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PHARMACOKINETICS STUDY OF NUTRI-PEPPER ENHANCER IN SPRAGUE DAWLEY RATS

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ABSTRACT: *The medicinal properties of curcumin and leonurine obtained from Curcuma longa L. and Leonuris sibiricus has demonstrated low efficiency due to their poor bioavailability and its rapid metabolism in the liver and intestinal wall. In this study, the effect of combining piperine, a known inhibitor of hepatic and intestinal glucuronidation, was evaluated on the bioavailability of curcumin and leonurine in rats. When curcumin was given alone, in the dose 200 mg/kg to rats, moderate serum concentrations were achieved over a period of 4 h. Concomitant administration of piperine 10 mg/kg increased the serum concentration of curcumin for a short period of 1-2 h post drug. Time to maximum was significantly increased (P < 0.02) while elimination half life and clearance significantly decreased (P < 0.02). The leonurine serum concentration also showed similar dissipation pattern with solely administration of leonurine alone demonstrated low accumulation as compared to co-administration with piperine. Result obtained also showed that the co-administration of both curcumin and leonurine with piperine enable to increase the bioavailability of curcumin and leonurine for about 261.11% and 154.81% respectively. In conclusion, this study has proven that the co-administration of piperine enhances the serum concentration, extent of absorption and bioavailability of curcumin and leonurine in rats with no adverse effects.*
KEYWORDS: pharmacokinetics study, nutri-pepper, sprague , dawley, rats

INTRODUCTION

Kacangma (*Leonurus sibiricus* L.) and turmeric (*Curcuma longa* L.) are a popular traditional herb that has been consumed for decades by the people of Sarawak as a herbal medicines in culinary ingredient (Chai et al., 1989, Teo and Chua 2001, Dayang *et al*., 2016). The role of kacangma and turmeric as an underutilized herb with potential economic value has been recognized (MOA 1995, Paulus and Lau 2004). Subsequently efforts are made to increase the utilization by developing kacangma and turmeric herb into various special herbal products with commercial significant such as mechanical kacangma, turmeric and kacangma confectionery jelly (Chua 2005), turmeric supplement etc.

Both kacangma and turmeric contain various type of bioactive compounds with significant therapeutically effect on human health. Curcumin, a yellow pigment isolated from turmeric has been used for the treatment of a diversity of disease (Agarwal *et al*., 2011). This compound has

also been cited as the main phytochemical responsible for the turmeric beneficial effect range from anti-inflammation, wound healing, antitumor activities and anticoagulant. In term of kacangma, this species has been reported containing leonurine medicinal properties. It has been used for the treatment of menstrual irregularities, amenorrhea, malaria, hypertension, and myocardial ischemia (Schmidt *et al*., 2013). This species is also known to have antibacterial, anti-inflammatory, and antioxidant activity (Islam *et al*., 2005; Ahmed *et al*., 2006; Shin *et al*., 2009) and has demonstrated a reduction of intracellular reactive oxygen species (Lee *et al*., 2010).

Pharmacokinetic properties of these 2 compounds, leonurine and cucurmin indicated that following oral administration, it is poorly absorbed (Ammon and WahI, 1991) and only traces of the compound appear in the blood, while most of its is excreted in the faeces (Ravindranath and Chandrasekhara, 1980). The transformation of curcumin and leonurine into unidentified compounds during absorption (Ravindranath and Chandrasekhara, 1981) and its glucuronidation in the liver (Holder *et al*., 1978) are probably responsible for its low concentration in blood.

Black pepper (*Piper nigrum* L.) has been in use as spice from ancient times throughout the world. A major component of this piper species is the alkaloid piperine (1-piperollpiperidine), which has been reported to enhance the bioavailability of drugs by inhibition of glucuronidation in the liver (Atal *et al*., 1985) and small intestine (Sirigh *et al*., 1985). In view of the potential therapeutic utility of curcumin and leonurine, it appeared pertinent to examine the effect of piperine, a known hepatic and intestine metabolic inhibitor, on the pharmacokinetic disposition of curcumin and leonurine in animals to provide a scientific rationale for assigning it a rightful place in the pharmacologist armamentarium.

Objective:
The objective of this study is to determine the bioavailability and plasma elimination kinetics of Nutri-pepper enhancer when the Nutraceuticals product is administered in an oral gavage dose to Sprague Dawley rats.

