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Biopharmaceutical profiling of sesamol: physiochemical characterization, gastrointestinal permeability and pharmacokinetic evaluation

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ABSTRACT

Sesamol has been studied extensively for its curative role in various diseases owing to its antioxidant potential, in the past two decades. In vitro and in vivo pre-clinical studies indicate diverse role of sesamol in ailments connected by a common denominator of oxidative stress. These include anti-oxidant, anti-mutagenic, neuroprotective, hepatoprotective, cardioprotective, chemopreventive and anti-ageing properties. However, in depth investigation of its important characteristics including its oral pharmacokinetics is warranted, before evaluating this molecule for clinical application. Present study was undertaken to determine physicochemical properties viz. solubility, log P, pKa and distribution coefficient of sesamol, coupled with its regional permeability through rat GIT. Single dose oral pharmacokinetic and tissue distribution studies of sesamol were also conducted in rats. The results indicate sesamol to be a molecule with appropriate aqueous solubility (~38.8 mg/mL) and log P (1.29); pKa of sesamol was found to be 9.79 and it exhibited a distribution coefficient >1. Sesamol was well absorbed throughout the GIT and showed an oral bioavailability of 95.61%. Sesamol is widely distributed to rat tissue with highest concentration in kidney followed by lung, brain, and liver. In spite of a favorable bioprofile, the wide distribution, small $t_{1/2}$ and fast clearance of sesamol indicate a need for packaging it into a suitable delivery system.

1. INTRODUCTION

Sesamol (1, 3-benzodioxol-5-ol) is an established antioxidant, which is extracted from roasted seeds of sesame (*Sesamum indicum Linn*.), belonging to the family Pedaliaciae[1]. The benzodioxole group of sesamol is known to scavange hydroxyl radical to produce 1,2-dihydroxybenzene, which is again an antioxidant. Sesamol has also been found to scavenge DPPH radicals[2], imidazoquinoxaline-type radicals and also superoxide anion, hydroxyl radical and singlet oxygen[3, 4]. It has also been reported to inhibit hydroxyl radical induced deoxyribose degradation and DNA cleavage[5, 6].

Furthermore, it has been reported to prevent generation of the imidazoquinoxaline-type heterocyclic amines in the heated model system composed of glucose/glycine/creatinine in aqueous diethylene glycol. ESR-spin trapping studies using 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and N-tert-butyl-alpha-phenylnitrone (PBN) showed that the heated model mixture of glucose/glycine or glucose/glycine/creatinine generated unstable carbon-centred radical(s), and their formation was effectively inhibited by sesamol[3].

Hence, it is likely that sesamol reacts equally with the basic reactive oxygen species (ROS) and the imidazoquinoxaline-type heterocyclic amines.

Earlier, we have comprehensively evaluated and reported the full spectrum of in vitro scavenging potential of sesamol[7] at a range of doses and in a variety of test systems including DPPH assay, $^{\bullet}O_2$ scavenging, H_2O_2 scavenging, $^{\bullet}OH$ scavenging, NO scavenging, and liver and brain lipid peroxidation. Sesamol exhibited high superoxide and NO scavenging which was even significantly better than catechin and epicatechin (IC_{50} for sesamol, catechin, epicatechin and ascorbic acid was 130.4, 188.3, 212.8, and 326.4 nmoles, respectively; unpublished work). Sesamol has so far been reported to exhibit numerous beneficial properties such as neuroprotective[8, 9], cardioprotective[10, 11], hepatoprotective[12], anti-mutagenic[10], renoprotective[13], radioprotective[14], anti-ageing[15, 16] and anti-inflammatory effects in various in vitro and pre-clinical models[17, 18].

