Pharmacokinetic-interaction of *Vitex negundo* Linn. & paracetamol

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Background & objectives: Currently, herbal preparations are clinically used as functional food, food supplements or as add on therapy, which affects the bioavailability and also the net therapeutic potential of co-administered allopathic drugs. Therefore, it is important to assess the interaction among these two classes of drugs. Here we studied the interaction between orally-administered ethanolic extract of leaves of *Vitex negundo* Linn. (*Verbenaceae*) (VN extract) and paracetamol in albino rats.

Method: Solvent free dried extract of VN leaves was orally given to experimental rats in different doses (62.5-1000 mg/kg/b.wt.), daily for six consecutive days. On days 3 and 6, paracetamol (100 mg/kg/b.wt.) was orally administered to these extract treated rats and control rats (drug vector). At various time intervals (5 min - 120 min), blood was collected from each animal and paracetamol concentration was determined in plasma by using HPLC with UV detector at 249 nm. Various pharmacokinetic parameters were calculated by non compartmental model.

Results: A significant decline in plasma concentration of paracetamol with time-gap was recorded with the increasing dose of VN extract, without affecting its T_{max} (maximum time to achieve peak plasma concentration). There was a significant decrease in the extent of absorption and decline in intensity of therapeutic response (as evidenced by reduced AUC value and decline in C_{max}). Further, compared to control, the relative bioavailability of paracetamol, in presence of VN extract, decreased significantly.

Interpretation & conclusions: VN extract or its ayurvedic formulation if co-administered with allopathic drug like paracetamol, the dose of allopathic drug needs to be adjusted in order to achieve desired therapeutic response of paracetamol.

Key words Bioavailability - herb-drug interaction - paracetamol - pharmacokinetics - *Vitex negundo*

Herbal preparations are in wide use as food supplement or as "add on therapy" for several chronic diseases. Most of the time, the herbal medicines are sold as non prescription medicine and the patients use it along with the prescribed conventional medicine at their own risk[1]. In this situation, herb-drug interaction is an important factor to be investigated because there is always a chance to get undesirable therapeutic effect of the prescribed allopathic medicine[2,3].

Paracetamol is widely used as analgesic and antipyretic agent in treatment of pain and fever. Its absorption is affected by surface area of orally co-administered drug[4], general gastrointestinal (GI) conditions such as inflammation and *p*H [5]. Paracetamol

is metabolized primarily in the liver (60-90 %), where it is converted to inactive compound by conjugation with sulphate and glucuronide for further excretion[6]. However, a small proportion (5-10 %) is metabolized via the hepatic cytochrome P450[7].

Vitex negundo Linn. (*Verbenaceae*), commonly known as Nirgundi, is already in clinical use in several traditional systems of medicine including Ayurveda, Unani and Siddha for management of pain, headache, inflammation, leucoderma, enlargement of the spleen, rheumatoid arthritis, gonerrhoea, bronchitis, fever, cold and cough, lactagogue and emmenagogue as juice, decoction and also as vapor[8-10]. It contains fragrant, volatile oil and resins with several reported phytomolecules *e.g.* nishindaside, negundoside (irridoid glycoside), and artemetin[11, 12]. Besides, several alkaloids, glycosides, flavonoids, reducing sugars, sterols, resin and tannins have also been reported[13].

Recently we have reported its antioxidant potential[14], but as no interaction study between extract of leaves of *V. negundo* and paracetamol has been carried out so far, this study was undertaken. The standardized ethanolic extract of *Vitex negundo* was orally given along with paracetamol to albino rats and pharmacokinetic changes were investigated in terms of AUC (area under curve), C_{max} (maximum plasma concentration), T_{max} (time to reach maximum plasma concentration), K_{el} (elimination rate constant), $t_{1/2}$ (elimination half life), RB% (relative bioavailability) of paracetamol.

Material & Methods

Materials: Albino rats, Charles foster strain of both sexes (150-200 g) were purchased from the Central Animal Facility of Institute of Medical Sciences, Banaras Hindu University (BHU), Varanasi, India and given free access to normal standard chow diet and tap water. Paracetamol powder was gifted by Swastik Pharmaceutical, Varanasi. HPLC grade methanol and water for chromatography LiChrosolv® were obtained from Merck Co. (Mumbai, India). Other chemicals were of analytical grade and purchased from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India). Leaves of *V. negundo* were collected from the Ayurvedic garden of Institute of Medical Sciences and authenticity was rechecked by pharmacognostical parameters in the Department of Botany, Faculty of science, BHU. Its voucher specimen was preserved in the department (Specimen# YBT / MC / 06-1) of Medicinal Chemistry.