Material and methods

Testing Laboratory
Industrial Biotechnology Research Center (IBRC), Bldg 19, SIRIM Berhad

Quality Assurance:
This study was performed in the spirit of the OECD Good Laboratory Practices (GLP) regulations but was not be subjected to Quality Assurance audit. All procedures not described specifically in this protocol was performed in accordance with current Standard Operating Procedures.

Test System

Strains/species	**:**	Sprague Dawley Rats
Supplier	**:**	SIRIM Berhad

Number of animals : 36
Age at study start : Young adult (9±1weeks)
Weight at study start : Mean± 20%
Animal Ethic Approval No : SIRIM-IACUC/IBRC/N19-27/0002

Test and Control Articles

3.4.1 Test Article: 1

Name	:	Nutri-pepper enhancer - 1
Supplier	:	Malaysian Pepper Board
Bulk storage conditions	:	Bulk nutraceutical product will be stored at room temperature
Compounds characteristic	:	Confirmation of the identify, purity and stability of the bulk product will be the responsibility of the client
Method of synthesis, fabrication or Derivation	:	responsibility of the client
Composition	:	250mg Curcumin, 150 mg leonurine, 10mg piperine and 90mg excipient (Total weight 500 mg/capsule)

3.4.2 Test Article: 2

Name	:	Nutri-pepper enhancer -2
Supplier	:	Malaysian Pepper Board
Bulk storage conditions	:	Bulk nutraceutical product will be stored at room temperature
Compounds characteristic	:	Confirmation of the identify, purity and stability of the bulk product will be the responsibility of the client
Method of synthesis, fabrication or Derivation	:	responsibility of the client
Composition	:	250mg Curcumin, 150 mg leonurine, and 90mg excipient (Total weight 500 mg/capsule)

3.4.2 Test Article: 3

Name	:	Control
Supplier	:	Malaysian Pepper Board

Composition	:	90 mg excipient and 410 mg water (Total weight 500 mg/capsule)

Experimental Design:

Randomization:

Rats was randomized according to SIRIM'S SOP on randomization Acceptable animal weight will be ± 20% of the mean weight at randomization. Animals outside this range will not be used without prior approval of the study director.

Group Assignment:

The 36 rats were randomly assigned to three dose group, with 12 rats per dose group, as shown below. Each group of rats wasreceive Nutri –pepper enhancer by oral gavage.

Group	Dose	Number of Rats
Test article 1	2000 mg/kg	12
Test article 2	2000 mg/kg	12
Control articles	2000 mg/kg	12

Dose procedure

All animals were receive a single oral gavage dose Nutri-pepper enhancer on day 1 of the study. Dose volumes will be based on animal's weight on the day of treatment, with uniform volume of 2000 mg/kg being used for all oral doses.

Body Weight

Body weights of all animals was determined on the day of randomization and on the day of dosing.

Blood samples collection

Blood samples was drawn through the retroorbital sinus from rats anesthetized with CO_2/O_2. Within each dose group, rats was subdivided into three collection groups, with four rats per collecting group. Blood samples for plasma drug analysis was drawn at the foll

Oral collection group	**Collection times (After dosing)**
1	45 minutes, 2, 6, and 24 hours
2	1, 3, 8 and 48 hours
3	15minutes, 1.5, 4, 12

The volume of blood to be collected at each time point was determined prior to initiation of the study and will depend on the requirements of the analytical method provided by the client. An aliquot of each blood sample was mixed with EDTA or other anticoagulant, and the samples will be centrifuged to separate plasma and blood cells. The plasma was be frozen at -20^{o}C or below until the time of analysis for bioactive compound concentration.

Analytical methods

Leonurine and curcumin standard was purchased from Sigma Aldrich. Analytical grade of sodium chloride, anhydrous magnesium chloride, dichloromethane and hexane were purchased from J.T. Baker, Philipsburgs, USA. Optima LC/MS grade of ACN were purchased from Fisher. The Z-Sept was purchased from Supleco. Leonurine and curcumin stock solution (500 $mgkg^{-1}$) was prepared by dissolving appropriate amounts of Leonurine and curcumin with methanol solvent. Appropriate aliquots of the stock solutions were diluted with methanol solvent to make solutions that contained 100, 50, 10, 5 and l.0 μgkg^{-1} of the standards.

Method development and validation

The recovery study was conducted by fortified known amount of Leonurine and curcumin active ingredient standards with serum sample. Appropriate 1ml of 1.0, 0.5, 0.1, 0.05 and 0.01 $mgkg^{-1}$ leonurine and curcumin standards was spiked onto 10 ml serum samples to obtain the recoveries at 100, 50, 10, 5 and l.0 $\mu g/kg^{-1}$ concentration. Each experiment of single concentration was conducted in triplicate. The Recovery percentage of leonurine and curcumin was calculated using the following equation:

Percentage of Recovery (%) = Detected residue (mg/kg) /spiked residue (mg/kg) *x* 100

Recovery between 70-120% indicated that the method is suitable in determining analyte quantitatively (Holland *et al*., 2000).

