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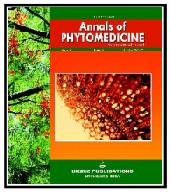

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Pharmacokinetics of protocatechuic acid following oral administration of protocatechuic ethyl ester alone and in combination with piperine in rats

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Abstract

The current study was carried out to estimate pharmacokinetics of protocatechuic acid following oral administration of protocatechuic ethyl ester alone and in combination with piperine in rats. Following oral administration of protocatechuic ethyl ester, the mean peak plasma protocatechuic acid concentration of 1.50 ± 0.08 µg/ml was achieved at 0.25 h. The pharmacokinetic parameters like mean absorption half-life ($t_{½\alpha}$) (0.16 ± 0.07), elimination half-life ($t_{½\beta}$) (0.43 ± 0.02 h), apparent volume of distribution (Vdarea) (102.4 ± 5.76), area under plasma drug concentration-time curve ($AUC_{0-\infty}$) (0.92 ± 0.02 µg h/ml), area under first moment curve (AUMC) (0.56 ± 0.01 µg h^2/ml), total body clearance (Cl_B) (163.7 ± 3.49 l/h/kg) and mean residence time (MRT) (0.61 ± 0.02) were observed for protocatechuic acid. Following oral administration of piperine with protocatechuic ethyl ester in rats, plasma concentrations of protocatechuic acid at all time point and pharmacokinetic parameters did not differ significantly in comparison to protocatechuic ethyl ester administered alone.

1. Introduction

Protocatechuic acid (3, 4-dihydroxybenzoic acid) is a phenolic acid, isolated from the dried flowers of *Hibiscus sabdariffa* (Tseng *et al*., 1998) and reported to possess 10 fold higher antioxidant properties than α-tocopherol (Li *et al*., 2011). It is reported to present in routine human diet like brown rice (*Oryza sativa* L.), onion (*Allium cepa* L.), plums (*Prunus domestica* L.), grapes (*Vitis vinifera*), almonds (*Prunus amygdalus*), olive oil, white wine, star anise (*Illicium verum*), rosemary (*Rosmarinus officinalis* L.), and cinnamon (*Cinnamomum aromaticum*) (Herrmann and Nagel, 1989; Das and Gezici, 2018; Malik *et al*., 2020). Literature shows that protocatechuic acid absorption is quite rapid following oral administration but bioavailability and mean residence time is quite low in rat body like other phytochemicals (Chen *et al*., 2012; Ma *et al*., 2015; Wang *et al*., 2016; Modi *et al*., 2019). So, in order to enhance systemic bioavailability, esterification of protocatechuic acid is one way (Biasutto *et al*., 2007) and to minimize *in vivo* metabolism, bioenhancer like piperine can be favorable phytochemical (Singh *et al*., 2009; Chauhan *et al*., 2017; Patel *et al*., 2019; Patel *et al*., 2020). Protocatechuic ethyl ester is alkyl ester derivative of protocatechuic acid and it has been widely explored for anticancer activity (Tanaka *et al*., 2011), antidiabetic activity (Scazzocchio *et al*., 2011), anti-ageing activity (Shi *et al*., 2006), anti-inflammatory activity and analgesic activity (Lende *et al*., 2011), antiatherosclerotic activity (Borate *et al*., 2011), hepatoprotective activity (Liu *et al*., 2002) and nephroprotective activity (Lee *et al*., 2009). Looking to the available literature and with the aim of evaluation of pharmacokinetics of protocatechuic acid following oral administration of protocatechuic ethyl ester in rats with special attention on effect of piperine co-treatment on pharmacokinetic profile of protocatechuic acid, the present study was undertaken.

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2. Materials and Methods

2.1 Experimental animals

The experiment was conducted in 48 Wistar rats (female) weighing between 300 to 400 g. Before commencement of the experiment, rats were subjected to clinical examination to exclude possibility of any disease and kept under constant observation for two weeks. Randomly, the animals were divided into two groups, housed in polypropylene cages with provision of *ad libitum* standard feed and water. Institutional Animal Ethics Committee, Veterinary College, Navsari, Gujarat approved use of animals for conducting the study with protocol number 062-VCN-VPT-2018.

