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Hepatoprotective Activity on *Vitex negundo* Linn. (Verbenaceae) by using Wistar Albino Rats in Ibuprofen Induced Model

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Abstract: The present study was intended to evaluate the hepatoprotective activity on ethanolic extract of leaves of *Vitex negundo* was determined by using Wistar Albino rats in male sex. The *V. negundo* Linn. is a natural plant product, in it's the leaves are used with the added advantage to revert Ibuprofen induced hepatotoxicity. Oral administration of ethanol extract of *V. negundo* (100 and 300 mg) produced a significant and dose dependent inhibition to the acute hepatotoxic induced rats and various parameters were analyzed, when compared with negative control *V. negundo* showed that the significant activity in 300 mg/kg/b.wt. They exhibited a significant inhibition of hepatic toxicity by using various marker enzymes and the histopathological analysis. The inhibitory effect of the *V. negundo* on hepatotoxicity was compared to that of positive control group. The various parameters such glucose, protein, triglycerides, bilirubin, urea, creatinine, ALP, ACP, SGPT, SGOT and histopathological parameters was measured by dissection the rats. A significant index and values were observed in the acute assays; an effective significant alteration in all biochemical and histopathological sections was observed. From these results, concluded that the *V. negundo* having the potential effectiveness at the dose of 300 mg/kg/b.wt., ($p<0.01$) significance in a dose dependent manner. These results suggest that leaves of *V. negundo* having the hepatoprotective activity, which support the hepatic cells protection.

Key words: *Vitex negundo*, hepatotoxicity, ibuprofen, Wistar rats

INTRODUCTION

Herbal remedies are a type of alternative medicine that originates from plants and plant extracts. Used to heal illnesses and disease and to address psychological concerns, herbal remedies have been around for centuries and were the precursor to modern medicine. Herbal remedies are obtained from a wide variety of natural resources including plant leaves, bark, berries, flowers and roots. Herbal medicine remains a popular alternative throughout China and the Far East and is growing in popularity throughout the United States. Botanicals have been used traditionally by herbalists and indigenous healers worldwide for the prevention and treatment of liver disease. Clinical research in this century has confirmed the efficacy of several plants in the treatment of liver disease. Basic scientific research has uncovered the mechanisms by which some plants afford their therapeutic effects. In recent years many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat diseases of the liver. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and modes of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies. Ibuprofen appears to be having the lowest incidence of gastrointestinal Adverse Drug Reactions (ADR's) of all the non-selective-NSAIDS. However this only holds true at lower doses of Ibuprofen, so, over the Counters (OTCs) preparations of Ibuprofen are generally labeled to advice a maximum daily dose of 1200 mg, which have to be taken in more than 3 doses. Ibuprofen is believed to work through inhibition of cyclo-oxygenase (COX), thus inhibiting prostaglandins synthesis. There are at least 2 variations of cyclo-oxygenase (COX-1 and COX-2), Ibuprofen inhibits both COX-1 and COX-2. It appears that its analgesic, antipyretic, achieved principally though COX-2 inhibition; whereas COX-2 inhibitions is responsible for its unwanted effects on platelet aggregation and the GI mucosa. The plant *Vitex negundo* was found to be an herb with medicinal values bizarre. Although, it has seen in the tropical hot region, it was used as female gonadal hormone remedy all over the world. The plant was highly focused for its essential oil composition. In Indian folklore medicinal practice (viz., Ayurveda) it was used as liver tonic to overcome some liver problem associated with liver metabolism and

