

**Pharmacokinetic Study of [Di (4-amino N-acetyl) phenoxy]methyl ketone as Compared to Paracetamol****\*Bhavna Shah<sup>1</sup>, Pravin Patil<sup>1</sup>, Hirva Shah<sup>2</sup>**

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**ABSTRACT**

In the present study, comparative pharmacokinetics study between [Di (4-amino N-acetyl) phenoxy] methyl ketone (DPMK) and paracetamol in rats were investigated. DPMK and paracetamol were orally given to experimental rats in dose 500 mg/kg/b.wt. At various time intervals, blood (1 h - 24 h) and urine (6 h - 84 h) sample was collected from each rat. The drug concentrations were measured in blood supernatants and urine samples by using validated UV-visible spectrophotometric method. Various pharmacokinetic parameters were calculated by non compartmental model for DPMK and paracetamol. DPMK reached a higher concentration  $C_{max} = 22.26 \pm 1.85 \mu\text{g/mL}$  after oral administration in rat as compared to standard paracetamol  $C_{max} = 5.18 \pm 0.57 \mu\text{g/mL}$ . There was a significant increased in the  $AUC_{(0-\infty)} = 85.82 \pm 8.67 \mu\text{g h/mL}$  and  $AURC_{(0-\infty)} = 338.86 \pm 53.76 \text{ mg}$  of DPMK as compared to the paracetamol  $AUC_{(0-\infty)} = 20.51 \pm 1.79 \mu\text{g h/mL}$  and  $AURC_{(0-\infty)} = 42.97 \pm 1.58 \text{ mg}$ . No significant differences were found in elimination rate constant during the PK study of DPMK and paracetamol. The apparent volume distribution ( $V_{ss} = 7.24 \pm 1.51 \text{ lit}$ ) and total clearance ( $CL = 171 \pm 0.24 \text{ lit/h}$ ) for DPMK were found to be less as compared to the paracetamol ( $V_{ss} = 29.40 \pm 4.17 \text{ lit}$ ,  $CL = 7.43 \pm 0.89 \text{ lit/h}$ ). Furthermore, the relative bioavailability of DPMK was found to be in the range 4.18-7.92 for both blood and urine sample.

**Keywords:** [Di (4-amino N-acetyl) phenoxy] methyl ketone, paracetamol, pharmacokinetic, wistar albino rats

Received 12 Feb 2014

Received in revised form 03 March 2014

Accepted 06 March 2014

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**INTRODUCTION**

Paracetamol (N-acetyl-p-aminophenol or acetaminophen) is widely used as an analgesic and antipyretic agent in the treatment of pain and fever. It is classified as non-steroidal anti-inflammatory drug (NSAIDs) in textbooks of pharmacology [1,2]. The pharmacokinetics of paracetamol has been extensively investigated, and the observed data indicate that an oral dose of 100 mg produces a maximum peak plasma concentration of about  $3.53 \mu\text{g/mL}$  in rat [3] whereas, 650 mg oral dose of paracetamol formulation produces a maximum peak plasma concentration in a healthy human being is  $5.90 \mu\text{g/mL}$  [4]. Maximal analgesic and antipyretic activity

occurs 1-2 h after peak plasma level [5,6]. Paracetamol is not significantly bound to plasma proteins, and has a volume of distribution of 0.7-1 L/kg. At therapeutic doses, paracetamol is metabolized primarily in the liver by conjugation to paracetamol glucuronide (60-90%) and paracetamol sulphate (35%). Paracetamol is not itself toxic drug, but its small fraction (5-10% paracetamol) is oxidized by the CYP450-dependent mixed-function oxidase enzyme pathway, formed a highly reactive toxic compound N-acetyl-p-benzoquinoneimine. N-acetyl-p-benzoquinoneimine is normally conjugated with glutathione and eventually

excreted as paracetamol cysteine and paracetamol mercapturate [7,8].