2.2 Drugs and chemicals

Protocatechuic acid, protocatechuic ethyl ester (3, 4-Dihydroxy-benzoic acid) and piperine were obtained from Sigma-Aldrich, St. Louis, USA. Caboxy methyl cellulose sodium salt (CMC), Acetonitrile and orthophosphoric acid were purchased from Merck Specialities Private Limited, Mumbai. Normal saline was obtained from local pharmacy located at Navsari, Gujarat.

2.3 Experimental design for pharmacokinetic study

Pharmacokinetic of protocatechuic acid was evaluated following oral administration of protocatechuic ethyl ester alone (150 mg/kg) and

in combination with piperine (40 mg/kg) in rats (n=48). Protocatechuic ethyl ester was dissolved in caboxy methyl cellulose sodium salt solution. Serial collection of blood samples at alternating time points was managed by use of multiple numbers of rats. Blood samples were collected in K_3EDTA vials, at different time interval *i.e.*, 0.083, 0.25 0.5, 1, 1.5, 2, 4, 6, 8 and 12 h from retro orbital plexus. Plasma samples were collected by the centrifugation (3000 rpm for 10 min) and stored at – 20°C in cryovials. Samples were processed and analyzed within 24 h for determination of protocatechuic acid concentration through high performance liquid chromatography (HPLC).

2.4 HPLC analysis of protocatechuic acid from plasma samples

Plasma protein was precipitated from plasma samples by adding methanol in plasma (1:1 ratio) in a clean microcentrifuge tube and mixed on a vortex mixer for 1 min. It was followed by centrifugation for 10 min at 10000 rpm and clean supernatant was collected into inserts of automatic sampler vial, from which 20 µl of supernatant was injected into HPLC system.

Plasma samples were analyzed to quantify protocatechuic acid using HPLC system by using procedure as described by Chen *et al.* (2012) and Wang *et al.* (2016) with minor modifications. HPLC system of Shimadzu (Japan) includes binary gradient delivery pump (model LC 20AP), auto sampler (model SIL 20A) and reverse phase C18 column (250 × 4.6 mm ID) and diode array detector (model SPD M20A). Mobile phase consisted of solvent A (5% orthophosphoric acid in lab grade water) and solvent B (100% methanol). Mobile phase was subjected to filtration by 0.2 µ size filter (Axiva N_{66}) and degassing by ultra-sonication. Gradient flow of mobile phase was pumped into column at a flow rate of 1.5 ml/min at ambient temperature as follows 0-5 min (10% solvent B) and 5.01-10 min (8% solvent B) and effluent was monitored at 210 nm wavelength (Patel *et al*., 2018; Modi *et al*., 2019).

For validation of HPLC method, initial stock solution of protocatechuic acid was prepared by dissolving 2 mg protocatechuic acid in 1 ml drug free plasma and standards were prepared and analysed to the resultant calibration curve (Figure 1).The calibration curves showed good linearity over the concentration ranges 0.09 to 25 µg/ml with a mean correlation coefficient (R^2) was 0.99. Representative chromatograms of blank plasma of rat, protocatechuic acid standard (25 µg/ml) in plasma, 15 min post oral administration of protocatechuic ethyl ester in rat and 15 min post oral administration of protocatechuic ethyl ester and in combination with piperine in rat depicted in Figure 2. The precision and accuracy of the assay were assessed using samples at concentration of 25, 6.25, 0.78 and 0.09 µg/ml. At all concentrations, the C.V. was less than 7.26 %. The lower limit of detection and limits of quantification of the drug was 0.04 and 0.09 µg/ml, respectively. Pharmacokinetic parameters were calculated using software PK solution (Version 2.0) which is based on non-compartmental model.

2.5 Statistical analysis

All data obtained for pharmacokinetic parameters of protocatechuic acid was presented as mean ± Standard error (SE). The data for plasma protocatechuic acid concentration suitably tabulated and analyzed by 't' test. The levels of significance to observe difference were 0.05 and 0.01. The *p* values < 0.05 and <0.01 were considered as statistically significant or highly significant, respectively.