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believed to have hepatoprotective capacity to extend. Any how there is no valid studies were carried to substantiate the actual medicinal worth. The *V. negundo* literally and in Tamil Nadu called as *Notchi* leaves; it's widely used in various pharmacological activity. *V. negundo* (VN) Linn., a large aromatic shrub with typical five foliolate leave pattern, is found throughout the greater part of India at warmer zones and ascending to an altitude of 1500 m in outer, Western Himalayas. It has been claimed to possess many medicinal properties. So, from the available data's hypothesized that the plant *V. negundo* has the hepatoprotective capacity, which in turn it ameliorates the hepatic damage made by the ibuprofen, an NSAID. The plant *V. negundo* has showed that the analgesic activity (Srivastava and Sisodia, 1970), anti-inflammatory (Dharmasiri *et al*., 2003) on methanolic extract, anti-bacterial activity (Perumal Samy *et al*., 1998; Zaidan *et al*., 2005), anti-oxidant activity (Munasinghe *et al*., 2001), anti-fungal activity (Sathiamoorthy *et al*., 2007), cardioprotectant (Ono *et al*., 2004), its clean up the heavy metals Fe, A1, Zn, Pb, Ni, Cr and As (Liu *et al*., 2005), anti-convulsant (Tandon and Gupta, 2005), anti-hyperglycemic activity (Villasenor and Lamadrid, 2006).

MATERIALS AND METHODS

Plates of *Vitex negundo* Linn. (Notchi) is shown in Fig. 1a and b.

Plant anatomy

Kingdom	-	*Plantae*	-	Plants
Sub Kingdom	-	*Tracheobionta*	-	Vascular plants
Super division	-	*Spermatophyta*	-	Seed plant
Division	-	*Magnoliophyta*	-	Flowering plants
Class	-	*Magnoliopsida*	-	Dicotyledons
Subclass	-	*Asteridea*		
Order	-	*Lamilales*		
Family	-	*Verbenaceae*		
Genus	-	*Vitex* Linn.		
Species	-	*Vitex negundo* Linn. (Chaste tree)		

Plant collection: The leaves of *Vitex negundo* Linn. which predominantly is a habitat in arid places were collected from Uthamaseeli, Near Kallanai Dam, Tiruchirappalli district, Tamil Nadu, India. during the month of December-January.

Authentication: The freshly collected plant was then authenticated by the botanist from PARC research centre of India, Chennai, Tamil Nadu, India and a voucher specimen of the plant were submitted in our laboratory (Voucher No. PARC/SC/08-09/Tech.854).

Fig. 1: Plates of *Vitex negundo* Linn., (a) stem with leaves and (b) flowers with buds

Garbling process: Garbling refers to the separation of that portion of the plant to be used from other parts of the plant, dirt and other extraneous matter; this step often during the collection process. Although, there are machines that perform garbling, usually garbling was performed by hand. After removing all such unwanted adhered materials; the collected materials were then spread over trays and dried under shade, with regular sifting of collected plant materials everyday to avoid growth of fungus. Such shade dried bark/leaves/aerial portions of plant were ground in grinder to powder and then subjected for extraction (Pulok Mukherjee, 2002).

Soxhlet extraction: The powdered bark of known quantity was taken in a soxhlet apparatus and extracted with absolute ethanol. The material was extracted continuously for 72 h. The crude ethanol extract was then concentrated

by distilling off the solvent under reduced pressure/vacuum and subjected for further studies (Pulok Mukherjee, 2002).

Animals selection and procurement: Healthy young adult animals of commonly used laboratory strains should be employed. Females (180-200 g) should be nulliparous and non-pregnant. At the commencement of its dosing, each animal should be between 8 and 12 weeks old and its weight should fall in an interval within ±20% of the mean initial weight of any previously dosed animals.

The temperature in the experimental animal room should be 22°C (±3°C). Although, the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 h light and 12 h dark. The animals are housed individually, for feeding, conventional rodent laboratory diets may be used with an unlimited supply of drinking water.

Wistar rats were obtained from the approved breeder of the animal house of Laboratory animal medicine, Veterinary University, Madhavaram, Chennai and King Institute, Guindy, Chennai, Tamil Nadu, India. with the proof of the CPSCEA acknowledgment.

Animal grouping: The acclimatized animals were grouped in to 4 groups, each having four animals a group.

