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PHARMACOKINETIC STUDY OF *ANDROGRAPHIS PANICULATA* ETHANOLIC EXTRACT TABLET

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ABSTRACT: *Andrographis paniculata* (Burm. F) Nees, commonly known as the "king of bitters," is an herbaceous plant belonging to the Acanthaceae and is found throughout tropical and subtropical Asia, Southeast Asia, and India. Andrographolide, a bitter diterpenoid, is the major active principle present in the plant. Authors have already worked on the phytochemical investigation, analgesic and antipyretic activities of ethanolic extract of *Andrographis paniculata*. Present study aimed to pharmacokinetic study of an ethanolic extract of *Andrographis paniculata* tablet in rat. Solid dispersion tablets of *Andrographis paniculata* extract equivalent to 50 mg/Kg was prepared and given to rats and various pharmacokinetic parameters such as maximum concentration of andrographolide in plasma (C_{max}), elimination rate constant (K), Elimination half-life ($t_{1/2}$) Area under the plasma andrographolide concentration- time curve up to measurable concentration ($AUC_{(0- t)}$), The area under the curve zero to infinity ($AUC_{0-\infty}$), mean residence time (MRT) and relative bioavailability (F_{rel}) were determined and also compared with pure andrographolide. Results showed that there significant increase in various pharmacokinetic parameters of solid dispersion tablet as compared with pure andrographolide.

INTRODUCTION: *Andrographis paniculata* (Burm. f.) Wall. ex Nees., (Family Acanthaceae) (English name-King of Bitters, Tamil name-Nilavempu) is an annual herbaceous plant and is extensively cultivated in Southern Asia, China and some parts of Europe.

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The plant mainly contains bitter diterpenoid lactone andrographolide **Fig. 1** and related compounds like deoxy andrographolide, 11, 12-didehydro-14-deoxy-andrographolide, neoandrographolide, and andropanoside. In traditional medicine, *A. paniculata* is widely used to get rid of body heat, dispel toxins from the body; prevent the common cold, upper respiratory tract infections including sinusitis and fever and as an antidote against poisons of snakes and insects [1 - 5]. The plant has been reported to exhibit a various mode of biological activities *in-vivo* as well as *in-vitro viz*., antibacterial, antiviral, anti-inflammatory, anti-HIV (Human immune-deficiency virus), immune-

modulating/ immunostimulatory and anticancer. The plant showed potential therapeutic action in curing liver disorders, common cough, and colds in human. The characteristic secondary metabolites encountered in this plant have considerably enhanced its importance in the arena of medicinal plants [6 – 11].

Authors have already done a phytochemical investigation, analgesic and antipyretic activities of ethanolic extract of *Andrographis paniculata*. Chemicals content in the extract was determined by high-performance thin layer chromatography and gas chromatography mass spectrometry investigations indicated presence of steroids, phenols, terpenoids, alkaloids, saponins, flavonoids *etc*. The various concentrations ethanolic extract showed significant analgesic activity in hot-plate method and acetic acid-induced writhing in mice. Mechanism of action was determined by studying prostaglandin inhibition.

Studies showed that KEE inhibits the synthesis of prostaglandin as evidenced by inhibition of diarrhea induced by Castor oil. The findings of the present study indicate ethanolic extract *Andrographis paniculata* shows significant analgesic and antipyretic activity [12].

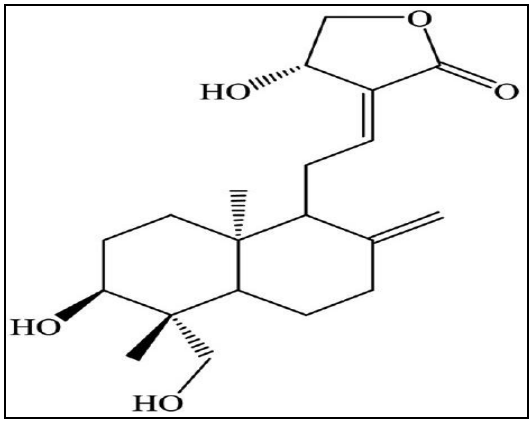

FIG. 1: CHEMICAL STRUCTURE OF ANDROGRAPHOLIDE [13]

Present study aimed to study various pharmacokinetic parameters such as maximum concentration of andrographolide in plasma (C_{max}), elimination rate constant (K), Elimination half-life ($t_{1/2}$) Area under the plasma andrographolide concentration-time curve up to measurable concentration ($AUC_{(0-t)}$), The area under the curve zero to infinity ($AUC_{0-\infty}$), mean residence time (MRT) and relative bioavailability (F_{rel}) of *Andrographis paniculata* ethanolic extract solid dispersion tablet in rats.