3. Results

Protocatechuic acid levels in plasma as a function of time schedule after oral administration of protocatechuic ethyl ester (150 mg/kg) and its combination with piperine (40 mg/kg) in rats are presented in Table 1, while semilogarithmic plots of the same have been presented in Figure 3. Pharmacokinetic parameters of protocatechuic acid following oral administration of protocatechuic ethyl ester (150 mg/kg) and its combination with piperine (40 mg/kg) in rats are shown in Table 2.

Following oral administration of protocatechuic ethyl ester alone or in combination with piperine, the mean peak plasma drug concentration of 1.50 ± 0.08 µg/ml vs 1.67 ± 0.03 µg/ml was achieved at 0.25 h which declined and detected as 0.12 ± 0.01 µg/ml vs 0.12 ± 0.01 µg/ml in plasma at 1.5 h and beyond then the drug was not detected in plasma. Plasma concentrations of protocatechuic acid at all time point did not differ significantly when protocatechuic ethyl ester administered alone and in combination with piperine.

Table 1: Plasma concentrations (µg/ml) of protocatechuic acid following oral administration of protocatechuic ethyl ester alone (150 mg/kg) and along with piperine (40 mg/kg) in rats (n = 6)

Time after drug administration (h)	Plasma concentration of protocatechuic acid	
	Protocatechuic acid (Mean ± SE)	Protocatechuic acid + piperine (Mean ± SE)
0.08	1.16 ± 0.09	1.22 ± 0.10
0.25	1.50 ± 0.08	1.67 ± 0.03
0.5	0.60 ± 0.02	0.58 ± 0.04
0.75	0.43 ± 0.02	0.42 ± 0.03
1	0.27 ± 0.02	0.25 ± 0.01
1.50	0.12 ± 0.01	0.12 ± 0.01

Means within rows did not differ significantly (p<0.05).

Table 2: Pharmacokinetic parameters of protocatechuic acid following oral administration of protocatechuic ethyl ester alone (150 mg/kg) and along with piperine (40 mg/kg) in rats (n=6).

Pharmacokinetic parameters	Unit	Protocatechuic acid	Protocatechuic acid after co-administration of piperine
K_a	h^{-1}	8.28 ± 2.06	8.99 ± 1.95
β	h^{-1}	1.62 ± 0.08	1.68 ± 0.08
$t_{½Ka}$	h	0.16 ± 0.07	0.11 ± 0.03
$t_{½β}$	h	0.43 ± 0.02	0.42 ± 0.02
C_{max}	µg/ml	1.50 ± 0.08	1.54 ± 0.09
T_{max}	h	0.25 ± 0.00	0.25 ± 0.00
$AUC_{(0-\infty)}$	µg.h/ml	0.92 ± 0.02	0.88 ± 0.01
AUMC	$\mu g.h^2/ml$	0.56 ± 0.01	0.53 ± 0.04
$Vd_{(area)}$	L/kg	102.4 ± 5.76	102.5 ± 6.27
$Cl_{(B)}$	L/h/kg	163.7 ± 3.49	169.9 ± 2.61
MRT	h	0.61 ± 0.02	0.59 ± 0.02

Means within rows did not differ significantly (p<0.05).