Experimental design: Ethanolic extract of *Vitex negundo* was prepared by direct extraction of dried leaves in a soxlet extractor (yield of extract = 15 g/100g dried leaves) and used for biological study. The experimental protocol was approved by the Institutional Animal Ethical Committee of Institute of Medical Sciences (BHU, India). The rats of both sexes were randomly divided into six (I – VI) groups of eight animals in each. The solvent free extract was dissolved in a drug vehicle (10% Tween-20 in water). The animals of control group (group I) received only the drug vehicle. In the remaining drug treated group, VN extract, dissolved in drug vehicle, was given in different doses *i.e.* 62.5, 125, 250, 500, 1000 mg/ kg body weight (BW) respectively, once daily for 6 consecutive days. In all groups (I – VI) paracetamol (100 mg /kg BW) was orally given on days 3 and 6, after 2 h of drug vehicle (group I) or VN extract (groups II – VI) administration. The blood was collected from supra orbital vein of eye[15] of each animal at various time points of 5, 15, 30, 40 ,60, 120 min into heparinized micro-centrifuge tube, and centrifuged for 15 min at 2000*g* to obtain plasma[16].

Analysis of paracetamol through HPLC: The HPLC system consisted of a LC-10-AT pump (Shimadzu, Kyoto, Japan), a SIL-10A auto-injector (Shimadzu, koyoto, Japan), ODS C-18 RP-18 Merck column (Intersile 250 x 4.6 mm I.D., 5 μm) and a SPD-10A UV-VIS detector (Shimadzu, Koyoto, Japan).The concentrations of paracetamol in plasma was determined by HPLC assay as per modified method of Peggy *et al*[16] and Prodan *et al*[17]. 100 μl of plasma was mixed with 100 μl of extraction buffer (1M potassium dihydrogen phosphate, 2M dipotassium hydrogen phosphate, *p*H = 7.4) and 200 μl of ethyl acetate, vortexed vigorously for 10 min and centrifuged at 1500 *g* at 4[0]C. The upper organic layer (100 μl) was separated out in a micro-centrifuge tube and evaporated to dryness at 75 [0]C in an oven. The dried residue was re-dissolved in 200 μl of mobile phase [mixture of methanol and water (80: 20 v/v)], centrifuged and 20 μl of supernatant was injected in HPLC column. It was eluted at the rate of 1ml/min and the detection was carried at 249 nm by UV detector at room temperature. The mobile phase was filtered through 0.45 μ Millipore membrane filter paper.

Pharmacokinetic analysis: Different pharmacokinetic parameters were calculated by non compartmental model[18]. Maximum drug concentration (C_{max}) and corresponding time (T_{max}) were measured directly from the paracetamol-concentration-time plot. Area under curve (AUC) was calculated by the

Trapezoidal method[18]. Elimination rate constant (K_{el}) was determined from the slope of the elimination part of the plasma paracetamol–concentration time plot and elimination half life ($t_{1/2}$) was obtained from the equation $t_{1/2} = 0.693/K_{el}$. The relative bioavailability (RB %) of paracetamol after oral administration was calculated as follows[19]

$$\text{Relative bioavailability (RB \%)} = \frac{\text{AUC pretreated}}{\text{AUC control}} \times 100$$

Statistical analysis: Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by post hoc test which includes Dennett's test, by using SPSS Software for Window 7.5 (Flipkart.com, Bangalore) statistical program (*P*< 0.05 was considered statistically significants).

Results & Discussion

Since there was no change in pharmacokinetic parameters of paracematol between control group (drug vehicle treated group) on day 3 and 6 and normal group (without drug vehicle treated group, data not shown), all comparisons were made with values, obtained from control group (Table). The concentration-time profiles of paracetamol (Fig) clearly indicated decline in plasma concentrations of paracetamol at different sampling time with the increase in dose of VN extract. It was interesting to note that the lowest dose of VN extract *i.e.* 62.5 mg/kg did not show any change in plasma concentration of paracetamol, but it has adopted the declining trend in paracetamol plasma concentrations on higher doses of VN extract in concentration dependent manner (Fig.). The value of AUC, which is indicative of extent of paracetamol absorption and clearance, declined significantly in VN-extract treated animals. Although this effect was more on 3rd day than on 6th day of treatment but the extent of absorption of paracetamol on 6th day is still significantly lower than the control animal. This could be due to lesser absorption of paracetamol on 3rd day as compared to 6th day from the intestinal mucosa in presence of VN extract, for which the exact mechanism is still to be explored. The reversal of changes in AUC/C_{max} on 3rd day verses 6th day could be attributed to adaptive nature of rat for longer duration of treatment. Further, T_{max}, which indicates the rate of absorption of paracetamol, remained unchanged, but C_{max} value, indicative of the intensity of therapeutic and toxic response, was found to be decreased significantly in VN extract treated groups, more on 3rd day than on 6th day. Thus, it could be suggested that without affecting the rate of absorption,