DPMK is new synthesized analgesic and antipyretic agent belonging to the class of paracetamol [9]. DPMK was synthesized by substitution of two paracetamol moieties, with methyl ketone linkage. In vivo study of DPMK in rat suggested that DPMK was more efficacious than paracetamol, particularly analgesic (66-68%) and antipyretic (93%) activity of DPMK is higher than paracetamol (analgesic 62-63% and antipyretic 80%) [9]. Although a good number of pharmacological activities possess the DPMK, the study of the pharmacokinetics and bioavailability properties of DPMK is yet to be conducted with Wistar albino rats. Hence the current research was focused on it.

The present study was designed to assess the pharmacokinetics of a single oral dose of 500 mg/kg of DPMK as compared with that of an equal dose of paracetamol in rats using blood and urine samples.

## MATERIALS AND METHODS

### Materials

The [4-amino N-acetyl] phenol (Paracetamol) (purity, 99.99%) was a generous gift sample from Cadila pharmaceuticals, Ahmedabad. Recrystallize form of [Di (4-amino N-acetyl) phenoxy] methyl ketone (DPMK) was used for the pharmacokinetic study. Commercial grade solvents and reagents were used without further purification. Double distilled water was used throughout the experimental work.

A double beam UV-visible spectrophotometer (Shimadzu-1700) equipped with a quartz cell of 1.0 cm path length was used to analyze DPMK and paracetamol concentration in blood and urine of rats.

Adult Wistar albino rats weighing between 250–320 g were used for the pharmacokinetic studies. The animals were maintained under normal laboratory condition and kept in standard polypropylene cages at  $30 \pm 2^\circ\text{C}$  temperature and 60 to 65% relative humidity. Rats were provided with standard diet and water *ad libitum*.

### Experimental design

Synthesized paracetamol derivative; [Di (4-amino N-acetyl) phenoxy] methyl ketone (DPMK) was used for pharmacokinetic study. The set of rules followed for animal experiment were approved by the Institutional Animal Ethical Committee (VBT/IAEC/10/12/40). Each group consists of four rats. All the animals were fasted for 18 h before the beginning of the experiment and water was given *ad libitum*. The animals of the group I was given paracetamol (500 mg/mL, orally) served as a control as well as reference standard. The animals of group II were orally administered with DPMK (500 mg/kg, orally). Blood samples (100  $\mu\text{L}$ ) were collected at the time intervals of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 10, 12 and 24 h from the tail vein of each rat. Immediately after, collection of 100  $\mu\text{L}$  blood sample from the tail vein of each rat was transferred in to in an Eppendorf test tube. A mixture of methanol and ethyl acetate solvents (1:2) was previously added in an Eppendorf test tube. The resulting mixture was shaken for about 5 minute by hand until the contents were properly mixed. Then after, the tubes were shaken for 5 minute on a vortex mixture and centrifuged at 2000 rpm for 5 minute at room temperature.

Urine samples (1.0 mL) were collected at the time intervals of 0, 6, 12, 24, 36, 48, 60, 72 and 84 h and measured the volume. 1.0 mL urine was diluted up to 100 mL with double distilled water. Blood supernatants and diluted urine samples were stored at  $-20^\circ\text{C}$  until the analysis.

### Standard preparation

1.0 mg/mL standard solution of DPMK was prepared in methanol. A 0.5 mL of standard solution of DPMK was properly mixed with 0.5 mL drug-free blood and 1 mL ethyl acetate. The resulting mixture was centrifuged at 2000 rpm for 5 min. The specific volume of blood supernatants was withdrawn and made the concentration of DPMK 1.25, 2.5 and 3.75  $\mu\text{g}/\text{mL}$ . 1.0 mL drug-free urine sample was mixed with 1.0 mL (1 mg/mL) of DPMK solution and finally diluted up to 100 mL with double distilled water. A certain volume of the diluted urine samples was sequentially withdrawn and made the concentration of DPMK 10, 15 and

20 µg/mL. Similarly, blood and urine samples of paracetamol were prepared by the above procedure. These samples were analyzed by the spectrophotometric method and used for precision, accuracy and stability testing. A calibration curve was constructed by spiking drug-free rat blood and urine in duplicate with a standard solution of DPMK and paracetamol and gave a concentration range between 5-65 µg/mL and 0.2-2 µg/mL respectively.