Lipinski's rule states that, in general, an orally active drug should not violate more than one of the following criteria, which include: a) molecular mass of <500 daltons, b) octanol-water partition coefficient i.e. log *P* not greater than 5, c) not more than 10 hydrogen bond acceptors and d) not more than 5 hydrogen bond donors. Sesamol fulfils all the criteria of Lipinski's rule and manifests

a wide spectrum of beneficial biological effects in the control of various disorders. However, inadequate information on the bioavailability and pharmacokinetic parameters of sesamol hamper its categorization as a therapeutic. Main objective of the present study was to gain preliminary pharmacokinetic information (in terms of blood/plasma concentration and tissue concentration) of sesamol in rats, coupled with its physicochemical characteristics (viz. organoleptic properties, solubility, pH-solubility profile, pKa determination, partition coefficient, and distribution coefficient), which monitor the biopharmaceutical performance of a drug.

For developing oral drug delivery systems, it is important to know the site of absorption, so that the formulation can be prepared and administered to provide better bioavailability and hence therapy. As no literature exists on the gastrointestinal permeability of sesamol, another objective of the present study was to determine regional permeability of sesamol through various segments of rat GIT, using ligated loop technique[19, 20]. Very few studies report on the oral pharmacokinetic profile of sesamol. Sesamol (100 mg/kg body weight in rats) is reported to be eliminated from the body within 0-4 hours of its oral administration, as conjugates[21]. The maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and area under curve (AUC) of sesamol was found to be 1.4±0.7 µg/mL, 563.7±36.9 min, and 501.3±200.8 min µg/mL, respectively, following oral administration of 50 mg/kg body weight dose of sesamol to rats[22]. A rapid decline in plasma concentrations and an oral bioavailability (BA) of 35.5 ± 8.5% has been reported for sesamol in Sprague Dawley rats[22]. Sesamol is reported to undergo reductive cleavage to 2-methoxybenzene-1,4-diol and benzene-1,2,4-triol[21]. Small quantities of these metabolites are observed in the urine of rats fed with sesamol. Moreover, sesamol or its metabolite were not detected when sesamol was incubated in vitro with rat fecal microbiota . Sesamol is excreted unchanged in the excreta while as a glucuronide in the urine. It is indicated that sesamol and its conjugated metabolites are rapidly eliminated from urine and feces in 0–4 h[21].

There is no information on the chronic toxicity or carcinogenicity of sesamol in humans. Sesamol when administered for a period of upto 2 years was reported to induce stomach cancer[23, 24] especially the forestomach of rodents. However, the TD_{50} in these experiments was determined to be as high as 16.2 and 41.3g (300 mmoles/day). Considering the high dose and long duration (2 years) and the fact that humans do not have forestomach, the observation to cause forestomach cancers is highly insignificant. Furthermore, sesamol, butylated hydroxyanisole and caffeic acid

are referred to as non-genotoxic carcinogens and the hyperplasia and papilloma induced by these agents readily regress after cessation of administration[25-28]. Moreover the dose at which sesamol has been reported to exhibit therapeutic potential in various models of diseases, lies between 2 to 20 mg/kg in rats, which is much below (<200 times) the 50% carcinogenic dose (TD_{50}). Based on the maximum effective dose of 20mg/Kg in rats, the human equivalent dose would be (human dose = 20/6.17) 3.24 mg/kg BW and for an adult weighing 60 kg (60 kg X 3.24= 194.4 mg) a dose of ~ 200 mg will be recommended which is much below the TD_{50} dose.

Plentiful of pharmacodynamic data on sesamol produced from our lab[7, 9, 13] and otherwise indicate 4 and 8 mg/Kg dose to show significant response. Hence, presently we performed single dose oral and i.v. pharmacokinetic and tissue distribution studies for sesamol at 4mg/Kg.

2 Experimental

2.1. Materials

Sesamol was obtained from Sigma Chemical Co., St Louis, MO, USA. All other chemicals and reagents were of analytical grade and were used without further purification. All protocols involving animal studies were duly approved by the Institute Animal Ethics Committee (IAEC) of Panjab University, Chandigarh, India.

2.2. Physicochemical characteristics

2.2.1. Organoleptic properties: Organoleptic properties such as, nature, colour and odour of sesamol were physically observed. The melting point was determined using melting point apparatus.

2.2.2. Solubility: For the determination of solubility, excess amount of sesamol was placed in 20 mL vials; 5 mL water was added and the contents were shaken in a water bath at 37 ± 1°C. Samples were withdrawn after 48h, filtered and analyzed spectrophotometrically at λ_{max} of 294nm after appropriate dilution. The study was conducted in triplicate.

