Naloxone at the dose of 1 mg/kg showed no significant increase in the tail-flick latency; however, it showed significant hyperalgesia at intervals of 15, 30 and 60 min with peak effect at 60 min (Table 1). Combined treatment consisting of NLE (50 mg/ kg) and naloxone (1 mg/ kg) produced no significant change in the tail-flick latency at all the time intervals except hyperalgesia seen at 60 min (Table 1). Hence naloxone pretreatment antagonized the antinociceptive effect of NLE suggesting the involvement of endogenous opioid peptides or opioid receptors in the mediation of the antinociceptive response of NLE.

The results of the present study revealed the antinociceptive effect of NLE in the pain model of the tail-flick test due to thermal stimulation. The present findings confirm those of previous investigators, who reported dose-dependent analgesic activity of NLE in mice and rats using various models of analgesia.[4,5] Neem leaves have been reported to relieve pain by opioidergic as well as other mechanisms.[4]

These results could thus have a potential clinical implication. Thus patients can benefit from relief of pain, using either morphine or NLE alone or their combination with lesser adverse effects.

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Effect of *Vitex negundo* on oxidative stress

Sir,

In vitro, the antiradical potential of the freeze-dried root extract of *Vitex negundo* (VN) was investigated by Munasinghe et al (2001)[1] by determining their abilities to scavenge DPPH(1,1-diphenyl-2-picrylhrazyl) free radical and to inhibit hydroxyl radical-mediated damage to deoxyribose. They however did not study the *in vivo* effects of VN on lipid peroxidation or endogenous antioxidant enzymes like superoxide dismutase (SOD). TThe leaves of VN are known to possess various antioxidant chemical constituents like flavonoids,[2] vitamin C and carotene[3] which may have a modulatory effect on oxidative stress or endogenous antioxidants. Moreover, no one has as yet investigated the *in vivo* effects of VN leaf extract on oxidative stress. Therefore, the present study was undertaken to investigate the effect of VN leaf extract on oxidative stress in albino rats.

The plant was collected from the local area of Sevagram (MS), and was authenticated by an expert. The fresh leaves of VN were shade-dried and powdered. The powder was macerated for 24 h in 70% v/v ethanol. Then, it was subjected to percolation using 70% v/v ethanol as solvent. The menstrum collected was again shade-dried. The final viscous yield (9.5%), was then suspended in 1% gum acacia and dissolved in distilled water to prepare suspension in desired concentrations just before use.

Albino rats (125-180 g) of either sex (Wistar strain) were used. Animals were housed and fed as per standard guidelines of CPCSEA. The animals were given a weeks time to get acclimatized to laboratory conditions and were fasted overnight before the experiment. The project was approved by the institutional ethics committee.

The animals were divided into four groups, each consisting of six rats. Group I served as control and received distilled water. The VN leaf extract was fed orally for 14 days in a volume of 10 ml/kg body weight in doses of 100 mg/kg/day (Group II), 250 mg/kg/day (Group III) and 500 mg/kg/day (Group IV) for assessment of the oxidative modulatory effect of VN. Dose selection was based on preliminary trials in our laboratory and estimated oral LD_{50} dose of VN leaf extract (7.58 g/kg, b.w.) in one of our previous reported study.[4]

For studying the effect of VN on ethanol-induced oxidative stress,[5,6] another set of animals were divided into five groups consisting of six rats each. Group I served as a disease control in which 1 ml/kg/day of 20%v/v ethanol was instilled intra-gastrically for 14 days. Groups II, III and IV were given the doses of VN extract orally for 14 days as mentioned earlier. After 30 minutes of this pre-treatment, each group received 1 ml/kg/day of 20%v/v ethanol orally for 14 days. Group V served as healthy control.

After completion of 14-day treatment, 3-5 ml of blood was collected from the inner canthus of the eye from each animal under light ether anesthesia using capillary tube, in a vial containing EDTA as anti-coagulant. Plasma was separated by centrifugation at 3000 rpm for 10 minutes. It was stored at –20° C and used to estimate malondialdehyde levels. Buffy coat was removed; remaining erythrocytes were washed three times in cold 0.9% saline and hemolysed by adding the same volume of ice-cold water to yield a 50% hemolysate. The hemolysate was stored in 500 μl aliquots at –20° C. It was used to estimate the activity of the enzyme SOD.