Analysis of bioactive compounds

Serum samples stored at -20°C were equilibrated to room temperature before analysis. A portion of 1 ml was transferred into a 10 ml volumetric flask and about 5 ml of methanol added. The mixture was shaken thoroughly and heated at 80°C on a water bath for half an hour. After cooling to room temperature, methanol was added to make up the volume to 10 ml and mixed well. The turbid solution was transferred into a 15 ml centrifuge tube and centrifuged at 4000 RPM for 10 minutes. The supernatant was collected by means of a 25 ml syringe and 10 cm needle (Luer lock) and the clear solution filtered through a 0.45 µm, 13mm millipore membrane filter, into a narrow end test tube. 20 µl of the solution were injected into the chromatograph for carrying out the LCMS analysis

Statistical analysis

Means and standard deviations will be calculated for body weight data.

Treatment of pharmacokinetic data

For calculation of pharmacokinetic parameters, curve fitting was carried out by a model independent method with non-linear least-square regression analysis using a computer designed programme "PHARMKIT". This programme uses analgorithm called "SIMPLEX" for calculating non-linear least squares. The various P1< parameters calculated were: absorp-tion half life ($t½(a)$), elimination half live ($t½_{el}$),volume of distribution (Vd); and clearence (Cl). Areas under the

concentration time curve (AUC_{0-tn}) was calculated using the trapezoidal method. Maximum concentration (Cmax) and time to max (Tmax) are the observed values. Relative bioavailability (F) was calculated using the formula:

$$F = \frac{AUC_{Curcumin\ +Piperine}}{AUC_{Curcumin}} \times 100$$

Animal care

This study was conducted in compliance with the Animal Welfare Act, and current Public Health Service regulations, and was consistent with the principles enunciated in the Animal Ethic Approval No: SIRIM-IACUC/IBRC/N19-27/0002. Animal care procedure was conducted in compliance with SIRIM Standard Operating Procedures

Caging

Rats was housed four per cage in polycarbonate cages with heat-treated hardwood chips for bedding. No contamination was present in the bedding which could interfere with and affect the results of the study. All rats in a given collection group /dose level was housed together in one cage.

Diet and water

Certified, commercial, dry, rodent chow and drinking water was available *ad libitum*. No known contaminants were present that could interfere with the outcome of the study.

RESULTS AND DISCUSSION

Recovery and detection limits

Recoveries rate for curcumin ranged between 92.5% - 118.5% and leonurine ranged between 96.1%-105.2% with both relative standard deviations (RSD) of <3.9 % were obtained from overall recovery data of 3 level of spiking suggested that the analytical method used for curcumin and leonurine were effective.

The limit of quantification (LOQ) of the analytical method for curcumin and leonurine serum were 0.01 mg/kg. The LOQ is the lowest level of spiking (0.01) mg/kg that gives acceptable recovery (92.5% - 118.5%) and precision (relative standard deviation of recoveries <15%). The example of calibration curve (for quantification of detected residue) with good linearity (R^2 = 0.9986 for curcumin and 0.9922 for leonurine) within 0.02 -0.1µl/ml is shown in Figure 2. Example of chromatogram of curcumin and leonurine peak in standard solution and serum extract samples are shown in Figure 3 and Figure 4.

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Table 1: Percentage recoveries of Curcumin and leonurine from spiked pepper berries

Active ingredient	Spike concentration	Percentage recovered (%)				SD	% RSD
		R1	R2	R3	Average		
Curcumin	0.01	97.6	92.5	93.5	94.5	1.9	2.3
	0.1	102.1	99.4	97.5	99.7	2.6	3.1
	0.5	118.5	103.3	106.7	109.5	3.1	3.4
Leonurine	0.01	98.6	97.2	96.1	97.3	3.8	3.9
	0.1	103.6	100.6	99.0	101.1	3.1	3.2
	0.5	105.2	101.2	99.6	102.0	3.0	3.1

