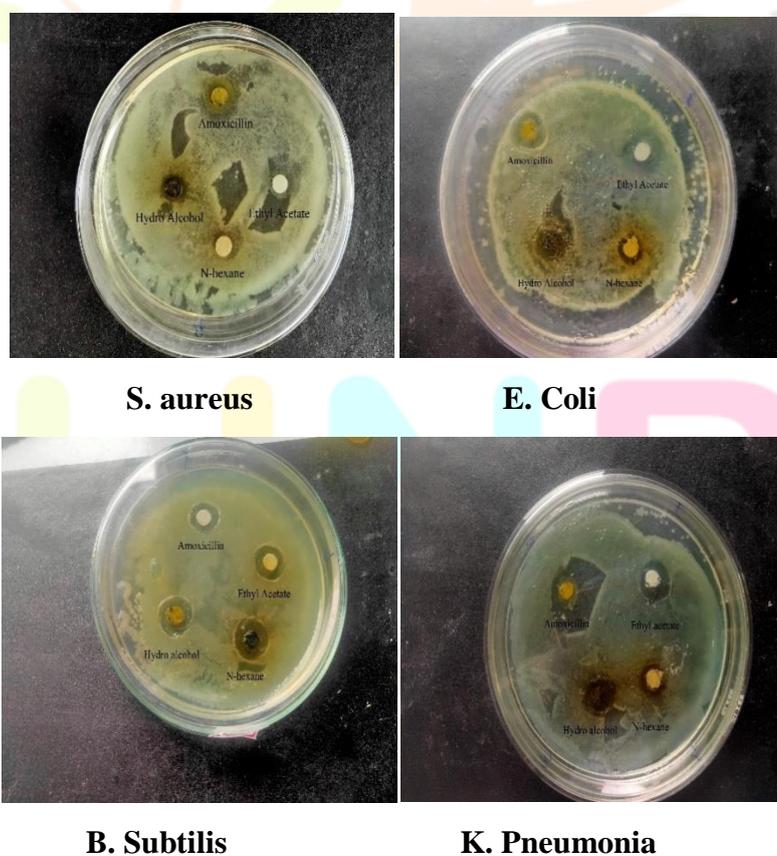
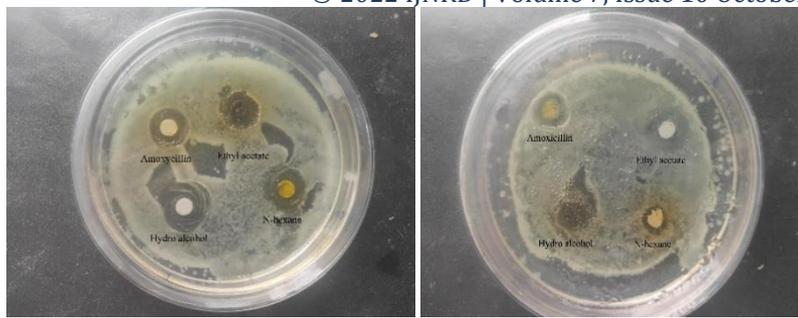
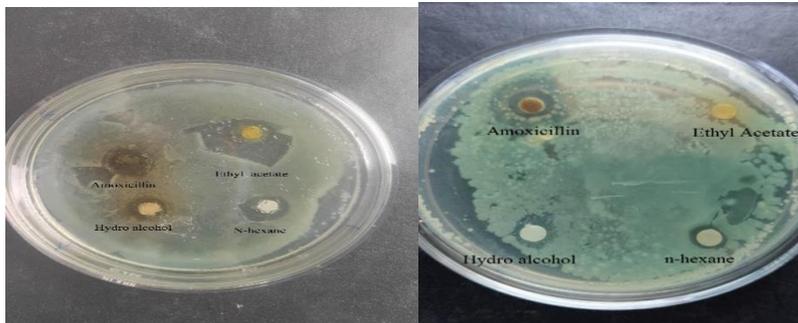


Fig 2 Disc diffusion method demonstrating inhibition zone by 500 µg/ml extract



S. aureus

E. Coli



B. Subtilis

K Pneumonia

Fig 3 Disc diffusion method demonstrating inhibition zone by 700 µg/ml extract

Evaluation of Hepatoprotective Activity

SGPT Activity

Table no. 6 showing absorbances

Sr. No.	Groups	Absorbances (340nm)			
1.	Control	0.3152	0.4091	0.3618	0.4132
2.	Paracetamol	0.05120	0.06442	0.09643	0.08783
3.	Paracetamol + n-hexane	0.0013	0.0400	0.0028	0.00801
4.	Paracetamol + Ethyl acetate	0.05040	0.08225	0.08571	0.09559
5.	Paracetamol + hydro alcohol	0.01105	0.07123	0.02309	0.09135

SGPT TEST:

Effect of different extracts on serum biochemical parameters in paracetamol induced hepatic damage in rats.