2.2.3. pH-Solubility profile: For the determination of pH-solubility profile, excess amount of the sesamol was placed in 20 mL vials containing 5 mL of respective buffers at ranging from pH 1-13. The contents were shaken in a water bath at 37 ± 1°C for 48 h. Samples withdrawn after saturation were filtered and analyzed by UV method after appropriate dilution. The study was conducted in triplicate.

2.2.4. Determination of ionization constant (pKa): Ionization constant means those constants which are used to measure the strength of acids and bases. The ionization constant (pKa) of sesamol was determined using Krebs and Speakman solubility method[29]. Solubilities of the respective neutral molecular species (Si) and the solubility at a pH (a closer range of pH values were selected) near the suspected pKa value (S_o) were determined. Then pKa values were calculated by employing the following equation:

$$pKa = pH - \log [(S_0/Si)-1]$$

2.2.5. Partition coefficient*:* Partition coefficient of sesamol was determined by shake-flask method. Equal volumes of n-octanol and water (1:1) were mixed and kept for mutual saturation, overnight. Then to the above mixture, a weighed quantity of sesamol was added and shaken horizontally 100 times in 5 min. The samples were centrifuged at 5,000 rpm for 10 min and the organic layer was analyzed by UV method. The drug concentration in aqueous medium (C*aq*) was calculated by subtracting the drug concentration in octanol layer (C*oct*) from the initial drug concentration. Partition coefficient was calculated as follows:

$$\text{Partition coefficient (P.C.)} = \frac{Concentrat\ \ ion \text{ of drug in n - octanol (Coct)}}{Concentrat\ \ ion \text{ of drug in water (Caq)}}$$

2.2.6. Distribution coefficient: The procedure was similar to that described above in section 2.2.5.; except that buffers of pH ranging from 1 to 7 were used instead of water as the aqueous phase.

2.3. Gastrointestinal permeability: The gastrointestinal permeability characteristics of sesamol were determined in Sprague-Dawley rats using the ligated-loop technique. The permeability studies in stomach were carried out using the procedure given by Fiese and Perrin[19]. For studies in intestinal segments, the procedure by Levine and Perikan[20] was adopted.

Briefly, the animals were fasted for 12 to 16 h before studies. They were anaesthetized by intra-peritoneal administration of urethane (1.5 g kg^{-1}) and kept on a wax tray. The temperature on the tray was maintained at 37 °C with the help of a table lamp. After making an abdominal incision, the GIT was exposed and divided into loops comprising of stomach, duodenum, jejunum and ileum. For preparing the loops, the exposed stomach was ligated immediately adjacent to the cardiac sphincter. Care was taken to avoid damage to any blood vessels during the experiment. A second ligature was placed next to the pyloric sphincter. A needle with silicone tubing was carefully introduced through the duodenum to project into the stomach *via* the pyloric sphincter, before the ligature was finally secured. Three intestinal loops of 10 cm each were prepared in the

same way as above. The duodenal loop was made about 1 cm from the pylorus. The jejunal loop was made immediately next to the duodenal loop. The ileal loop was made about 5 cm above the ileo-cecal junction. The bile duct was also ligated to avoid the entero-hepatic drug circulation. The intestinal loops were washed with 10 mL saline solution and a needle with silicone tubing was inserted into each of them.

Using a syringe, 1 mL of drug solution, prepared in a pH corresponding to respective loop (Table 1), was injected slowly through the silicone tubing attached to each needle. After an hour, the loops were cut off and rinsed in ice-cold saline. The contents of each loop were emptied individually into respective 25 mL volumetric flasks. Stomach loops showing any food material were rejected. The mucosal side of each loop was rinsed with the buffer for that segment and added to the respective flask and the volume was made up to 25 mL. The resulting solution was filtered and analyzed by HPLC. Additionally, mucosa of each loop was scraped and homogenized in 5 mL saline to determine the accumulation of sesamol in various gastrointestinal tissues. Methanol (5 mL) was added to each homogenate, mixed thoroughly and centrifuged at 10,000 rpm for 5 min. The supernatant was filtered and analyzed by HPLC. During analysis, all the samples were stored at 4°C by keeping them in HPLC auto sampler.