#### **Spectrophotometric conditions**

The spectrophotometric method was used for the determination of DPMK in blood and urine samples [9]. After treatment, blood supernatant or diluted urine sample was mixed with 1.0 mL, 0.5 N sodium nitrite, 1.0 mL 1 N hydrochloric acid and subsequently diluted up to 10 mL with 0.5 N sodium hydroxide solution, respectively. Then the resulting solutions were examined by spectrophotometrically at  $\lambda_{max}$  433 nm, after 5 min and 30°C temperature.

The paracetamol was measured from blood supernatants and diluted urine samples by spectrophotometrically [10]. After treatment, blood supernatant or diluted urine sample was mixed with 1.0 mL 1 M hydrochloric acid and 2.0 mL 1 mM ferric sulphate. The resulting solution was heated at 100°C in water bath for 10 min. Then after adding 2.0 mL 1 mM potassium ferricyanide and was diluted up to 10 mL with double distilled water. The resulting samples were analyzed by spectrophotometrically at  $\lambda_{max}$  700 nm, after 24 min.

#### **Method validation**

Method validation was performed according to the protocol using a nominal concentration range of standards spanning from 5-65 µg/mL and 0.2-2 µg/mL for DPMK and paracetamol in blood and urine to demonstrate the linearity, precision, accuracy and stability of the method. Stability was confirmed in blood and urine samples exposed to intra-day sample stored at - 20°C and was analyzed by spectrophotometrically day to day for a week.

#### **Pharmacokinetic analysis**

Pharmacokinetic parameters were calculated by non-compartmental analysis, according to the standard method with the

use of MS-Excel. Maximum drug concentration ( $C_{max}$ ) and corresponding time ( $T_{max}$ ) were measured directly from the drug-concentration-time plot. AUC from 0 to the last drug concentration ( $AUC_{0-t}$ ), AUC from 0 to infinity ( $AUC_{0-\infty}$ ), area under the first moment curve from 0 to the last drug concentration and infinity ( $AUMC_{0-t}$ ,  $AUMC_{0-\infty}$ ) were calculated by the Trapezoidal method. Elimination rate constant ( $K_{el}$ ) was determined from the slope of the elimination part of the drug-concentration time plot and elimination half life time ( $t_{1/2}$ ) was obtained from the equation:

$$t_{1/2} = 0.693 / K_{el}$$

Mean residence time (MRT) was calculated using the formula:

$$MRT = AUMC_{inf} / AUC_{inf}$$

The total clearance (CL) and apparent volume distribution ( $V_{ss}$ ) was calculated from the equation:  $V_{ss} = (Dose \times AUMC_{0-\infty}) / (AUC_{0-\infty})^2$

$$CL = K_{el} \times V_{ss}$$

The relative bioavailability was calculated as follows:

$$F = [AUC_{0-\infty}]_{test} / [AUC_{0-\infty}]_{Ref.}$$

From the urinary data: initiate and end time of each urine collection interval ( $\Delta t$ ), urine concentration (C), and urine volumes (V) from which the midpoint of each collection interval and the renal excretion rate for each interval (R) was computed according to equation:

$$R = CV / \Delta t$$

The maximal renal excretion rate ( $R_{max}$ ) was observed and the midpoint of the respective collection interval associated with the maximal observed excretion rate ( $T_{max}$ ) was also determined by visual inspection of the urinary excretion rate versus time profile curve. Area under the rate of drug excretion versus time curve ( $AURC_{0-tz}$ ) was calculated by the Trapezoidal method.  $AURC_{0-\infty}$  was determined by the equation:

$$AURC_{0-\infty} = AURC_{0-tz} + R_{tz} / K_{el}$$

The percentage fraction of drug excreted (fe) was calculated from the equation:

$$fe = [U_{inf} / Dose] \times 100$$

Where,  $U_{inf}$  = cumulative amount of drug at infinite time. Elimination rate constant ( $K_{el}$ ) was calculated from the curve of amount of

drug remaining to be excreted at a time (t) versus endpoint time.