The estimation of malondialdehyde (MDA)[7] was carried out by the TBARS method. Thiobarbituric acid (TBA) reacts with MDA, one of the aldehyde products of lipid peroxidation, to give a colored product which was extracted in butanol and absorbance measured spectrophotometrically at 530 nm. MDA (Sigma chemicals) was dissolved in 0.05 M sulphuric acid to prepare 10 μM stock solution. By diluting the stock solution, different concentrations (1 to 5 nm/ml) of MDA were obtained for preparing a standard curve. To 0.5 ml each of plasma and MDA standards, 2.5 ml of 20% TCA was added and centrifuged at 3000 rpm for 10 minutes. The supernatant was de-

canted and the precipitate was washed once with 0.05M sulphuric acid and 3 ml of 0.2 g/dl TBA reagent (Loba chemie, India) was added to the precipitate. The mixture was heated in a boiling water bath for 30 minutes. After cooling, the resultant chromogen was extracted with 4 ml of n-butyl alcohol and the separation of the organic phase was done by centrifugation at 3000 rpm for 10 min. Absorbance of the butyl alcohol extract of samples was measured at 530 nm against distilled water as blank. The standard curve was plotted and the concentration of the sample was calculated and expressed as nmol/ml malondialdehyde. An elevated level of MDA was taken as the index of oxidative stress.

The estimation of superoxide dismutase (SOD) activity in erythrocytes was carried out by the method of Marklund and Marklund (1974)[8] utilizing the inhibition of auto-oxidation of pyrogallol by SOD enzyme. The final assay mixture contained 3 ml of triscacodylate buffer (50 mM, pH 8.2) containing air equilibrated 0.2 mM pyrogallol [E merck, India], 1 mM EDTA, 1 mM DTPA(diethylene triamine penta acetic acid) and 100 ml of enzyme source. The amount of hemoglobin in the hemolysate was estimated by Drabkin's method.[9] Hemoglobin was then precipitated and SOD was extracted in Tsuchihasi extract containing chloroform and ethanol (1.5:1). This was used to estimate the enzyme activity. Three sets of reaction mixture were prepared. *Standard*: Known amounts of SOD (Sigma St. Louis, USA) from pure bovine source, in different concentrations were added to the assay mixture to achieve 30% to 50% inhibition of pyrogallol auto-oxidation. *Test:* The clear supernatant from Tsuchihasi extract was added instead of standard SOD. *Control*: Neither test sample, nor standard was added to assay mixture. The crude extract was replaced by 50 mM of phosphate buffer (0.1 Mm, pH 5.6) to obtain uninhibited auto-oxidation of pyrogallol.

In all sets, the reaction was started by the addition of pyrogallol (0.2 mM) and the change in optical density was recorded for 4 min at 420 nm. The initial 10 sec were considered as the induction period of the enzyme. Change of absorbance/minute ("A) was calculated from the reading. The %inhibition of the test system was calculated from the standard graph. The SOD activity was expressed in units/g hemoglobin. One unit of SOD activity being defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation. The raised levels of SOD, an endogenous antioxidant was taken as an indicator of the antioxidant property of the test drug.

The data were analyzed using one-way (ANOVA) followed by Dunnett's test. P <0.05 was considered significant. The data are represented as mean ± SEM.

The results are shown in Tables 1 and 2.

Vitex negundo in the present study produced significant ($P<0.05$) reduction in MDA levels after 14-day treatment in only the higher dose (500 mg/kg/po). Although a marginal rise of SOD in this dose was observed it was not significant. Usually, induction or activation of enzymes leads to a several-fold rise in enzymatic activity. From this marginal rise, it is difficult to infer whether the enzyme activity is really altered. Moreover, serum MDA levels in control rats seem slightly towards the higher side, which may probably be due to the fact that blood was collected under light ether anesthesia. Therefore, it is difficult to comment that continuous VN therapy can reduce oxidative stress. Whereas, in the ethanol-induced oxidative stress model, it significantly ($P<0.05$ and $P<0.01$) reduced only MDA levels in both moderate and higher doses and the effects on SOD were non-significant. Moreover, in the lower doses it failed to show any modulatory effect on lipid peroxidation or SOD enzyme activity.

Ethanol-induced lipid peroxidation and reduction of SOD activities are well-known facts.[5] Although a report contrary to this is also available showing a significant increase in SOD activity.[6] In our study however, ethanol treatment has produced greater alteration in lipid peroxidation in comparison to SOD activity. The findings of the present study suggest that VN leaf extract can produce reduction of oxidative stress by reducing lipid peroxidation whereas it has failed to modulate endogenous antioxidant enzyme (SOD) activity. However, these findings are contrary to the findings of Munasinghe *et al* (2001).[1] Their study did not show anti-radical activity of VN. On the contrary they observed pro-oxidant role of VN. This discrepancy might be due to a different experimental set-up in the form of different methods used, different part of the plant used and different method of extraction used in their study. The leaves of VN are known to possess various antioxidant chemical constituents like flavonoids,[2] vitamin C and

Table 1

Oxidative modulation by *Vitex negundo* therapy

Drug	*Dose (mg,ml/kg p.o./daily X 14 days)*	*MDA levels n mole/ml*	*SOD levels U/g Hb*
DW (control)	10	3.1±0.6	728.0±54.3
VNE	100	3.0±0.2	762.0±59.0
VNE	250	3.0±0.9	774.0±48.0
VNE	500	2.2±0.3*	798.5±53.3
One-way ANOVA	F	52.5	2.01
	df	3, 20	3, 20
	P	< 0.001	NS

DW= distilled water, VNE = *Vitex negundo* extract, n=6 in each group, values are mean±SEM. *$P<0.05$ when compared to control (Dunnett's test).