The extent of absorption was considered as the total drug disappearing from the loop. The latter was obtained by deducting the sum of the amount present in the loop and the amount accumulated in the mucosa from the actual amount injected.

Table 1. Physiological pH of regions of human GIT in the fasting state[30] and the pH and buffer composition[31]employed in the present study for various segments of GIT

Segment	Fasting state pH	pH employed in the present study	Buffer composition
Stomach	1.0 – 2.1	2.0	Concentrated hydrochloric acid – 6.7 mL Citric acid – 6.3 g Sodium hydroxide – 2.4 g Sodium chloride – 4.2 g Water – q.s. to 1 L
Duodenum	4.9 – 6.4	5.5	Sodium chloride – 5.0 g Disodium hydrogen phosphate – 1.2 g Potassium dihydrogen phosphate – 8.6 g Water – q.s. to 1 L
Jejunum	4.4 – 6.4		
Ileum	6.5 – 7.4	7.0	Sodium chloride – 4.3 g Disodium hydrogen phosphate – 14.3 g Potassium dihydrogen phosphate – 3.6 g Water – q.s. to 1 L

The buffers of various pH (corresponding to different segments of GIT) were prepared according to the formulae given in Table 1 and the osmolality was adjusted to 280-300 mmol kg^{-1} with sodium chloride. The concentration of sesamol used in the study was 1 mg/mL. The drug solutions of sesamol at various pH were prepared by dissolving the required quantity in the respective buffer.

2.4. Pharmacokinetic Studies: For in vivo pharmacokinetic studies, male Wistar rats (200–250 g, 3 months old) were used. The protocol was duly approved by the Institute Animal Ethics Committee (IAEC) of Panjab University, Chandigarh, India. The animals were divided into two groups (n=6). Each group consisted of six animals. Sesamol (4 mg/Kg) was administered both intravenously (Group I) and orally (Group II) as a solution. Blood samples (500 µl) were collected from the cannulated femoral artery into heparinized tubes (containing 50µl of heparin) at 5 min, 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h and 24 h both for intravenous and oral (except for 5 min sample) routes. The plasma was obtained after centrifugation for 10 min at 5000 rpm. The plasma samples were stored at -80ºC until used.

2.4.1. Tissue sample collection*:* Rats were divided randomly into 18 groups and were given sesamol (4 mg/kg) by i.v. route and orally, as a solution. The animals were sacrificed at 15min, 30 min, 1h, 2h, 4h, 6h, 8h, 12h, and 24h after drug administration in each case. The liver, kidneys, lungs and brain were removed in all cases. The tissues were homogenized with phosphate buffer (0.1 M, pH-7.4) and subsequently centrifuged at 10,000g for 10 min. The supernatants were separated and stored at -80ºC until used.

2.4.2. Quantitation of drugs*:* Plasma and tissue samples were analyzed for sesamol by employing a validated HPLC method (Column: Spherisorb ODS2 (4.6x250mm, 5µ); flow rate: 1.0ml/min; λ_{max} : 294nm; injection volume: 50µL; mobile phase: acetonitrile:water=60:40; limit of detection (LOD): 10ng/ml and limit of quantification (LOQ): 50ng/ml). For the analysis of drug in plasma, 100 µl of plasma sample was mixed with 100 µl of methanol to precipitate the proteins. The tubes were centrifuged at 5000 rpm for 15 min and the clear supernatant obtained was injected into the HPLC. In case of tissue samples, 200 µl of supernatant was taken and 200 µl of methanol was added to precipitate tissue proteins. Then the tubes were centrifuged at 5000 rpm for 15 min and analysed for the drug content in the same way as that for plasma samples.

2.4.3. Data analysis*:* Plasma concentration-time data was used to calculate the elimination rate constant, t½, absorption rate constant and volume of distribution from plasma by regression

analysis. Maximum concentration (C_{max}) and the time at which the maximum concentration (t_{max}) is achieved were calculated for each group. Plasma concentration from 0 min to the time at which maximum concentration was achieved was used for absorption calculations. The area under the plasma time curve (AUC) was calculated by the trapezoidal rule using individual replicate data and is reported as mean ± S.D.