MATERIALS AND METHODS:

Materials: Ethanolic extract of *Andrographis paniculata* aerial part, pure andrographolide was purchased from Maysar Herbals. Lactose and starch were procured from Loba Chemie Pvt. Ltd., Mumbai. HPLC grade methanol was obtained from Thermofisher Mumbai. All other chemicals used were of analytical and HPLC grade.

Collection and Authentication of the Plant Material: The aerial parts of plant of kariyat were collected from surrounding areas of Taluka Shirol, District Kolhapur and Maharashtra. The plant was washed with distilled water, dried at room temperature under shade. The sample was identified and authenticated by "Nikhil analytical & research laboratory (ISO 9001-2008 certified, central Government Approved for Agmark), Sangli, Maharashtra, India as well as by Head, Department of Botany Dattajirao Kadam Arts, Science & Commerce College, Ichalkaranji, Taluka Hatkanagale, Dist. Kolhapur, Maharashtra, India.

Methods:

Extraction of *Andrographis paniculata*: Dried aerial part of the *Andrographis paniculata* was ground to obtain coarse powder using an electric grinder. Powdered drug was extracted with ethanol in 25% concentration (25 gm of weighed powder) and 100 ml of solvent at 60 °C - 70 °C), in a continuous hot extraction method using Soxhlet extractor. The extract was concentrated in a rotary flash evaporator (Hahnvapor, Hahnshin Scifintic Korea) and the residue was dried in vacuum desiccators over anhydrous calcium chloride to yield ethanolic extract (KEE). The extract was stored in a refrigerator at 4ºC for further use.[12] Solid dispersion Tablet of ethanolic extract of andrographolide was prepared using Soluplus and was used to study various pharmacokinetic parameters in rat.

Pharmacokinetic study of an ethanolic extract of *Andrographis paniculata* tablet in rats: A simple, selective and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method was selected for the analysis of *Andrographis paniculata* extract [14, 15].

Instrumental Specification: A sample was resolved on a Shimadzu [(UV2075plus)/(Jasco UV-1575)] ODS Hypersil C18 column (4.6 mm x 250 mm5µm).

Mobile Phase: 65 volumes of methanol and 35 volumes of water were used.

Preparation of Standard Solution: Standard working solution was prepared by dissolving 10mg of *Andrographis paniculata* extract with 100mL of methanol to give a concentration of 100 µg/mL.

Preparation of Serum Samples for Calibration Curve: 0.2 mL of rat serum samples were vortexed (extracted) with methanol using 1mL v/v for 2 min and centrifuged up to 5min. After centrifugation supernatant layer up to 0.9 mL was separated and evaporated. Finally, dry residue was reconstituted with mobile phase.

Calibration curve range: 10, 20, 30, 40, and 50 µg/mL in rat serum.

Pharmacokinetic Study in Rats: Albino rats of either sex weighing (250-300 g) were divided into 3 groups, each consisting of 6 animals. First group received ethanolic extract solid dispersion tablet (equivalent to 50 mg/Kg). Second group received a pure sample of Andrographolide (50 mg/ Kg) and third group was kept under control. The rats were restrained in a rat holder during blood sampling.

The initial blood sample was taken by clipping the end of the rat's tail while the subsequent blood samples were collected by removing the clotted blood with cotton rinsed with 100 IU of heparin solution. After each blood sampling, the wound was monitored for approximately 3 min to ensure there was no excessive bleeding. Blood samples were immediately transferred to a heparinized micro centrifuge tube and centrifuged at 3000 rpm for 10 min at 5 ºC.

The resulting plasma samples (0.2 mL supernatant) was transferred into 1.5 mL Eppendorf tubes and stored at - 80 ºC until HPLC analysis. Blood samples were collected at time intervals of 0 min (predose), 0.5, 1, 2, 4, 8, 12, 16 and 24 hrs after dosing [16]. The Institutional Animal Ethical Committee approved the protocol for this study (Ref. No. SETCP/IAEC/2017-2018/022).

Determination of Various Pharmacokinetic Parameters: [17, 18] The pharmacokinetic parameters such as a maximum concentration of andrographolide in plasma C_{max}, the time for the andrographolide to reach maximum concentration in plasma after administration t_{max}, were computed directly from measured plasma concentration data.
The elimination rate constant (K) was estimated from the terminal slope of the plasma concentration-time curve values.

$$K = C7 – C8 / T8 – T7$$

Elimination half-life $t_{1/2}$ was calculated from quotient 0.693/K

$$t_{1/2} = 0.693/K$$

Area under the plasma andrographolide concentration-time curve up to measurable concentration, $AUC_{(0- t)}$ was calculated using the trapezoidal rule. The area under the curve zero to infinity, $AUC_{(0- \alpha)}$ was calculated by the equation:

$$AUC_{0- \alpha} = AUC_{0- t} + C_t / K$$

Where C_t is the last measurable concentration.