Group I: Normal control animals
Group II: Toxic control/Ibuprofen Induced control (200 mg/kg/b.wt.)
Group III:EEVN (100 mg/kg/b.wt.)
Group IV:EEVN (300 mg/kg/b.wt.)

Phytochemical screening: The methods of Harborne (1984), Trease and Evans (1983), Ikhiri *et al.* (1992) and Dahou *et al.* (2003) were used to screen the chemical constituents of EEVN. The presence of alkaloid (Dragendroff reagent and Mayer's reagent), flavonoids (Shinoda test), steroids (Liberman Burchard test) and terpenes (Vanillin-sulfuric acid reagent) were assessed.

Pharmacological activity: *In vivo* anti-hepatoprotective activity: The male Wistar albino rat animals were kept at polypropylene cage with humidity and further the animals separated in to 4 groups. In that particular the first group was served as Negative control as without any induction acclimatized with room temperature and given normal animal feed with solvent sodium chloride AR (1%). The second group was served as the positive control with induced Ibuprofen (200 mg kg^{-1}) with CMC solvent at once. Third and fourth group were served as the Ibuprofen with EEVN (100 and 300 mg/kg/b.wt.) respectively treated up to one month. Finally the animals were sacrificed and dissected the organs such liver for histopathological analysis. The blood was analyzed with various marker enzymes present in hepatic cells such protein, triglycerides, bilirubin, urea, creatinine, ALP, ACP, SGPT, SGOT.

RESULTS AND DISCUSSION

Vitex negundo Linn. (Fig. 1a, b) (Notchi) is a short plant of meditarranian region and used as herbal remedy of folklore usage. An ethanolic extract of *V. negundo* leaves were prepared in a way which remained more close to traditional extract preparation method. Experiments were carried to study biologically measurable proof for the hepatoprotectivity of EEVN in Wister albino rats. An experimental liver damage was induced with Ibuprofen at the single dose 200 mg kg^{-1} of body weight. The preliminary phytochemical (Table 1) study also showed that the presence various medicobiological chemicals such alkaloids, flavonoids, glycosides, reducing sugars, starch, tannins, terpenoids. Blood samples and liver were collected and evaluated biochemically for glucose, protein, triglycerides, billirubin, urea, creatinine, ACP, ALT, SGPT and SGOT further the liver was histopathologically studied for hepatocyte protection level. The administration of ibuprofen to the rats resulted in a marked increase in total bilirubin, serum amino transaminases (AST and ALT). Ibuprofen induced group was reduced the glucose level in a single dose of 200 mg/kg/b.wt. and further the EEVN treated group showed that recover the glucose level to normal condition (Table 2). However, the serum total protein level was decreased. The toxic effect of ibuprofen was controlled in the animals treated with the EEVN by way of restoration

Table 1: Phytochemicals screening of EEVN

Chemicals/plant name	EEVN
Alkaloids	+
Amino Acids	-
Anthraquinones	-
Flavonoids	+
Glycosides	+
Proteins	-
Reducing sugars	+
Saponins	-
Starch	+
Steroids	-
Tannins	+
Terpenoids	+
Gums	-
Resin	-
Mucilages	-
Volatile Oil	-

+: Presence, -: Absence

Table 2: Biochemical parameters on Ibuprofen induced hepatotoxicity studies on EEVN

Groups	Protein (g dLG^{1})	Glucose	Triglycerides	Bilirubin	Urea
		----------(mg dLG^{1})----------			
Negative control	3.40±0.18	84.91±1.63	51.18±1.6	2.03±0.09	16.94±2.02
Ibuprofen (200 mg kgG^{1})	1.76±0.16^{c}	105.35±12.11^{c}	70.8±1.09^{b}	4.38±0.14^{c}	17.31±1.84^{c}
EEVN (100 mg kgG^{1})	3.63±0.16^{b}	107.68±3.73^{b}	48.6±0.21^{c}	1.72±0.9^{b}	18.31±1.42^{b}
EEVN (300 mg kgG^{1})	2.41±0.21^{a}	99.56±8.04^{a}	53.41±1.6^{a}	2.2±0.08^{a}	18.01±1.45^{a}