### Statistical analysis

The pharmacokinetic parameters were calculated as a mean value  $\pm$  standard deviation (SD) (n = 4). Statistical analysis was performed by student t-test and one way ANOVA. A value of  $P < 0.05$  was considered to be statistically significant.

## RESULTS AND DISCUSSION

### Method validation

The DPMK from the blood and urine samples were determined by spectrophotometric method [9]. The DPMK was estimated from the blood samples after pretreatment with 1:2 mixture of methanol and ethyl acetate [9]. The DPMK from the urine samples were measured by using diluted urine (1:100) and no further treatment was required [9]. The spectrophotometric method was used for quantification of DPMK and provided the appropriated sensitivity and specificity. The experimental limit of quantification of the method was 0.85  $\mu\text{g/mL}$  and 0.05  $\mu\text{g/mL}$  for DPMK and paracetamol respectively. The limit of detection was 2.85  $\mu\text{g/mL}$  and 0.2  $\mu\text{g/mL}$  for DPMK and paracetamol respectively. Analysis of replicate blank blood and urine samples indicated no interference peaks.

The paracetamol was successfully estimated from the blood and urine samples by spectrophotometric method [10]. The similar pretreatment methods were used in blood and urine samples of paracetamol. The calibration curve was linear over the concentration range of 5-30  $\mu\text{g/mL}$  ( $r^2=0.999$ ) and 10-60  $\mu\text{g/mL}$  ( $r^2=0.999$ ) in blood and urine sample of DPMK respectively. The calibration range of 0.2-1.2  $\mu\text{g/mL}$  ( $r^2=0.996$ ,  $r^2=0.998$ ) in blood and urine sample of paracetamol. The calibration curves of DPMK and paracetamol in blood and urine samples are depicted in supporting information (**Fig. S1-4**). The inter-day and intra-day accuracy of the method was in ranged from 98.9-101.6% and 98.2-100.2% in blood and urine of DPMK respectively. The inter-day and intra-day accuracy of the method was in ranged from 98.7-101.4% and 98.7-101.1% in blood and urine of paracetamol respectively. The inter-day and intra-day

precision of the method was in ranged from 0.55-3.25% and 0.57-4.11% for DPMK in blood and urine, respectively. The inter-day and intra-day precision of the method was in ranged from 0.59-2.43% and 0.61-4.73% for paracetamol in blood and urine, respectively. The intra-day and inter-day, accuracy and precision of DPMK and paracetamol in blood and urine are summarized in (**Table S1 - 4**). No significant degradation of DPMK and paracetamol in blood and urine samples, were observed during one week under the storage condition.