The oral absolute bioavailability was calculated by dividing AUC of the plasma concentration-time

$$F = \frac{AUC_{extravascular} \times D_{I.V.}}{AUC_{I.V.} \times D_{extravascular}} \times 100$$

profile obtained upon oral administration with that obtained for the intravenous dose, using above mentioned equation,where, F is the percent absolute bioavailability; $AUC_{extravascular}$ is the area under the curve of plasma concentration-time profile of extravascular (oral) administration; $AUC_{I.V.}$ is the area under the curve of plasma concentration-time profile of intravenous administration; $D_{I.V.}$ is dose administered through intravenous route and $D_{extravascular}$ is dose administered through extravascular route and was 4mg/Kg in either case.

The volume of distribution (Vd) is the amount of drug in the body divided by the concentration in the plasma. Drugs that are highly lipid soluble, have a very high volume of distribution. Drugs which are lipid insoluble, remain in the blood, and have a low Vd. The clearance (Cl) of a drug is the volume of plasma from which the drug is completely removed per unit time. The amount eliminated is proportional to the concentration of drug in plasma. The fraction of sesamol in the body eliminated per unit time is determined by the elimination constant (Kel). This was represented by the slope of the line of the log plasma concentration versus time. The clearance was calculated by using the following formula:

$$Cl = Kel \times Vd$$

3. RESULTS AND DISCUSSION

3.1. Physicochemical characterisation

The drug research can be divided functionally into two stages: drug discovery/design and development. Drug development depends on the biopharmaceutical and pharmacodynamic properties of the drug since these properties control the rate and extent of drug reaching its site of action. Of the various physicochemical properties, solubility and ionization constant (pKa) are two important factors which monitor drug liberation and absorption. Partition coefficient is another

important factor, since it determines the ability of a molecule to cross the biological membranes. Partition coefficients (log P) measured at a given pH are known as distribution coefficients (log D). Both log P and log D values for various drugs, measured using n-octanol as organic phase are reported to correlate well with permeability, distribution and other pharmacokinetic parameters of the respective drug molecules[32]. The above mentioned parameters were studied for physicochemical characterisation of sesamol.

3.1.1. Organoleptic Properties: Sesamol when evaluated for various organoleptic properties was observed as pale brown crystalline needles with a characteristic odour. The melting point of sesamol was found to be 64±1°C with no signs of hygroscopicity.

3.1.2. Solubility: The solubility of sesamol was found to be 38.8 ± 1.2 mg/mL (n = 3) at 37ºC in water. It is generally recognized that solubility plays an important role in governing the overall biological performance of the drugs[33]. To correlate with the in vivo dissolution in stomach fluids the solubility studies were conducted at 37 ºC (same as body temperature). A minimum solubility of 10 μg/mL[34] is considered to be fit for effective bioavailability after oral administration. In addition to depending on the aqueous solubility, the bioavailability of drugs also depends on the human dose. Generally, a drug is considered as highly soluble, when the highest human dose is soluble in 250 mL of liquid at pH 1-7.5[35]. Although, the human dose of sesamol is not yet reported or defined, however considering 4-8 mg/Kg dose in rodents, the human dose comes out to be 1.296mg/Kg (8X0.162mg/Kg)[36], and the ideal solubility should thus be greater or equal to 0.0052 mg/ml[36]. Presently obtained results indicate that the aqueous solubility of sesamol is suitable.

3.1.3. pH-Solubility Profile: The pH-solubility profile of sesamol is shown in Fig. 1. It is evident that sesamol shows solubility of ~10 mg/mL (9.50-11.08 mg/ml) at all the pH<9. Further, there is a sharp increase in solubility at more alkaline pH>10, with the value being 41.83 mg/mL at pH 13.