Values are Mean±SD of the six numbers of animals. Rats were used for study with EEVN 100 and 300 mg/kg/i.p. of drug administration with Ibuprofen. $^{a}p<0.001$, $^{b}p<0.01$, $^{c,d,e}p<0.05$ vs. control group with one way analysis, i.e., DMRT

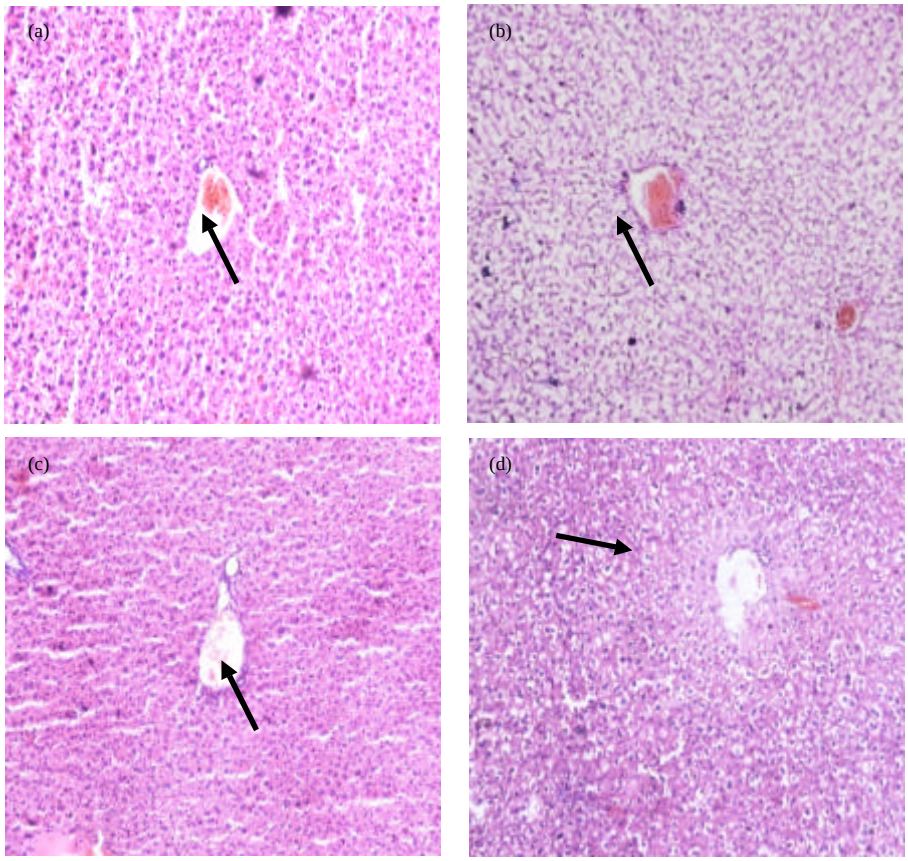

Fig. 2: Histopathological Sections of Liver hepatotoxicity studies by treating upon EEVN, (a) Liver section of control rat showing a normal hepatic architecture well brought out from the central vein. No inflammation and infiltration was found without any vesicular damage. (Hematoxycylin and Eosin 20X), (b) liver section of Ibuprofen (200 mg/kg/b.wt) rat showing abnormal hepatic architecture well brought out central vein with cells; the hepatocytes had prominent vesicular nuclei with damage and granular cytoplasm; while the sinusoids were dilated. (Hematoxycylin and Eosin 20X), (c) liver section of rat treated with EEVN (100 mg kgG^{1}) showing mild lymphatic infiltration besides the central vein and some cells show dead nuclei and dark stained hepatocytic nuclei indicating hepatocytic nuclear death or cell pycnosis and congestion of the central vein. (Hematoxycylin and Eosin 20X) and (d) liver section of rat treated with EEVN (300 mg kgG^{1}), showing hepatic vesciles which was diffusely distributed throughout the liver lobule congestion of the central vein without any inflammation. (Hematoxycylin and Eosin 20X)