To assess the degree of retardation of drug release mean residence time (MRT) was calculated.

$$MRT = 1 / K$$

Relative bioavailability (F_{rel}) of TEL from TSM1 and TSM4 to that of plain TEL suspension was calculated using formula: [19]

$$F_{rel} = \frac{AUC_{test} / Dose_{test}}{AUC_{reference} / Dose_{reference}} \times 100$$

All results were expressed as mean ± SD. The data from different formulations were compared for statistical significance by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION:

Extraction of Andrographolide from *Andrographis paniculata*: Extraction of Kariyat was carried out by continuous hot extraction method by Soxhlet apparatus using ethyl alcohol. The temperature of apparatus was kept around 60 - 70 ºC to avoid degradation of chemical constituents. Thus obtained ethanolic extract was further concentrated by rotary evaporator to get greenish brown colored slurry and around 8.13 % yield was obtained.

Pharmacokinetic Study of Andrographolide Ethanolic Extract Tablet in Rats: So as to determine various pharmacokinetic parameters of andrographolide ethanolic extract solid dispersion tablet in rat a selective and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method was used. Chromatogram of andrographolide in rat plasma is shown in **Fig. 2**. Peak overlay of different concentration of andrographolide in rat plasma is shown in **Fig. 3**.

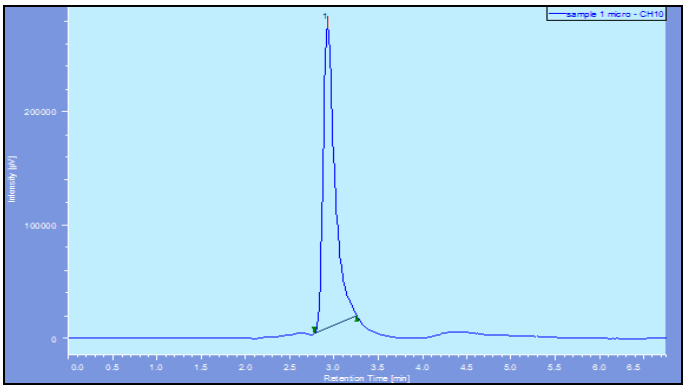

FIG. 2: CHROMATOGRAM OF ANDROGRAPHOLIDE IN RAT PLASMA

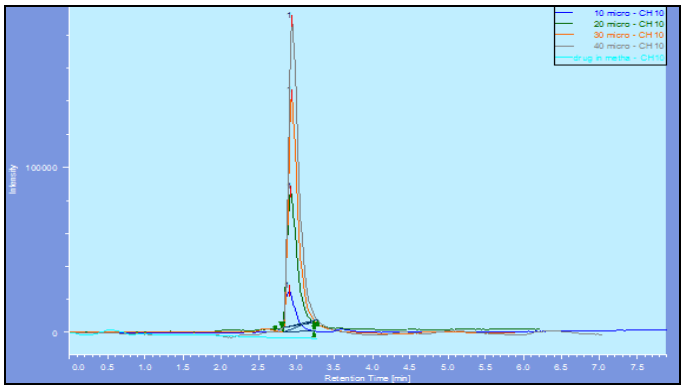

FIG. 3: PEAK OVERLAY OF DIFFERENT CONCENTRATION OF ANDROGRAPHOLIDE IN RAT PLASMA

Fig. 4 shows calibration curve of andrographolide in rat plasma with linearity equation and R2 value of 0.997. Hence this calibration curve was found to be linear. Plasma concentration of andrographolide extract solid dispersion tablet and pure andrographolide versus time profile was determined after application of a single oral dose (50 mg/kg) of andrographolide in rats. The concentration versus time profile is shown in **Fig. 5**.