### Pharmacokinetics studies

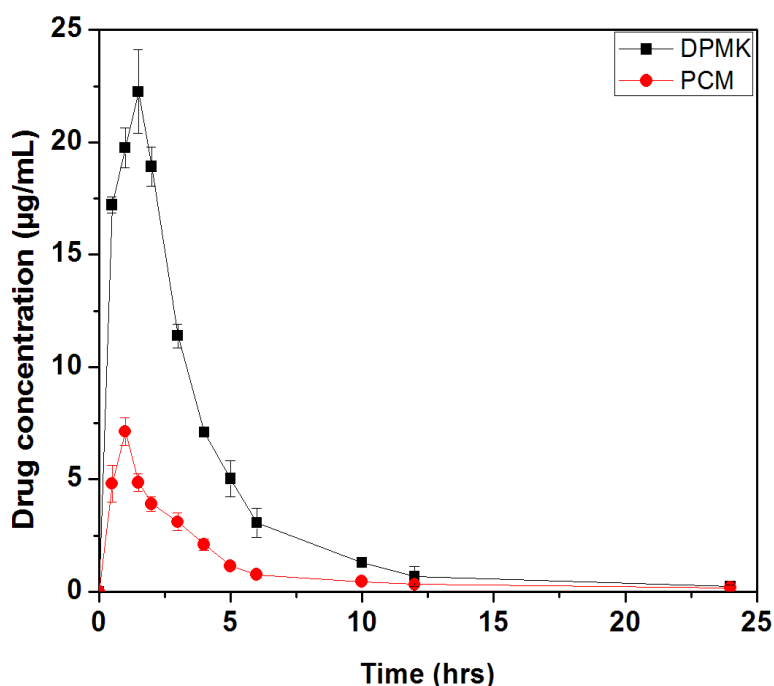
The pharmacokinetics study of DPMK was evaluated by non-compartment animal model and trapezoidal rule [11]. The pharmacokinetic parameters in blood sample are summarized in (**Table 1**). The mean concentration profile of DPMK is elucidated in (**Fig. 1**). The concentration-time profiles of DPMK clearly indicated declines in plasma concentrations of DPMK at different sampling time. The area under the curve  $AUC_{(0-t)}$ ,  $AUC_{(0-\infty)}$  and area under the first moment curve  $AUMC_{(0-\infty)}$  values were calculated from the curve of concentration of DPMK vs. time (**Fig. 1**) and the concentration of DPMK and time vs. time (**See in SI, Fig. S5**). The value of the AUC, which is indicative of the extent of drug absorption. DPMK showed higher AUC value ( $85.82 \pm 8.67 \mu\text{g h/mL}$ ) as compared to standard paracetamol ( $20.51 \pm 1.79 \mu\text{g h/mL}$ ) that indicates DPMK showed significantly more absorption than paracetamol. Further,  $T_{max}$ , which indicates the rate of absorption of the drug, DPMK was found to be higher rate of absorption, because of higher  $T_{max}$  (1.5 h) value than paracetamol (1 h). Moreover,  $C_{max}$  value, indicative of the intensity of therapeutic and toxic response, was found to be higher in DPMK ( $C_{max} = 22.26 \pm 1.85 \mu\text{g/mL}$ ) as compared to paracetamol ( $C_{max} = 5.18 \pm 0.57 \mu\text{g/mL}$ ). The mean residence time (MRT) is indicative of average total time drug molecules of given dose remain in the body, was found to be  $4.29 \pm 0.96 \text{ h}$  in DPMK that was significantly higher than the paracetamol ( $3.95 \pm 0.16 \text{ h}$ ). The elimination rate constant ( $K_{el}$ ) of DPMK did not show more significant differences as

compared to the paracetamol, but elimination half life ( $t_{1/2}$ ) was found to be significantly higher than paracetamol. The apparent volume distribution ( $V_{ss}$ ) and a total clearance of DPMK were found to be significantly lower than paracetamol. The relative bioavailability of DPMK in terms of  $AUC_{(0-\infty)}$  and  $AUMC_{(0-\infty)}$  was obtained 4.18 and 4.62, respectively. The results suggest that the DPMK has lower bioavailability as compared to the previous reported bioavailability of paracetamol (60-90%).