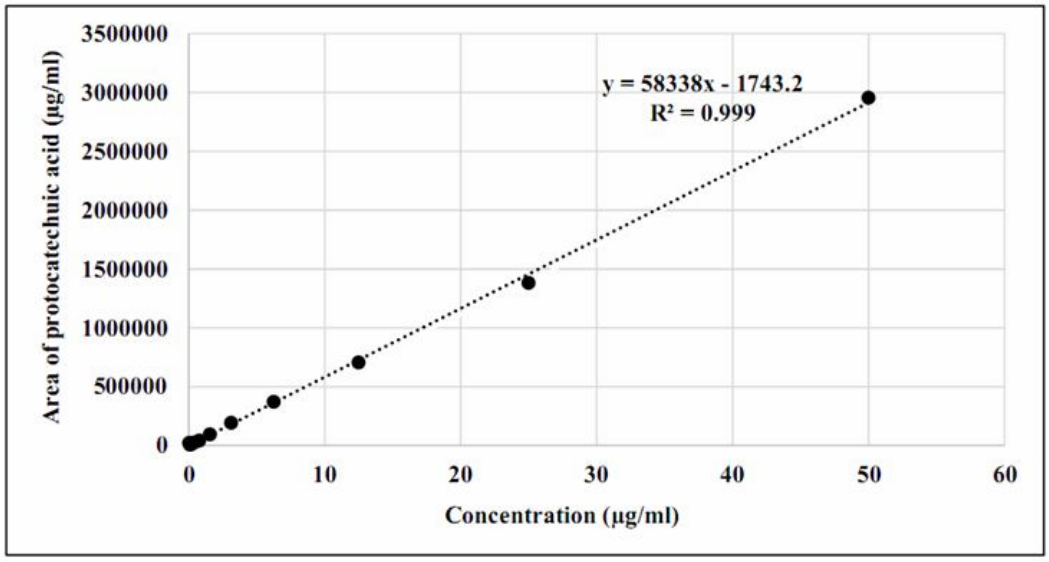
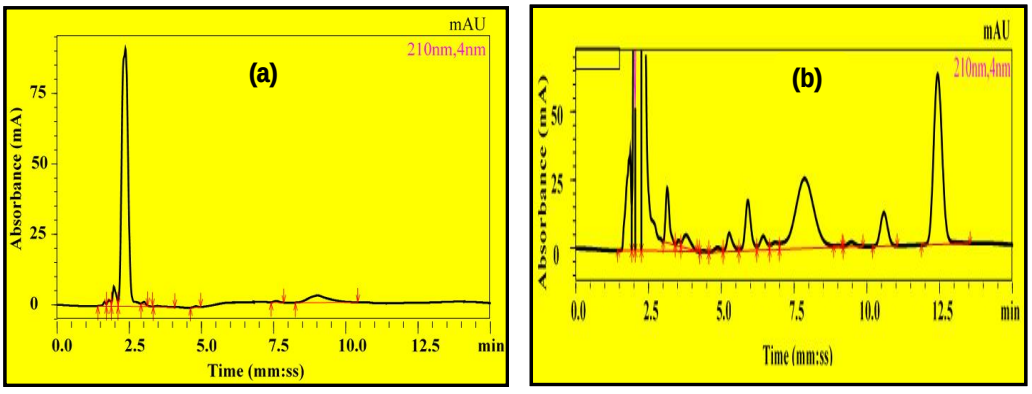


Figure 1: Calibration curve of protocatechuic acid drug-free plasma of rats.



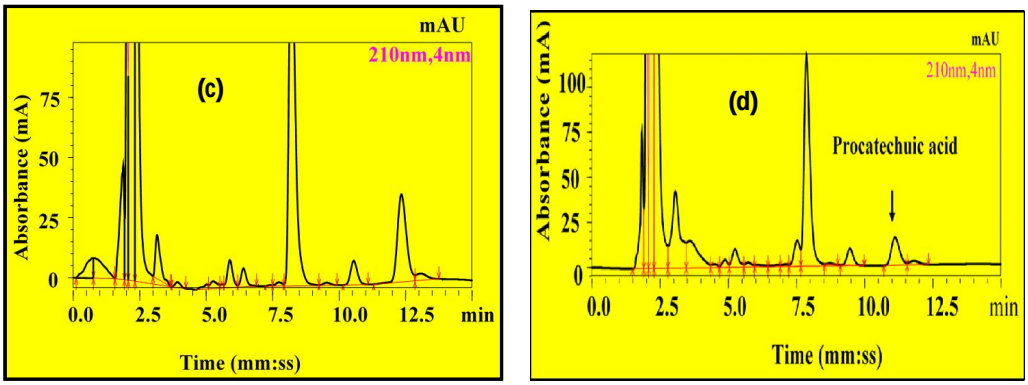


Figure 2: Representative chromatograms of (a) blank plasma of rat, (b) protocatechuic acid standard (25 µg/ml) in plasma (c) 15 min post oral administration of protocatechuic ethyl ester in rat, and (d) 15 min post oral administration of protocatechuic ethyl ester and its combination with piperine in rat.