Table 2

Effect of *Vitex negundo* on ethanol-induced oxidative stress

Drug	*Dose (mg,ml/kg p.o./daily X 14 days)*	*MDA levels n mole/ml*	*SOD levels U/g Hb*
Ethanol (disease control)	1	6.5±0.49	680.6±56.6
VNE+Ethanol	100+1	6.1±0.23	708.2±43.7
VNE+Ethanol	250+1	4.3±0.07*	718.0±39.0
VNE+Ethanol	500+1	3.3±0.20**	728.6±53.3
DW (normal healthy control)	10	3.1±0.18***	748.8±37.0*
One-way ANOVA	F	146.2	2.96
	df	4, 25	4, 25
	P	< 0.001	< 0.05

VNE = *Vitex negundo* extract; DW= Distilled water; n=6 in each group, values are mean±SEM. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ when compared to disease control (Dunnett's test).

carotene,[3] which might possibly be responsible for the reduction of lipid peroxidation produced by it in the present study. As there are several limitations in the measurement of MDA by the TBA method, GSH, GPx, GR, GST and catalase along with MDA and SOD are needed to be evaluated in future studies to prove the effect of *Vitex negundo* on oxidative stress. The limitation of the present study was that no positive control was taken. This is the first report which has indicated that VN can produce reduction of oxidative stress mainly by reducing lipid peroxidation, which needs to be substantiated by a detailed study.

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Self-medication among urban population of Jammu city

Sir,

Self-medication can be defined as obtaining and consuming drugs without the advice of a physician either for diagnosis, prescription or surveillance of treatment.[1] There is a lot of public and professional concern about the irrational use of drugs. In developing countries like India, easy availability of a wide range of drugs coupled with inadequate health services result in increased proportions of drugs used as self medication compared to prescribed drugs.[2] Although, OTC (over the counter) drugs are meant for self medication and are of proved efficacy and safety, their improper use due to lack of knowledge of their side effects and interactions could have serious implications, especially in extremes of ages (children and old age) and special physiological conditions like pregnancy and lactation.[3,4] There is always a risk of interaction between active ingredients of hidden preparations of OTC drugs and prescription medicines, as well as increased risk of worsening of existing disease pathology. As very few studies have been published regarding self medication pattern in our community, therefore, we conducted this cross-sectional study in 600 urban families living in Jammu city to evaluate their self medication practices.

Nuclear families were selected randomly by stratified sampling and Jammu city was arbitrarily divided in 10 sections and 60 families were randomly selected from each section. The families under study were further classified into two classes; class-A with head of the family having qualification more than 12th standard and class-B with qualification less than 12th standard. A family having another member with qualification more than 12th standard was also included in the class-A even if the head was not qualified up to the 12th standard. 380 families were included in class-A and 220 families in class-B. A simple questionnaire was prepared and each family was interviewed only once in the local language. At least two members of the family (including head of the family and excluding children below 12 years and mentally sick persons) were interviewed together after obtaining their consent. The questionnaire was filled by a qualified assistant. The questionnaire contained the questions pertaining to identifications data (name of head of the family, number (no.) of children, no. of adults, address, qualifications, employment, income), practice of self medication by the family, commonly used drugs as self medication, knowledge of the family regarding dose, duration, side effects and interactions of the drugs in use, source of information about the drug, attitude towards allopathic, ayurvedic and homeopathic medicines and a recall period of one year was kept. In the end of the study all the data was collected and analysed (Table 1 and Table 2). All the parameters were compared between class-A and class-B using Chi-square test and P values <0.05 were considered statistically significant.

A trend towards self medication in adults was seen both in class-A and class-B. However, use of self medication was significantly more in class-B than class-A (Table 2). Previous studies have shown the prevalence of self-medication as 37% in urban population and 17% in rural population in India;[5] where as 12.7% to 95% in other developing counteries.[2,6,7] In class-A NSAIDS (non-steroidal antiinflammatory drugs) were widely administered as self medication followed by ORS (oral rehydration solution) /antimotility drugs, cough/cold remedies and antibiotics; whereas, cough and cold remedies were widely used drugs in class-B followed by NSAIDS, vitamins, ayurvedic drugs, H_2 blockers/proton pump inhibitors/antacids and ORS/antimotility drugs.

Present study also indicated low knowledge about dose/