SD= Standard deviation
RSD= Relative standard deviation

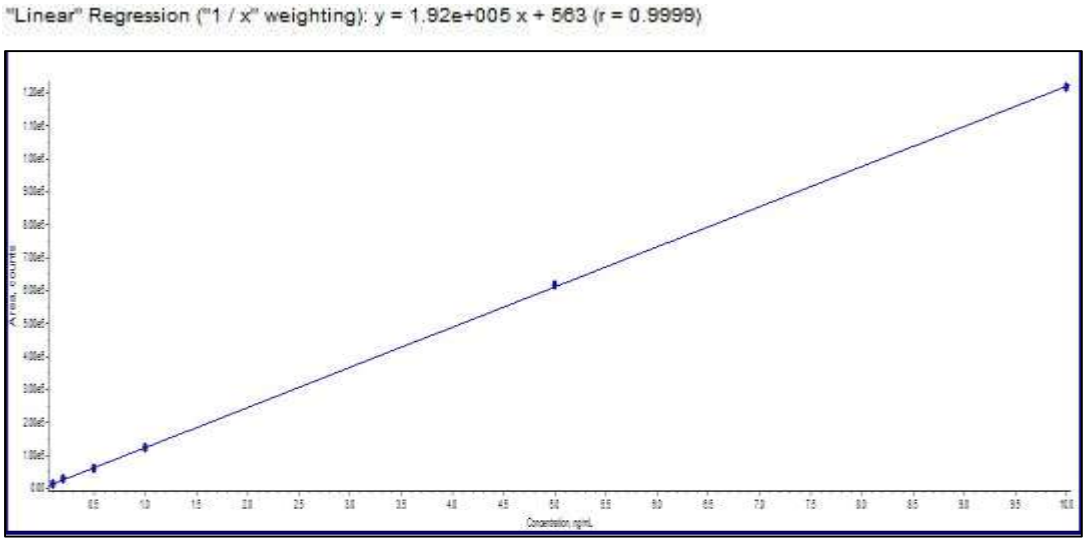
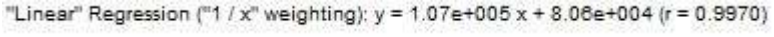


Figure 2A: Calibration curve of Curcumin



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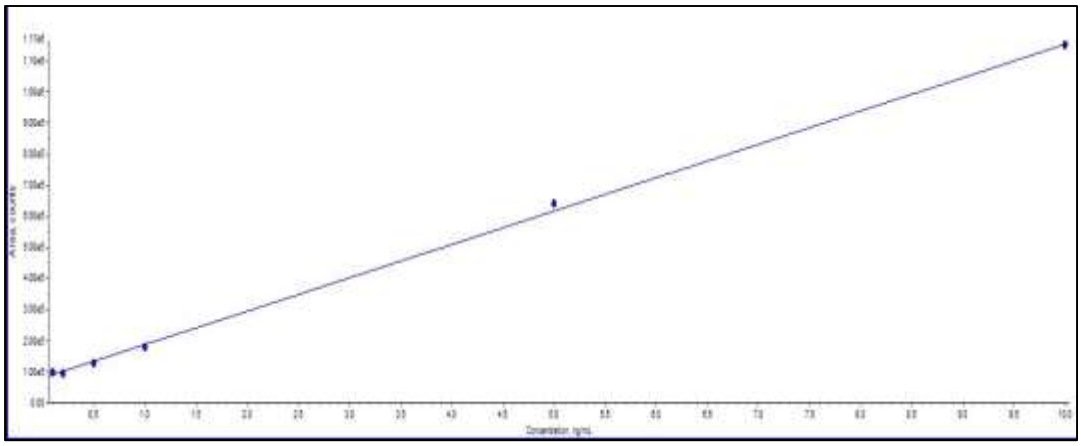