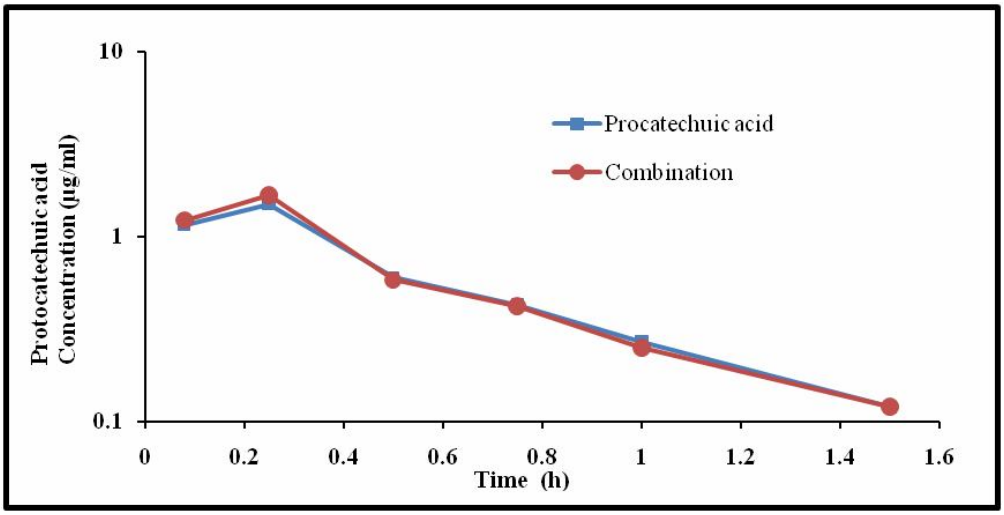


Figure 3: Semi logarithmic plot of comparison of protocatechuic acid concentration in plasma versus time following oral administration of protocatechuic ethyl ester (150 mg/kg) alone and along with piperine (40 mg/kg) in rats. Each points represents Mean ± SE.

4. Discussion

Protocatechuic acid peaks were well separated from endogenous substances in the blank plasma as shown in Figure 2, which imply that the bioanalytical method developed herein provide acceptable selectivity without endogenous interferences occurring at the appearance of protocatechuic acid peaks. The calibration curves for protocatechuic acid in plasma were observed to be linear from 0.09 to 25.0 µg/ml. A representative equation for the calibration curves is as follows: y = 58338x – 1743.2. The correlation coefficients (r2) is 0.99, indicating an acceptable linearity of method. The intra-day and inter-day accuracy and precision were determined for protocatechuic acid and at the LLOQ (0.09 µg /ml) and four quality control (QC) levels 25, 6.25, 0.78, and 0.09 µg/ml. The mean precision of the method was determined to be 7.26 %, and its mean accuracy was 94.50 %. These values were within the acceptable range, indicating that the present method is reproducible, accurate, and precise. Notably, present method with a simple deproteinization procedure achieved an equivalent LLOQ (0.09 µg /ml) in a previous studies involving liquid–liquid extraction (Chen *et al.*, 2012; Wang *et al.*, 2016).

To the best of our knowledge, we carried out the pharmacokinetic study of protocatechuic acid following oral administration of protocatechuic ethyl ester (150 mg/kg) along with piperine (40 mg/kg) in rats first time. The mean peak plasma drug concentration (C_{max}) of 1.50 ± 0.08 µg/ml was achieved at 0.25 h. In accordance to present study, similar peak plasma concentration of protocatechuic acid (4.8 µg/ml at 0.18 h) was observed following oral administration (20 mg/kg) of protocatechuic acid aldehyde in rats (Wang *et al.*, 2016). In addition, Ma *et al.* (2015), Guo *et al.* (2008) and Zhao *et al.* (2011) observed C_{max} and T_{max} as 0.03 µg/ml at 0.6 h, 2.90 ± 1.73 µg/ml at 0.38 h and 2.6 ± 0.3 µg/ml at 0.8 h, following oral administration of *Polygonum capitatum* extract (120 mg/kg), following intravenous administration of guanxinning lyophilizer (800 mg/kg) and following intravenous administration of Danshen injection (10 ml/kg) in rats,

respectively. Variation in plasma concentration of protocatechuic acid may be due to variation in dose of drug administration, formulation and other ingredients of plant extracts. In present study, following oral administration of piperine with protocatechuic ethyl esterin rats, plasma concentration of protocatechuic acid did not differ significantly (p<0.05).