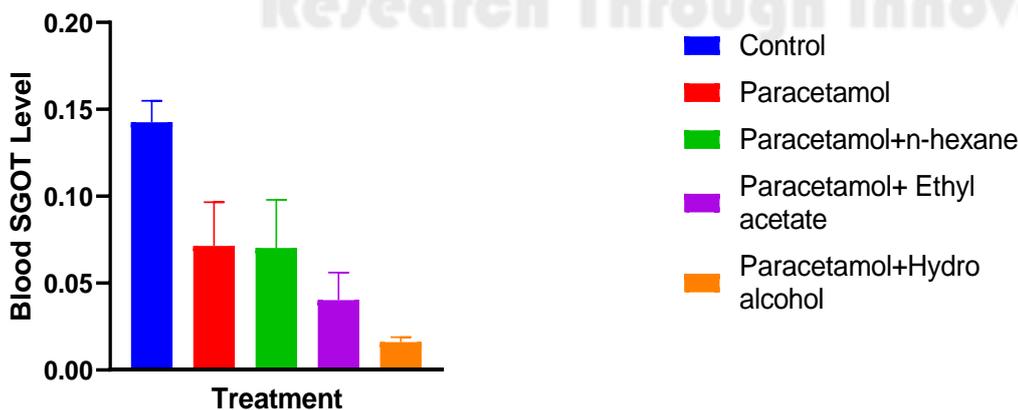


Fig 4- SGPT Graph

Data expressed as mean SEM of ethyl acetate showed significant effect with the P value of <0.0002 when compared with the control group (One-way ANOVA followed by post hoc Bonferroni multiple comparison test)

SGOT Activity

Table no. 7 showing absorbances

Sr. No.	Groups	Absorbances (340nm)			
1.	Control	0.1389	0.1268	0.1551	0.1493
2.	Paracetamol	0.03539	0.08109	0.07654	0.09292
3.	Paracetamol + n-hexane	0.03362	0.06451	0.09593	0.08682
4.	Paracetamol + Ethyl acetate	0.02133	0.03501	0.04664	0.05812
5.	Paracetamol + hydro alcohol	0.01610	0.01231	0.01852	0.01771

SGOT TEST:

Effect of different extracts on serum biological parameters in paracetamol induced hepatic damage in rats.

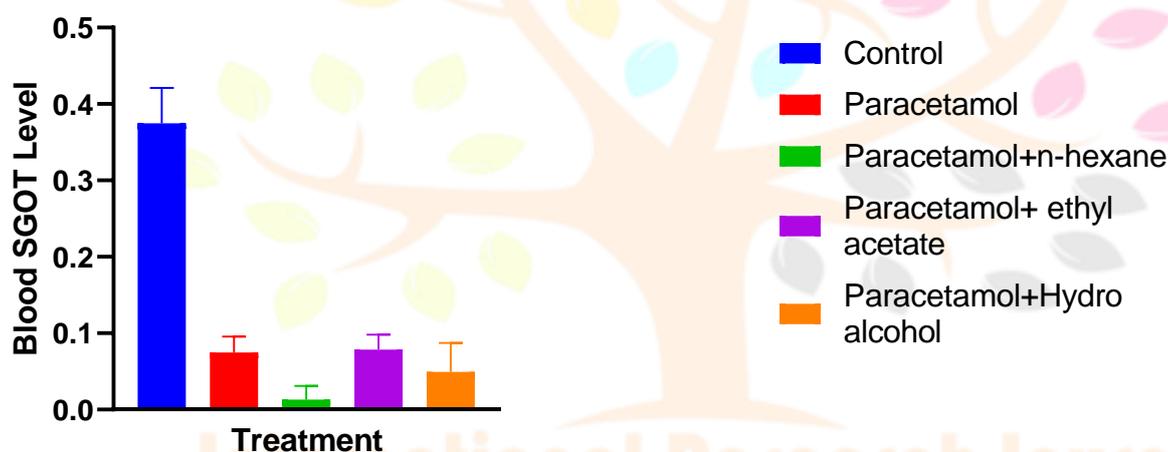


Fig 5-SGOT Graph

Data expressed as mean SEM of ethyl acetate showed significant effect with the P value of <0.0002 when compared with the control group (One-way ANOVA followed by post hoc Bonferroni multiple comparison test)

CONCLUSION:

In current study attempts have been made of explore hither to unreported scientifically hepatoprotective activity of *Leonotis nepetifolia*. After authentication to plant was collected and successive extraction was carried out using solvents like n-hexane, ethyl acetate, hydro alcohol extract in increasing order of polarity of solvent using Soxhlet apparatus. The Preliminary phytochemical investigation shows the presence of alkaloids, flavonoids, glycosides in the extracts. TLC was also performed on all extract and Rf values were also noted for component of each extract. Beside this antimicrobial activity of all extract was evaluated on various gram +ve & gram -ve bacterias (*S. aureus*, *E. Coli*, *B. Subtillis*, *K. pneumoniae*) by disc diffusion method. In this study Ethyl acetate showed significant anti-microbial activity than all the extract. The hepatoprotective activity of *Leonotis nepetifolia* was carried out with the hepatotoxic inducer paracetamol to check the activity of different isolated extracts. Each extract was subjected to hepatoprotective activity results indicate the significant hepatoprotective activity in ethyl acetate extract when compared with other extracts.

The present study also describes the significance of *Leonotis nepetifolia* as an important medicinal plant exhibiting diverse biological activities. Since the number of phytochemical constituents identified from the *Leonotis nepetifolia* is limited it would be a rich opportunity to isolate more bioactive chemical constituents.

For this plant, furthermore, it would also be valuable to determine the structure-activity relationship of the identified compounds.

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