Figure 2A: Calibration curve of leonurine

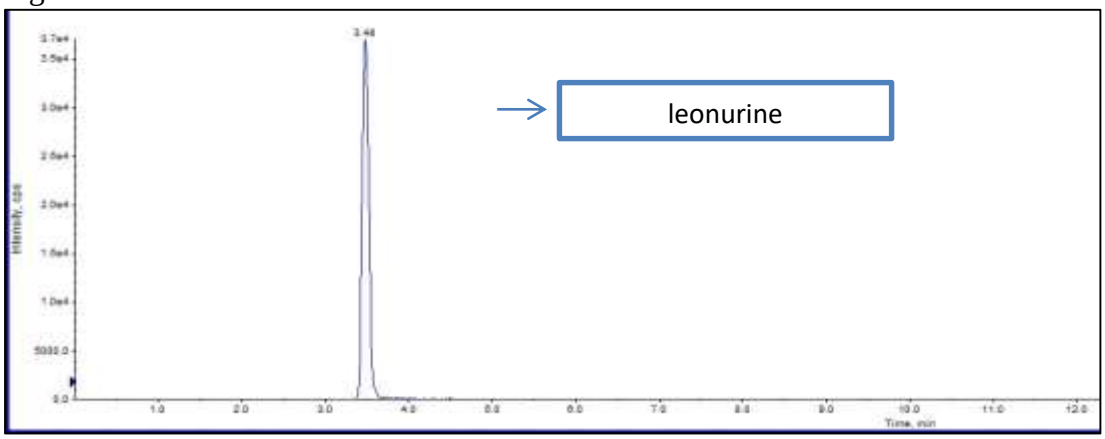

Figure 3A. LCMS chromatogram of leonurine standard at retention time of 3.48 min

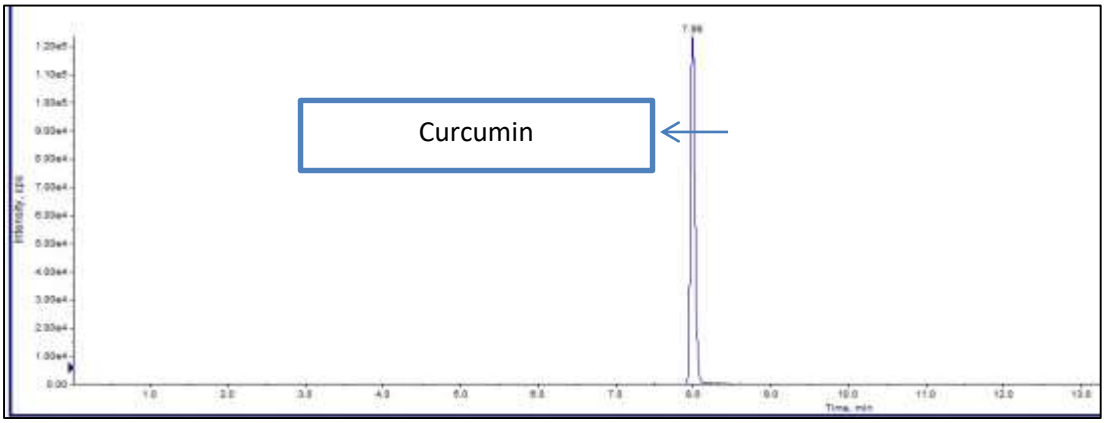

Figure 3B. LCMS chromatogram of curcumin standard at retention time of 7.99 min

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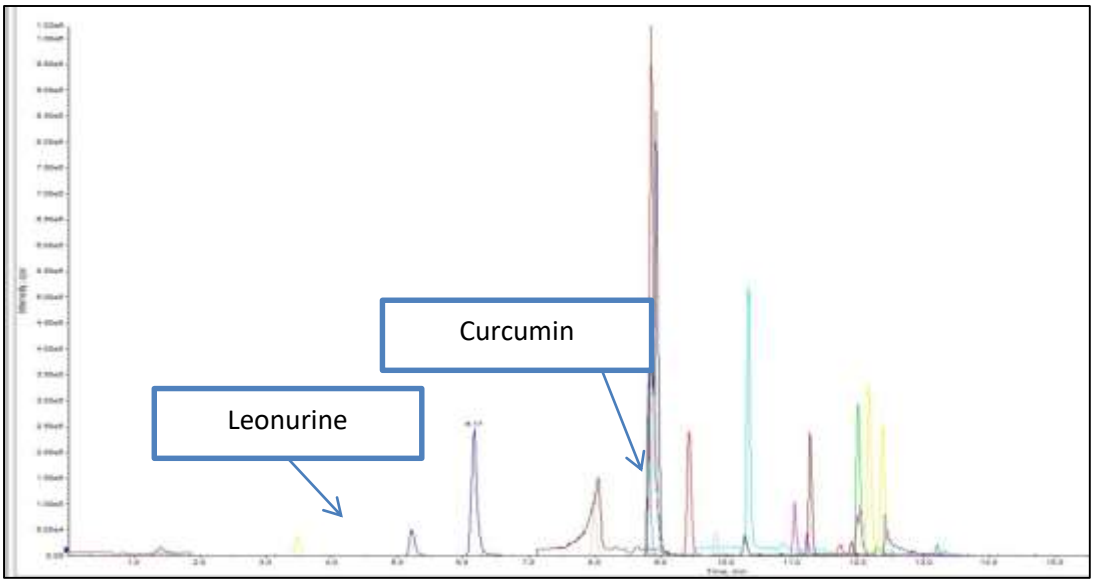