The elimination half-life ($t_{1/2\beta}$) of protocatechuic acid following oral administration of protocatechuic ethyl ester in the present study was 0.43 ± 0.02 h. In accordance to present study, Chen *et al*. (2012) observed terminal half-life ($t_{1/2}$) of 0.04 h following oral administration (50 mg/kg) in mice; Ma *et al.* (2015) observed elimination half-time of 0.08 h for protocatechuic acid following oral administration of *Polygonum capitatum* extract (120 mg/kg) in rats and Guo *et al*. (2008) observed elimination half-life of 0.38 h for protocatechuic acid following intravenous administration of guanxinning lyophilizer (800 mg/kg). Mean apparent volume of distribution (Vd_{area}) of protocatechuic acid following oral administration of protocatechuic ethyl ester was 102.4 ± 5.76 l/kg in rats. In accordance to present study, higher volume distribution (Vd) *i.e.*, 128.8 l/kg and 2.5 l/Kg were observed for protocatechuic acid following oral administration of *Polygonum capitatum* extract (120 mg/kg) in rats (Ma *et al.*, 2015) and following intravenous administration of danshen injection (10 ml/kg) (Zhao *et al.*, 2011). However, lower values for volume of distribution (V_d), *i.e.*, 0.03 l/kg was observed following oral administration of protocatechuic acid (50 mg/kg) in mice (Chen *et al.*, 2012).

The total body clearance of protocatechuic acid was observed to be 163.7 ± 3.49 l/h/kg following oral administration (150 mg/kg) of protocatechuic ethyl ester in rats. In accordance to present study, high total body clearance of protocatechuic acid, *i.e.*, 67.68 l/h/kg, 267.6 l/h/kg and 608.33 l/h/kg were observed following oral administration of *Polygonum capitatum* extract (120 mg/kg) in rats (Ma *et al*., 2015), following by oral administration (50 mg/kg) (Chen *et al*., 2012) and following intravenous administration of danshen injection in rats (Zhao *et al*., 2011). The MRT values calculated following oral administration of protocatechuic acid in present study was 0.61 ± 0.02 h. Similarly, Wang *et al.* (2016) observed MRT of 1.32 ± 0.79 h and 0.81 ± 0.17 h for protocatechuic acid following oral (20 mg/kg) and intravenous administration (1 mg/kg) of protocatechuic aldehyde (PAL). In addition to this, Chang *et al*., (2010) and Zhao *et al*., (2011) also observed similar MRT value of 0.358 h and 0.686 h for protocatechuic acid following intravenous administration of danshen injection in rats.

Pharmacokinetic analysis of protocatechuic acid indicates faster absorption and clearance of drug from the body following oral administration of protocatechuic ethyl ester in rat. This result is supported by observations of Xu *et al*. (2007), *i.e.*, protocatechuic aldehyde (PAL) metabolic products (free and conjugate forms) was detected at 5 min in the plasma and reached the peak level at 15 min after protocatechuic aldehyde administration and Ma *et al.* (2015), *i.e.*, protocatechuic acid were excreted more than 80% in the first 4 h after oral administration of *Polygonum capitatum* extract. All pharmacokinetic parameter of protocatechuic acid did not differ significantly following co-administration of piperine with protocatechuic ethyl ester in comparison to administration of protocatechuic acid alone in rats.

5. Conclusion

In conclusion, following oral administration of protocatechuic ethyl ester alone and along with piperine in rats, effective concentrations were maintained up to 1 h post drug administration. In rats, protocatechuic acid remains for a shorter time after oral administration in rats due to rapid clearance from the body. Oral administration of piperine with protocatechuic ethyl ester did not affect overall pharmacokinetic profile of protocatechuic acid.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Biasutto, L.; Marotta, E.; De Marchi, U.; Zoratti, M. and Paradisi, C. (2007). Ester-based precursors to increase the bioavailability of quercetin. J. Med. Chem., **50**(2):241-253.