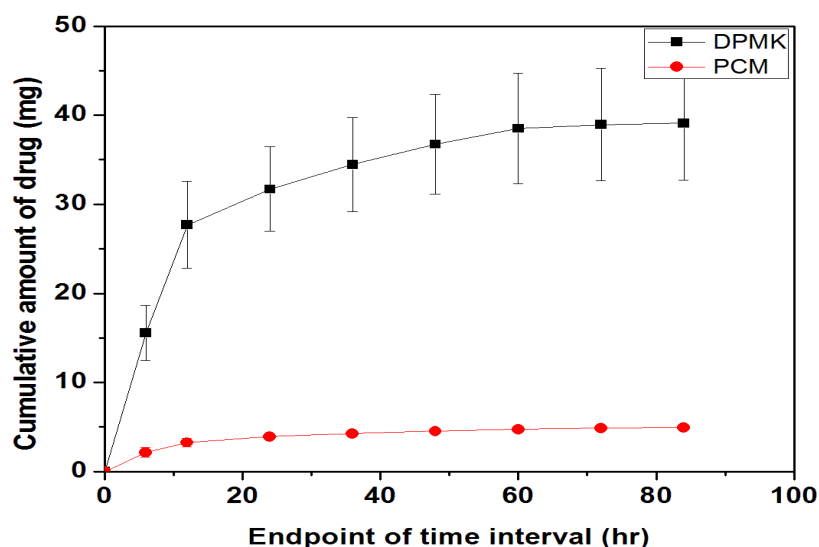
No clinical or biological side effects were reported during the urinary study. The plots of the mean cumulative amount of DPMK over a period of 84 h versus endpoint of time intervals and rate of excretion versus the midpoint of time intervals for all rats are shown in (Fig. 2 & 3). From these figures, it is evident that both DPMK and paracetamol show a different excretion pattern, which in turn, indicates differences in their bioavailability.

**Table 1: The pharmacokinetics parameters (Mean  $\pm$  SD) of DPMK and paracetamol (blood sample)**

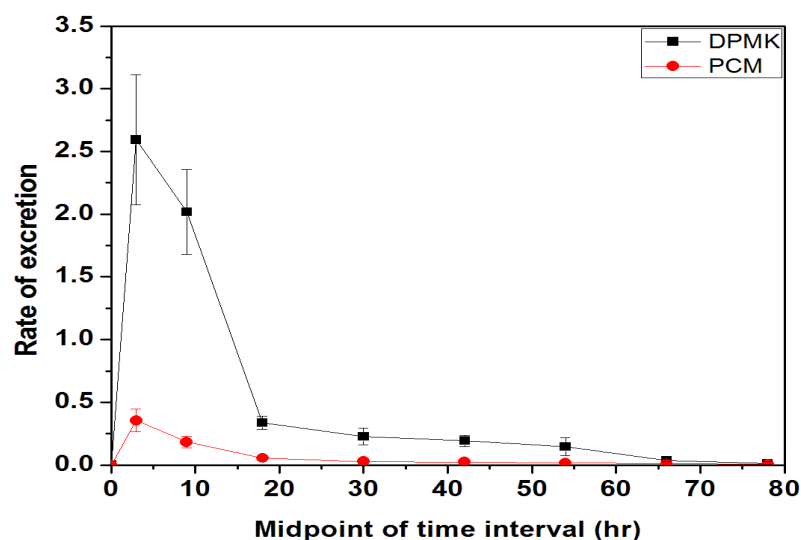
Pharmacokinetic parameter	DPMK	Paracetamol	Relative bioavailability (F)
$T_{max}$ (h)	1.5 $\pm$ 0.00*	1.0 $\pm$ 0.00*	-
$C_{max}$ ( $\mu\text{g}/\text{mL}$ )	22.26 $\pm$ 1.85*	5.18 $\pm$ 0.57*	-
MRT (h)	4.29 $\pm$ 0.96*	3.95 $\pm$ 0.16*	-
Elimination rate constant ( $K_{el}$ ) ( $\text{h}^{-1}$ )	0.24 $\pm$ 0.06*	0.25 $\pm$ 0.01*	-
Elimination half life ( $t_{1/2}$ ) (h)	2.98 $\pm$ 0.67*	2.74 $\pm$ 0.11*	-
$AUC_{(0-\infty)}$ ( $\mu\text{g h}/\text{mL}$ )	85.82 $\pm$ 8.67*	20.51 $\pm$ 1.79*	4.18
$AUMC_{(0-\infty)}$ ( $\mu\text{g h}^2/\text{mL}$ )	374.01 $\pm$ 111.86*	80.97 $\pm$ 6.72*	4.62
Total clearance (CL) (lit/h)	1.71 $\pm$ 0.24*	7.43 $\pm$ 0.89*	-
Apparent volume distribution ( $V_{ss}$ ) (lit)	7.24 $\pm$ 1.51*	29.40 $\pm$ 4.17*	-