of the levels of the liver function biochemistry (Table 2). Among the treated groups, significant hepatoprotective activity was observed in those EEVN treated in higher doses, i.e., (300 mg kgG^{1}) (Table 3). Histological profile of the control animals showed normal hepatocytes (Fig. 2a), group II that is Ibuprofen induced animals exhibited intense centrilobular necrosis (N), vacuolization (F) and macrovesicular fatty change (Fig. 2b). The animals treated with ethanol extract, i.e., EEVN exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty change and infiltration (Fig. 2c, d).

Table 3: Biochemical parameters on Ibuprofen induced hepatotoxicity studies on EEVN

Groups	Creatinine (mg dL^{-1})	ALP	ACP	SGPT	SGOT
		----------	($IU L^{-1}$)	----------	
Negative control	0.59±0.08	3.19±0.18	1.23±0.06	0.37±0.17	0.27±0.03
Ibuprofen (200 mg kg^{-1})	0.88±0.12^{b}	3.27±0.29^{a}	1.86±0.25^{c}	0.62±0.30^{e}	0.34±0.04^{b,c}
EEVN (100 mg kg^{-1})	0.95±0.07^{c}	4.62±0.65^{c}	1.64±0.30^{b,c}	0.58±0.03^{d}	0.38±0.02^{d}
EEVN (300 mg kg^{-1})	0.93±0.03^{a}	4.26±0.41^{b}	1.64±0.30^{b}	0.44±0.04^{b,c}	0.31±0.02^{a,b}

Values are Mean±SD of the six numbers of animals. Rats were used for the study treated with EEVN 100 and 300 mg/kg/i.p. of drug administration with Ibuprofen. $^{a}p<0.001$, $^{b}p<0.01$, $^{c,d,e}p<0.05$ vs. control group with one way analysis, i.e., DMRT

The Ibuprofen has been used as a tool to induce hepatotoxic in experimental animals. This toxic chemical caused peroxidative degradation in the adipose tissue resulting in fatty change and infiltration of the hepatocytes. The increase in levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphatase was the clear indication of cellular leakage and loss of functional integrity of the cell membrane (Sarawat *et al*., 1993) Administration of leaves of EEVN showed significant hepatoprotective activity; while qualitative phytochemical investigations on the EEVN also showed positive for flavonoids by ferric chloride, alkaline reagent and Shinoda tests. Further, it has been reported that the flavonoid constituents of the plant possess antioxidant properties (Hesham *et al*., 2002) and was found to be useful in the treatment of liver damage (Maurya *et al*., 2004). The administration of hepatoprotective drugs may induce the hepatocytes to resist the toxic effect of Ibuprofen. The results indicated that the EEVN has significant hepatoprotective activity. This may be probably due to the higher content of flavonoids. The earlier investigators (Janbaz *et al*., 2002) have screened the hepatopro*tective activity of the flavonoid compound, rutin, isolated from *Artemisia scoparia*, which is also claimed to have free radical scavenging and anti-lipid peroxidant activities against CCl_4-induced hepatic toxicity. The isolation and characterization of the flavonoids from the leaves of EEVN and screening of the pharmacological action against the liver damage is being in an investigation.

CONCLUSIONS

From the available data's and the experimental results suggest the plant *Vitex negundo* Linn., as a hepatoprotectant. However the mechanism of action and the active component which is responsible for the actual hepatoprotectivity is not well known. The present study was suggested that the EEVN could have a preventive activity towards ibuprofen induced hepatotoxicity in albino rats. From the above study we can conclude that the plant have medicobiological properties in a dose dependent manner. However, it is necessary to continue the investigations in order to establish the active component responsible for hepatoprotectivity.

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