Borate, A.R.; Suralkar, A.A.; Birje, S.S.; Malusare, P.V. and Bangale, P.A. (2011). Antihyperlipidemic effect of protocatechuic acid in fructose induced hyperlipidemia in rats. Int. J. Pharma. Bio. Sci., **2**(4):456-460.

Chang, B.B.; Zhang, L.; Cao, W.W.; Cao, Y.; Yang, W.L.; Wang, Y.; Chen Y.C. and Liu, X.Q. (2010). Pharmacokinetic interactions induced by content variation of major water-soluble components of Danshen preparation in rats. Acta. Pharmacol. Sin., **31**(5):638.

ChauhanV.B.; ModiC.M.; Patel U.D.; Patel H.B.;KalariaV.A.; FefarD.T.; BhadarkaD.H.;SolankiS.L. and Ahmed S.R. (2017). Safety profile of marbofloxacin following repeated intramuscular administration alone and piperine pretreated rats. Ann. Phytomed., **6**(2):88-92.

Chen, W.; Wang, D.; Wang, L.; Bei, D.; Wang, J.; See, W.A.; Mallery, S.R.; Stoner, G.D.and Liu, Z. (2012). Pharmacokinetics of protocatechuic acid in mouse and its quantification in human plasma using LC-tandem mass spectrometry. J. Chromatogr. BBiomed. Appl., **908:**39-44.

Das Kuntal and Gezici Sevgi (2018).Plant secondary metabolites, their separation, identification and role in human disease prevention. Ann. Phytomed., **7**(2): 13-24.

Guo, X.; Chen, X.; Li, L.; Shen, Z.; Wang, X.; Zheng, P.; Duan F.; Ma. Y. and Bi, K. (2008). LC-MS determination and pharmacokinetic study of six phenolic components in rat plasma after taking traditional Chinese medicinal-preparation: Guanxinning lyophilized powder for injection. J. Chromatogr. BBiomed. Appl., **873**(1):51-58.

Herrmann K. and Nagel C. W. (1989).Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. Crit. Rev. Food. Sci. Nutr., **28**(4):315-347.

Lee, J.H.; Lee, H.J.; Lee, H.J.; Choi; W.C.; Yoon, S.W.; Ko, S.G.; Ahn K.S.; Choi S.H.; Ahn K.S; Lieske J.C. and Kim, S.H. (2009). Rhusverniciflua Stokes prevents cisplatin-induced cytotoxicity and reactive oxygen species productionin MDCK-I renal cells and intact mice. Phytomedicine, **16**(2-3):188-197.

Lende, A.B.; Kshirsagar, A.D.; Deshpande, A.D.; Muley, M.M.; Patil, R.R.; Bafna, P.A. and Naik, S.R. (2011). Anti-inflammatory and analgesic activity of protocatechuic acid in rats and mice. Inflammopharmacology, **19**(5):255.

Li, X.; Wang, X.; Chen, D. and Chen, S. (2011). Antioxidant activity and mechanism of protocatechuic acid *in vitro*. Funct. Food Health Dis., **1**(7):232-244.

Liu, C.L.; Wang, J.M.; Chu, C.Y.; Cheng, M.T. and Tseng, T.H. (2002). *In vivo* protective effect of protocatechuic acid on tert-butyl hydroperoxide -induced rat hepatotoxicity. Food Chem. Toxicol., **40**(5):635-641.

Ma, F.; Gong, X.; Zhou, X.; Zhao, Y. and Li, M. (2015). An UHPLC-MS/MS method for simultaneous quantification of gallic acid and protocatechuic acid in rat plasma after oral administration of *Polygonum capitatum* extract and its application to pharmacokinetics. J. Ethnopharmacol, **162:**377-383.

Malik T.; Madan V. K. and Ram Prakash(2020). Herbs that heal: Floristic boon to the natural healthcare system. Ann. Phytomed., **9**(2):6-14.

Modi Falguni, D., Bhavsar, S. K., Patel, J. H.; Varia, R.D.; Modi, L.C.; Modi, Megha A. and Kale, N. (2019). Pharmacokinetic profile of rutin after intramuscular administrationin rats favours its *in vivo* anti-inflammatory activity in carrageenan induced rodent model of inflammation. Ann. Phytomed., **8**(1):185-192.