Table. Pharmacokinetic parameters of paracetamol after oral administration (100mg/kg/b.wt.) on 3rd and 6th day to rats pretreated with *V. negundo* (62.5 – 1000mg/ kg /b.wt for 6 days)

Parameters	control group Day 6th	Pre treated with VN extract (Mean ± SD)										
		62.5 mg/kg + PCM		125 mg/kg + PCM		250 mg/kg + PCM		500 mg/kg + PCM		1000 mg/kg + PCM	
		3rd	6th	3rd	6th	3rd	6th	3rd	6th	3rd	6th
$AUC_{0-\infty}$ (µg-h/ml)	5.95±1.56	5.93±1.13	5.94±1.12	3.35±0.218*	4.25±1.180	3.29±0.604*	3.45±1.29*	1.65±0.452**	3.14±1.87*	1.49±0.542**	3.05±0.166*
C_{max} (µg/ ml)	3.53±0.185	3.56±0.052	3.55±0.042	2.67±0.184*	3.26±0.306	2.32±0.469**	2.62±0.77*	1.26±0.235**	2.57±0.591*	0.64±0.096**	2.24±0.101*
$t_{1/2}$ (h)	0.99±0.301	0.94±0.265	0.93±0.343	0.69±0.079	0.65±0.263	0.76±0.140	0.71±0.133	0.915±0.335	0.77±0.352	1.52±0.522	0.80±0.046
K_{el} (per h)	0.75±0.264	0.78±0.246	0.71±0.233	1.01±0.115	1.06±0.316	0.93±0.177	0.98±0.141	0.83±0.325	0.90±0.534	0.51±0.212	0.86±0.054
RB %	100	99	99	56	71.4	55	58.8	27	52.7	25	51.2

Mean ± SD (N=8 in each group); *P**<0.05 **<0.01 compared to control; AUC , area under the plasma concentration-time curve from 0 to ∞ ; C_{max} , peak concentration; t ½ , elimination half life; K_{el}, elimination rate constant ; RB %, relative bioavailability expressed as percentage ; PCM, paracetamol (100 mg/kg); N, no. of animals
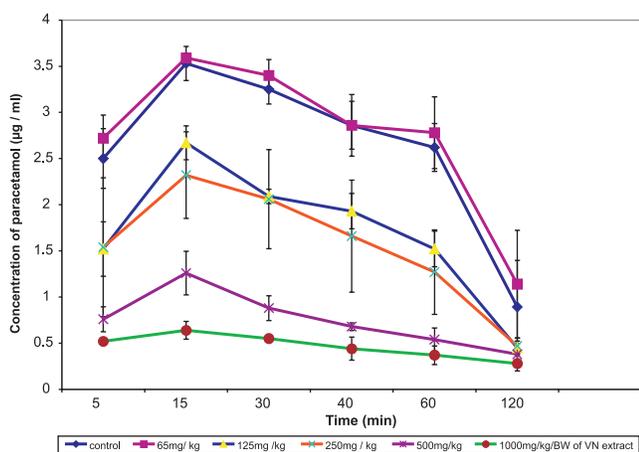
Fig. Effect of oral administration of different dose of *V. negundo* extract on the pharmacokinetics of paracetamol on 3rd day in rats: Bars on each point represent the standard deviation (Mean ± SD, N = 8 in each group). *P* < 0.05; *P*< 0.01 compared to control value.

VN extract has significantly affected the overall extent of absorption of paracetamol.

The elimination half life (t ½) and elimination rate constant (K_{el}) of paracetamol were almost unaffected in the VN extract treated groups. The difference in values of t ½ and Kel were insignificant. The relative bioavailability (RB) of paracetamol was declined to 25 per cent on 3rd day, and about 50 per cent on 6th day in higher doses of VN extract treatment as compared to control group. Thus it could be inferred that (*i*) the dose of drugs like paracetamol needs to be adjusted (increased) if administered with VN extract, (*ii*) the toxicity is expected not to increase with the enhancement in dose of paracetamol because C_{max} was significantly suppressed by VN extract, and (*iii*) since T_{max} was unchanged *i.e.*, the rate of paracetamol absorption and also the onset of paracetamol effect is expected to remain the same in either the presence or absence of VN extract. Though the above inference has been drawn on the basis of single dose (acute) study, more realistic picture of pharmacokinetic interaction may be visible if paracetamol is administered as repeated doses, which has been planned for future studies. However, it can be concluded that for obtaining desired therapeutic response without any significant side effect/toxicity it may be necessary to adjust the dose of paracetamol if it is being administered along with VN extract. If the active principle of an herbal drug is known, its pharmacokinetic changes can determined in the presence of allopathic drugs so that, if needed, the dose of herbal drug can be appropriately adjusted.

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