Figure 4A. LCMS chromatogram of rat serum extract sample at retention time of 3.48 min (leonurine) and 7.99 min (curcumin)

Animal studies

Curcumin alone at 250 mg/kg, or when combined with piperine, 10 mg/kg, was well tolerated by the rats as they showed no untoward effects for 48 h. Yellow coloured faecal pellets appeared at 30 h postdrug and continued upto 48 h. Perusal of Figure 1 indicates that when curcumin was given alone, peak serum concentrations of 0.067± 0.006 µg/mL were attained rapidly within 45 minutes and plateaued till 1 hour. Thereafter, the levels declined gradually reaching zero at 5 h. The plasma concentration time curve of curcumin in combination with piperine followed a similar pattern from 0 to 45 minutes and 3 to 5 h. However, piperine produced higher serum concentrations of curcumin at 1 and 2h (0.158 ± 0.006 and 0.162 ± 0.009 µg/mL) respectively, being significantly higher ($P < 0.02$) at 2 h. Thus piperine significantly enhanced the serum concentration of curcumin, albeit for a limited duration (although serum samples were collected up to 6 h, values are depicted till 4 h only, since the 5-6 hour value was also "0" in all animals).

Table 1 shows the values (mean SEM) of the pharmacokinetic parameters of curcumin per se and when combined with piperine. Cmax was increased from 1.35 ± 0.23 to 1.80 ± 0.16 µg/mL, while Tmax was significantly increased from 0.83 ± 0.05 to 1.29 ± 0.23 h. The $t½_{(el)}$ showed decreasing pattern from 1.70 ± 0.58 to 1.05 ± 0.18 h ($P < 0.02$). Results obtained also showed that both $t½_{(a)}$ and AUC reading increased from 0.310.07 to 0.470.03 h and from 2.36 ± 0.28 to 3.64 ± 0.31 pg/h/ml, respectively whereas both Cl and Vd significantly decreased from 713.00 ± 12.00 to 495.00 ± 37.00 L/h ($P < 0.02$), and from 1366.00 ± 248.70 to 782.60 ± 193.90 L/kg respectively which indicated that the curcumin enable to retain in human body for longer period of times. In summary, the relative bioavailability of curcumin when combined with piperine has increased 261.11% as compared to application of curcumin singly.

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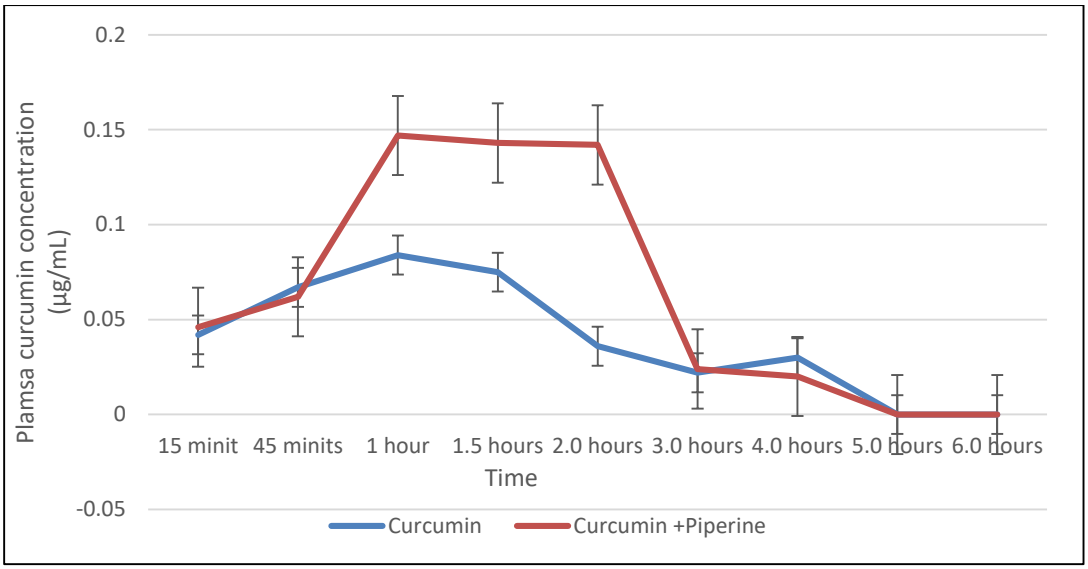

Table1 Pharmacokinetic parameters of oral curcumin 200 mg/kg alone and in combination with piperine 10mg/kg in rats (n=12/drug/time cut).