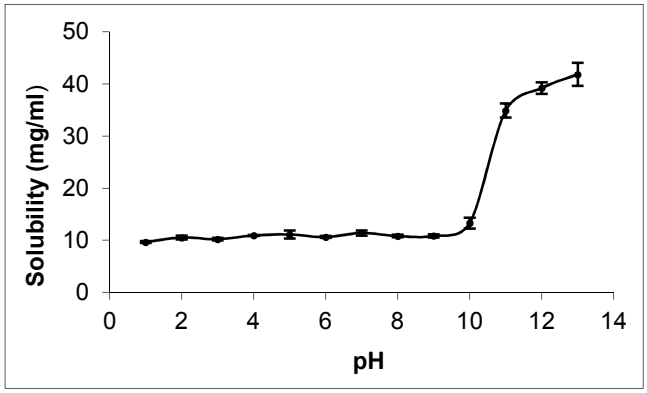

Fig. 1. pH-solubility profiles of sesamol

3.1.4. Determination of Ionization Constant (pKa): The gastrointestinal/blood barrier is impermeable to ionized form of weakly acidic or basic drugs, although the unionized form of any drug invariably shows significant permeability. Thus ionization constant plays an important role in deciding the extent of absorption of drugs. The ionization constant (pKa) values of sesamol determined by Krebs and Speakman[29] method are shown in Table 2. The predicted and experimental pKa of sesamol is 8.72 and 9.79 ± 0.06, respectively (n=3). From the pKa values of sesamol, it can be concluded that a majority of the sesamol present in the gastric fluid (i.e., pH 1.2-4) is expected to exist in the unionized (absorbable) form.

Table 2. Calculation of pKa of sesamol from the solubility data using Krebs and Speakman method[29]

pH	S_0 (mg mL^{-1})	$(S_0/S_i^*) - 1$	Log $[(S_0/S_i) - 1]$	pKa	Antilog (pKa)
9.31	11.71	0.35	-0.446	9.75	5707619328
9.48	11.78	0.42	-0.373	9.85	7468699738
10.12	13.30	1.94	0.288	9.83	6786767946
10.49	16.36	5.00	0.699	9.79	6260225828
10.62	19.07	7.71	0.887	9.73	5402701831
11.02	28.87	17.51	1.243	9.77	5977560770
Average of antilog (pKa)					6267262574
Average pKa**					9.79
Scatter***					0.06

*The intrinsic solubility (S_i) was 10.36. **The average pKa was calculated by taking logarithmic of the average antilog (pKa). ***The scatter was the highest difference obtained by deducting the highest or lowest pKa from the average pKa value.

3.1.5. Partition Coefficient: The experimental and predicted log P (as per Pallas 20 software) values for sesamol are 1.29 ± 0.01and 1.34, respectively (n=3). Lipophilicity plays an important role in overall biological performance of drugs[33]. Drugs need to partition towards the lipid biomembranes, from the aqueous biological fluids, in which they should be soluble, to reach the site of action. Thus, for a favourable bioavailability, a drug molecule should have a balance of hydrophilic and lipophilic characteristics and a log P value between 0-3[37] is considered suitable for an effective oral bioavailability of any given drug molecule. The log P value of 1.3 obtained

presently for sesamol thus indicates that it will show a good oral bioavailability. The experimental log P value for sesamol coincides well with the predicted value.

3.1.6. Distribution Coefficient: Distribution coefficient (log D), measured in two-phase bulk solvent systems at different pH, is one of the main descriptors for prediction of *in vivo* drug permeation[32]. Fig. 2 shows the log D profile of sesamol and the value is found to vary between 1.0-2.0. though pH-solubility profile of sesamol was found to be fairly constant between pH 1.0-7.0, it may be noted here that the observed variation in log D value may be due to variable partitioning of sesamol into octanol at various pH; however the value match well with its log P value of 1.3. Both the log P and log D values of >1.0 indicate a favourable partitioning of sesamol across biological membranes indicating its good in vivo permeability. It is evident that the log D value for sesamol decreases as the pH increases.