**Figure 1: Mean drug concentration-time curves in rat after single oral administration of 500 mg/kg of DPMK and Paracetamol**



**Figure 2: Mean cumulative amount of drug vs. endpoint of time curves in rat after single oral administration of 500 mg/kg DPMK and Paracetamol**



**Figure 3: The rate of excretion vs. midpoint of time curves of DPMK and paracetamol after 500 mg/kg oral administration**

Urinary recovery of DPMK and paracetamol are provided in (Table 2). About 39.11 mg and 4.95 mg of DPMK and paracetamol were excrete in urine ( $Ae_{0-t}$ ) at 85 h after oral administration in rats, respectively. Mean  $AURC_{0-t}$  values were found to be 338.57 mg and 42.75 mg for DPMK and standard paracetamol, respectively. The maximum amount of DPMK and paracetamol excreted in respective time

intervals ( $R_{max}$ ) was 2.59 mg/h and 0.36 mg/h, respectively. It was also observed that both DPMK and paracetamol showed maximum excretion rates in the interval of 3 h ( $T_{max}$ ) in term of midpoint of time (Fig. 3). The elimination rate constant ( $K_{el}$ ) was calculated from the semi-log graph of the amount of drug remaining to be excreted at a time versus endpoint time (See in SI, Fig. S6). The elimination rate constant of DPMK

and paracetamol did not show a significant difference, but elimination half life ( $t_{1/2}$ ) of DPMK was found to be lower than paracetamol. Fraction of DPMK unchanged in urine was found to be 2.7 % that were

lower than paracetamol (3.30 %). The relative bioavailability of DPMK in terms of  $AURC_{0-t}$ ,  $AURC_{0-\infty}$  and  $Ae_{0-t}$  for urinary study were found to be 7.92, 7.89 and 7.9, respectively.

**Table 2: The pharmacokinetics parameters (Mean  $\pm$  SD) of DPMK and paracetamol (urine sample)**

Pharmacokinetic Parameter	DPMK	Paracetamol	Relative bioavailability (F)
Tmax (h)	3.0 $\pm$ 0.00*	3.0 $\pm$ 0.00*	-
Rmax (mg/h)	2.59 $\pm$ 0.52*	0.36 $\pm$ 0.09*	-
Elimination rate constant (Kel) ( $h^{-1}$ )	0.05 $\pm$ 0.01*	0.04 $\pm$ 0.01*	-
Elimination half life ( $t_{1/2}$ ) (h)	14.23 $\pm$ 2.03*	19.26 $\pm$ 4.15*	-
$AURC_{(0-t)}$ (mg)	338.57 $\pm$ 53.54*	42.75 $\pm$ 1.66*	7.92
$AURC_{(0-\infty)}$ (mg)	338.86 $\pm$ 53.76*	42.97 $\pm$ 1.58*	7.89
fe (%)	2.7 $\pm$ 1.00*	3.30 $\pm$ 0.3*	-
$Ae_{(0-t)}$ (mg)	39.11 $\pm$ 6.41*	4.95 $\pm$ 0.26*	7.90

## CONCLUSION

In conclusion, Pharmacokinetic study of [Di (4-amino N-acetyl) phenoxy] methyl ketone was evaluated in rats and this study may be the first evaluation of pharmacokinetics of DPMK. DPMK showed high extent of absorption, high rate of elimination and more intensive therapeutic effect as compared to the paracetamol. The present pharmacokinetic results of DPMK were indicated lower bioavailability than the standard paracetamol.

## ACKNOWLEDGEMENT

The authors acknowledge the Vidyabharti Trust College of Pharmacy, Umrakh providing the facility to carry out the research work. The authors thank Dr. A. V. Shah for helping in the research work.

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