Parameter	Curcumin alone	Curcumin + Piperine
C_{max} (µg/mL)	0.091	0.154
T_{max} (h)	0.83	1.29
$t½_{(a)}$ (h)	0.31	0.47
$t½_{(el)}$ (h)	1.73	1.05
AUC (0-tn) (µg/mL)	2.16	5.64
Vd (L/kg)	1489.02	382.35
CI (L/h)	713.00	489.56

Cmax: Maximum serum concentration.
Tmax: Time to reach maximal serum concentration.
t½(a): Absorption half-life.
$t½_{(el)}$: Elimination half-life
AUC(0-tn): Area under the concentration time curve.
Vd: Volume of distribution
Cl: Total clearance.

In term of leonurine, this compound alone or when combined with piperine was well tolerated by all the tested subjects and there were no adverse or untoward reactions. Figure 2 shown the serum concentration of leonurine per se and when given with piperine. Although serum samples were collected upto 48 h, we have depicted values till 6 h, since the 8, 12, 24, and 48 h values were also "0" in all tested subjects. Serum levels of leonurine followed the similar pattern with curcumin with the rapid accumulation of leonurine within 1 hours with the maximum concentration was detected on 1.5 hours after treatment. Thereafter, the concentration of the leonurine in serum was dropped gradually and reach limit at 6 hours with the concentration value of 0. However, when piperine was

added, the serum concentrations of leonurine were significantly increased at the time points upto 1.5 h. Subsequently there was a rapid decline upto 1 h and thereafter a gradual decline to zero by 6 h.

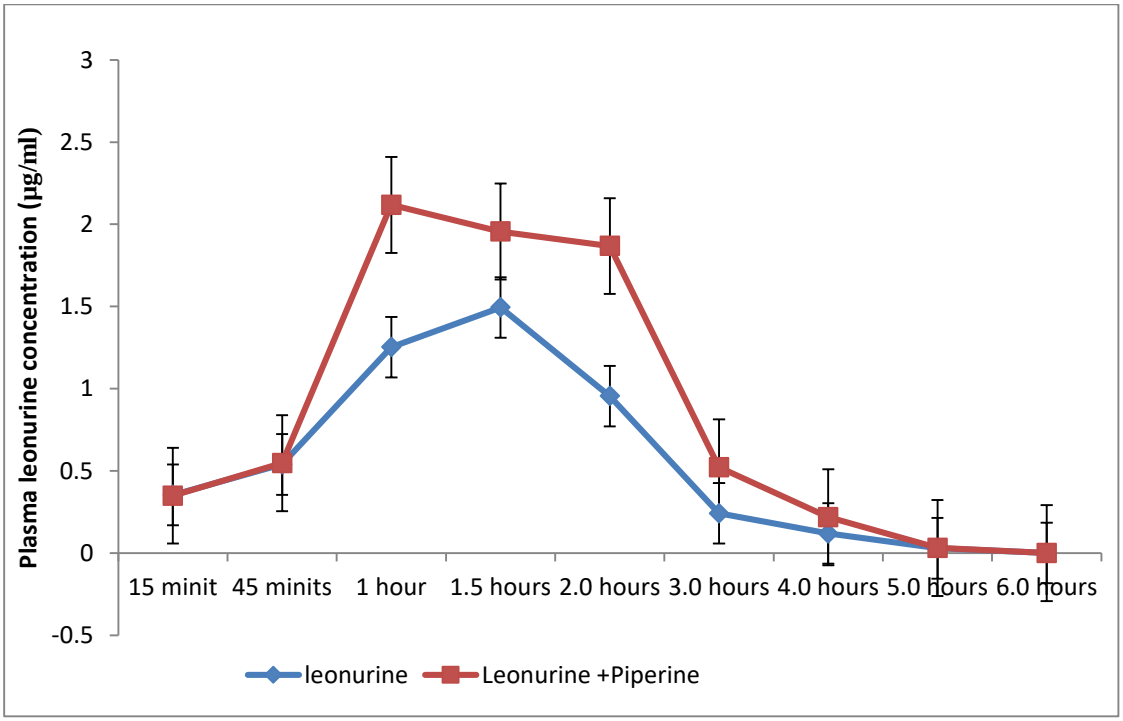

Table 2: Pharmacokinetic parameters of oral leonurine 150 mg/kg alone and in combination with piperine 10mg/kg in rats (n=12/drug/time cut).