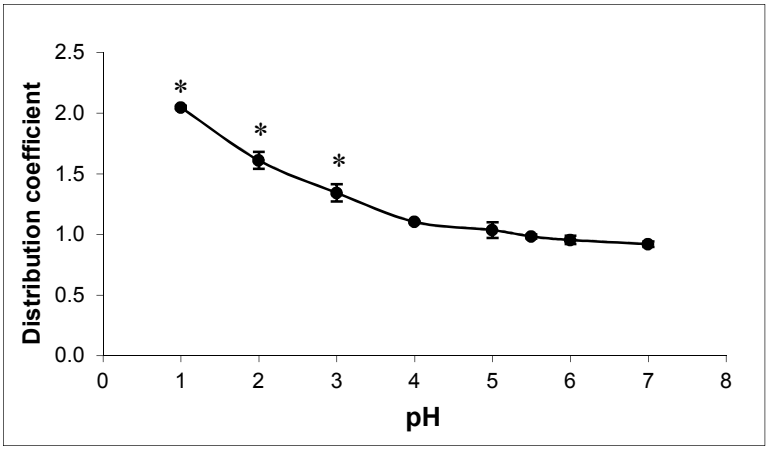

Fig. 2 Log D profile of sesamol at pH 1-7. * significant difference ($p<0.001$).

3.2. Gastrointestinal permeability

Presently we used ligated loop technique to determine the intestinal permeability. The permeability behaviour of sesamol in the GI tract of rats is shown in Fig. 3. The results indicate that sesamol is almost completely absorbed from the stomach (~85%). Further it is ~65-70% absorbed from each segment of the intestine. The reason for a significantly high permeability of sesamol through stomach ($p<0.05$) at acidic pH could be explained in terms of its high log D value at the acidic pH (pH 2) corresponding to that of the stomach as compared to the intestinal pH conditions (pH 5-7). Furthermore, no statistically significant difference between the permeability of sesamol through various intestinal regions, i.e., duodenum, jejunum and ileum was observed. Considering the high permeability of sesamol through all the segments of GIT (as also indicated by log P and log D

values); coupled with a sufficiently high aqueous solubility (38.8mg/mL), a low molecular weight (138.34g) and small size, it may be considered that sesamol holds promise for oral delivery. Though at the face of it, it might appear that these studies had no significance for sesamol, however this is confirmed only from the data obtained and presented herein for the first time that sesamol can be absorbed from all regions of GIT. The suitable physicochemical properties coupled with a good antioxidant activity (vitamin C equivalent value is 1.48 in DPPH assay[7]) make sesamol all the more interesting. However, a high absorption through GIT does indicate a need to develop a controlled release formulation which can ensure a slow and steady release of sesamol over a prolonged period of time to maintain its required concentration in plasma for sustained times. Former will also take care of any irritation caused by high local concentrations of free sesamol when administered as such.

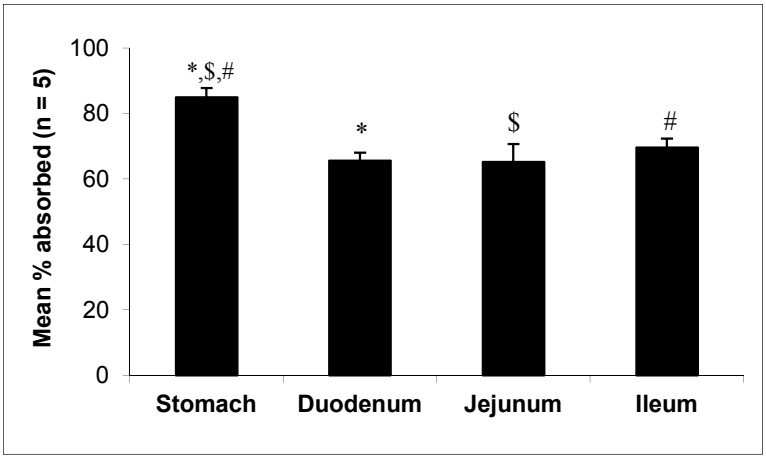

Fig. 3. Absorption behaviour of sesamol from different segments of GIT in rats. *Groups marked similarly are significantly different from one another.*