Modi, M.A.; Kale, N.V.; Patel, J.H.; Varia, R.D.; Modi, F.D. and Vihol, P.D. (2019). Pharmacokinetics of ferulic acid following oral administration ethyl ferulate alone and in combination with piperinein rats. Ann. Phytomed., **8**(2):194-198.

Patel , J.H.; Vihol, P.D.; Sadariya, K.A.; Patel, U.D., Varia, R.D.; Bhavsar, S.K.; Thaker, A.M. and Raval, J.K. (2019). Pretreatment with trikatu augments pharmacokinetic profile and bioavailability of orally administered levofloxacin in goat. Ann. Phytomed., **8**(1):172-177.

Patel Harshad, B.; Patel Urvesh, D.; Modi Chirag, M.; Fefar Dhaval , T. and Bhadaniya Amit R. (2020). Effect of piperine and quercetin pretreatment on safety profile of marbofloxacin after repeated oral administration in broiler chickens. Ann. Phytomed., 9(2):283-292. http://dx.doi.org/10.21276/ap.2020.9.2.28.

Patel Harshad, B.; Patel Urvesh, D.; Modi Chirag, M.; Ahmed Shaul and Solanki Shivani, L. (2018). Pharmacokinetic profiles of marbofloxacin following single and repeated oral administration in broiler chickens. Ann. Phytomed., **7**(2):174-179.

Scazzocchio, B.; Varì, R.; Filesi, C.; D'Archivio, M.; Santangelo, C.; Giovannini, C., Iacovelli, A.; Silecchia, G.; Volti G.L.; Galvano F.; and Masella, R. (2011). Cyanidin-3-O-β-glucoside and protocatechuic acid exert insulin-like effects by upregulating PPAR γ activity in human omental adipocytes. Diabetes, **60**(9):2234-2244.

Shi, G.F.; An, L.J.; Jiang, B.; Guan, S.; and Bao, Y.M. (2006). Alpinia protocatechuic acid protects against oxidative damage *in vitro* and reduces oxidative stress *in vivo*. Neurosci. Lett, **403**(3):206-210.

Singh, R.; Sarita Devi; Patel, J.H.; Patel, U.D.; Bhavsar and S.K.; Thaker, A.M. (2009). Indian herbal bioenhancers: A review. Pharmacogn. Rev., **3**(5):80-82.

Tanaka, T.; Tanaka, T. and Tanaka, M. (2011). Potential cancer chemopreventive activity of protocatechuic acid. J. Exp. Clin. Med., **3**(1):27-33.

Tseng, T.H.; Hsu, J.D.; Lo, M.H.; Chu, C.Y.; Chou, F.P.; Huang, C.L. and Wang, C.J. (1998). Inhibitory effect of Hibiscus protocatechuic acid on tumor promotion in mouse skin. Cancer Lett, **126**(2)**:**199-207.

Wang, X.; Yan, K.; Ma, X.; Li, W.; Chu, Y.;Guo, J.; Li, S.; Zhou, S.; Zhu, Y. and Liu, C. (2016). Simultaneous determination and pharmacokinetic study of protocatechuic aldehyde and its major active metabolite protocatechuic acid in rat plasma by liquid chromatography-Tandem mass spectrometry. J. Chromatogr. Sci., **54**(5)**:**697-705.

Xu, M.; Zhang, Z.; Fu, G.; Sun, S.; Sun, J.; Yang, M.; Liu, A.; Han, J. and Guo, D.(2007). Liquid chromatography-tandem mass spectrometry analysis of protocatechuic aldehyde and its phase I and II metabolites in rat. J. Chromatogr. BBiomed. Appl., **856**(1-2):100-107.

Zhao, D.; Han, D.E.; Li, N.; Lu, Y.; Li, T.T.; Yang, S.Y.; He, J.K. and Chen, X.J. (2011). Simultaneous determination of six phenolic constituents of danshen injection in rat plasma by liquid chromatography electrospray ionization mass spectrometry and its application to a Pharmacokinetic Study. Eur. J. Mass Spectrom., **17**(4):395-403.

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