Table 2 are depicted the Pharmacokinetic parameters of leonurine when given alone and with piperine. Cm, (observed values) when leonurine was given alone was only 0.006 ± 0.005 µg/mL at 1 h whereas when piperine was added the Cmax (observed value) was increased to 0.180 ± 16 µg/mL.). The elimination half live ($t½_{(e)}$) of this compounds was increase from 1.93 to 2.63 respectively form leonurine and leonurine + piperine. Similar to accumulation half live ($t½_{(el)}$), The ($t½_{(a)}$) was also increased from 1.93h to 2.63 h indicated that that leonurine compound can retain more longer time is rat body to provide efficient therapeutic effect for health enhancement. Result obtained also showed that both Cl and Vd value demonstrated decreased pattern by showing decreasing value from 844 L/h to 574.29 L/h and1943.24 L/kg to 982.35 L/kg for both Leonurine and Leonuride + piperine treatment respectively. Excitingly, the AUC value has shown a decreased pattern with AUC leonurine alone was 1943.24 µg/mL and AUC leonurine + piperine was 982.35 µg/mL. This finding further supports our hypothesis that the co-administration of leonurine with piperine relatively increases the leonurine bioavailability for about 154.81% when compared to application of leonurine individually.

Table1 Pharmacokinetic parameters of oral curcumin 200 mg/kg alone and in combination with piperine 10mg/kg in rats (n=12/drug/time cut).

Parameter	Leonurine alone	Leonurine + Piperine
C_{max} (µg/mL)	1.625	2.374
T_{max} (h)	1.50	1.50
$t½_{(a)}$ (h)	0.82	0.87
$t½_{(e)}$ (h)	1.93	2.63
AUC (0-tn) (µg/mL)	3.12	4.83
Vd (L/kg)	1943.24	982.35
CI (L/h)	844.00	574.29

Cmax: Maximum serum concentration.
Tmax: Time to reach maximal serum concentration.
t½(a): Absorption half-life.
$t½_{(e)}$: Elimination half-life
AUC(0-tn): Area under the concentration time curve.
Vd: Volume of distribution
Cl: Total clearance.

Discussion

The results obtained in the study demonstrate that piperine enhances the oral bioavailability of both curcumin and leonurine in rats at doses that were devoid of adverse side effects. Curcumin per se attained overall moderate serum concentrations over a 4 h period in rats with peak levels occurring between 45 minutes to 1 h. Further in rats with the addition of piperine, curcumin and leonurine compound achieved higher concentrations albeit for a short period, took a slightly longer time to peak and declined slowly. This rapidity in decline is more apparent probably because of the higher levels of curcumin and leonurine achieved with piperine as compared to curcumin and leonurine alone. There was an increase in the AUC which indicated an increase in bioavailability of curcumin and leonurine by about one and a half times as compared to curcumin given alone in rats. In rats when piperine was added to curcumin and leonurine, both Vd and Cl decreased which may have also contributed to the higher concentration. Our findings concerning absorption of curcumin in rats are in agreement with data obtained by Wahlstrom and Blennow (Wahlstroin and Blennow, 1978), who showed that when Sprague Dawley rats were given curcumin 1 g/kg, measurement of blood plasma levels and biliary excretion indicated some absorption from the gut with no apparent toxic effects upto 5 g/kg. Likewise, Khanna *et al* (Khanna *et al*., 1981) found that after curcumin consumption at the rate of 100 mg/kg, 74% was absorbed from the gastrointestinal tract within the first 5 h, while complete elimination occurred within 48 h. There is evidence that piperine is a potent inhibitor of drug metabolism, and glucuronidation altering the disposition and bioavailability of a large number of drugs (Atai *et al.*, 1985). This property of piperine suggests that it may be involved in inhibiting the metabolism of curcumin and leonurine which enhancing its bioavailability. In conclusion, the study shows that piperine enhances the serum concentration

and bioavailability of curcumin and leonurine in rats probably due to increased absorption and reduced metabolism.

CONCLUSION

Curcumin and leonurine has been proven to have a variety of biological activities, particularly antioxidant and anti-inflammatory properties, which are beneficial for the treatment of various diseases. Moreover, the therapeutic effect of curcumin and leonurine has been investigated in preclinical studies, showing that it is safe, even when used at high doses. However, the drawbacks of curcumin and leonurine are its low aqueous solubility, instability, and poor bioavailability. Thus, as summarized in this review, the co-administration of piperine with both leonurine and curcumin have shown greater bioavailability than when the drugs were used singly which suggested that this newly developed Nutri-pepper enhancer can be served as a potential dietary supplement to improve human health with no adverse effects.

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