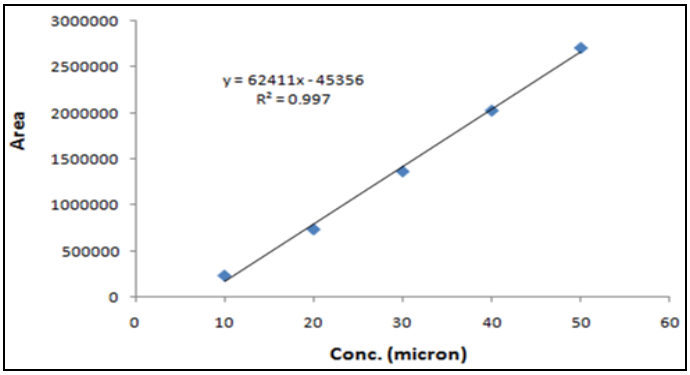

FIG. 4: CALIBRATION CURVE OF ANDROGRAPHOLIDE IN RAT PLASMA

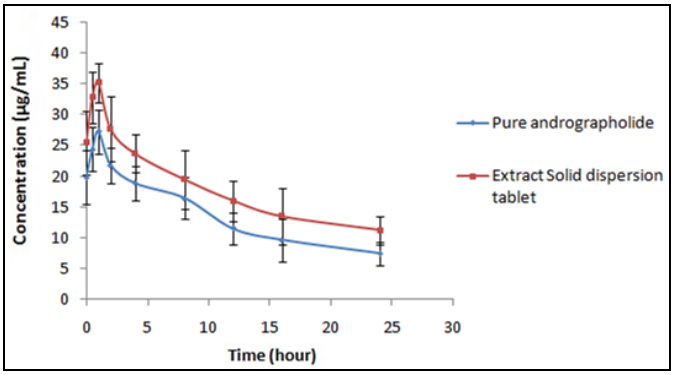

FIG. 5: PLASMA CONCENTRATION VERSUS TIME PROFILE OF *ANDROGRAPHIS PANICULAT* ETHANOLIC EXTRACT SOLID DISPERSION TABLET AND PURE ANDROGRAPHOLIDE

From **Fig. 5**, maximum plasma drug concentration (C_{max}) of pure andrographolide and extract tablet was found to be 27.24±3.23µg/mL and 35.22±3.54µg/mL respectively. Similarly, time required to reach maximum concentration (t_{max}) was found to be 1 h for both. From trapezoidal rule, AUC_{0-t} was calculated. AUC_{0-24} was found 84.3±10.04µg.h/mL for pure andrographolide and 99.00±11.45µg.h/mL for extract tablet. The elimination rate constant (K) was estimated from the terminal slope of the plasma concentration-time curve values. Elimination rate constant was found to be 0.059±0.0011h^{-1} for pure andrographolide and 0.047±0.002 h^{-1} for extract tablet. From the value of K, elimination half-life $t_{1/2}$ was calculated and it was found to be 17.74±0.02 h and 11.74±0.04 h for extract tablet and pure andrographolide respectively. $AUC_{0-\alpha}$ i.e. area under the curve for zero to infinity was calculated and it was found 429.7±43.4 µg.h/mL of extract tablet and 420.0±32.2 µg.h/mL for pure andrographolide. Mean residence time (MRT) of extract tablet and pure andrographolide was found to be 21.27±3.1 h and 16.94±4.3 respectively. All these pharmacokinetic parameters are summarized in **Table 1**. All results of pharmacokinetic parameters

of andrographolide extract tablet were found to be near about similar to that of pure andrographolide.

TABLE 1: PHARMACOKINETIC PARAMETERS OF ANDROGRAPHOLIDE EXTRACT TABLET AND PURE ANDROGRAPHOLIDE

Parameters	Extract tablet	Pure andrographolide
C_{max} (µg/mL)	35.22±3.54	27.24±3.23
T_{max} (h)	1	1
Slope K (1/h)	0.047±0.002	0.059±0.001
Plasma half-life $T_{1/2}$ (h)	17.74±0.02	11.74±0.04
AUC_{0-t} (µg.h/mL)	99.00±11.45	84.3±10.04
$AUC_{0-\alpha}$ (µg.h/mL)	429.7±43.4	420.0±32.2
MRT (h)	21.27±3.1	16.94±4.3
F_{rel} (%)	117.43±14.6	-

Results of a pharmacokinetic study of ethanolic extract tablet and pure andrographolide showed that there was 1.29 fold improvement of C_{max} of ethanolic extract solid dispersion tablet as compared to that of pure andrographolide without a change in T_{max}. It reveals that there was a significant increase in absorption of andrographolide from solid dispersion tablet as compared to pure andrographolide. The difference was very significant ($p < 0.01$) as compared to pure andrographolide.

It was also observed that AUC_{0-t} of ethanolic extract solid dispersion tablet was 1.17 fold higher than that of pure andrographolide and difference was highly significant ($p < 0.01$) as compared to AUC_{0-t} of pure andrographolide. The relative bioavailability of andrographolide from solid dispersion tablet to that of pure andrographolide was found to be 117.43±14.6.

CONCLUSION: Present study was aimed to study various pharmacokinetic parameters such as maximum concentration of andrographolide in plasma (C_{max}), elimination rate constant (K), Elimination half-life ($t_{1/2}$) Area under the plasma andrographolide concentration-time curve up to measurable concentration ($AUC_{(0- t)}$), The area under the curve zero to infinity ($AUC_{0-\infty}$), mean residence time (MRT) and relative bioavailability (F_{rel}). From results, it can be concluded that maximum concentration of andrographolide in plasma is reached within 1h and there are 1.29 fold improvements in C_{max} of ethanolic extract solid dispersion tablet as compared to that of pure andrographolide. The study concluded that relative bioavailability of andrographolide can be increased when it is prepared in the form of solid dispersion tablet.

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CONFLICTS OF INTEREST: No conflicts of interest.

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