3.3. Pharmacokinetics of sesamol after i.v. and oral administration

The plasma-concentration time curves of sesamol obtained by oral and i.v. route are shown in Fig. 4. The best fit for the analysed data was achieved with a two-compartment model, which is described by the following mathematical equations for i.v. and oral routes respectively:

$C_{(t)} = B.\ e^{-\beta t} + A.\ e^{-\alpha t}$ (for i.v. administration)

$C_{(t)} = B.\ e^{-\beta t} + A.\ e^{-\alpha t} - C_{(0)}.\ e^{-Kat}$ (for oral administration)

Where $C_{(t)}$ = concentration at time t; $C_{(0)}$ = concentration at time 0; β = elimination rate constant; α = distribution rate constant; B = intercept of back-extrapolated monoexponential elimination slope

β with the ordinate; A = intercept of distribution slope α with ordinate; Ka = absorption rate constant.

The obtained pharmacokinetic parameters of sesamol for both the routes are listed in Table 3. The elimination half-life for sesamol, when administered by i.v. route was 4.10 ± 0.15 h, and it increased to 10.9 ± 0.06 h for oral route, with a t_{max} of 2h. Oral bioavailability of sesamol was found to be significant at 95.61%, as expected based on its physicochemical parameters. Log P and log D values of >1.0 and significant GIT absorption indicate that sesamol enters quickly into systemic circulation from where it will be distributed into the tissues in high amounts within 2 h.

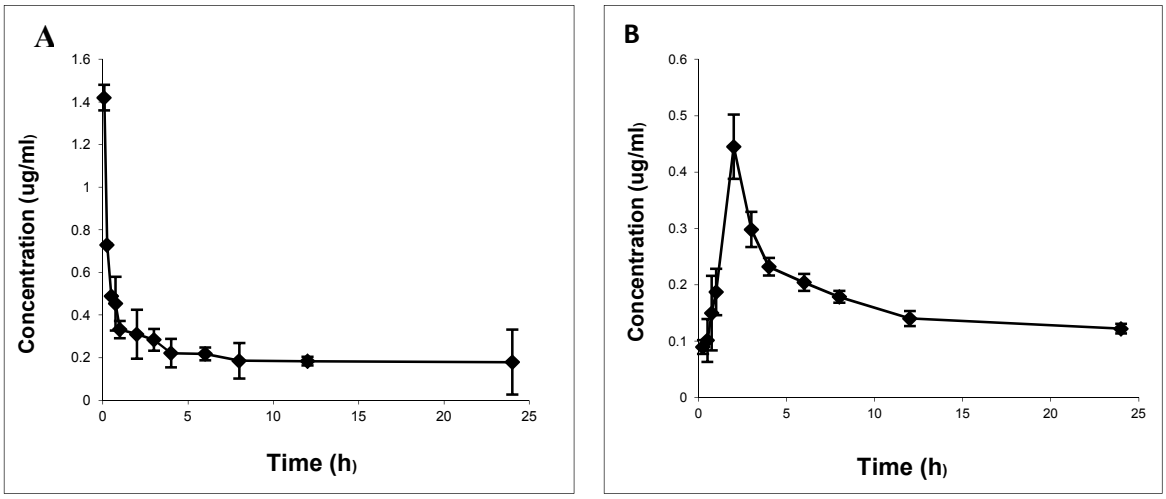

Fig. 4. Plasma concentration-time profiles of sesamol when administered by i.v. (A) or oral (B) route

Table 3. Pharmacokinetic parameters of sesamol (4 mg/kg) when administered by intravenous and oral routes, in rats

Parameters	Intravenous	Oral solution
t½ (h)	4.10 ± 0.15	10.9 ± 0.06
C_{max} (µg/ml)	1.52 ± 0.001	0.44 ± 0.001
t_{max} (h)	0	2.00 ± 0.04
F (%)-upto 24h	-	95.61± 1.65
K_a (h^{-1})	-	5.15 ± 0.016
Vd_{ss} (L)	1.75 ± 0.005	2.02 ± 0.002
AUC (µg/ml).h-24h	6.29 ± 0.002	6.01 ± 0.004
CL_{tot} (L/h)	0.295 ± 0.005	0.127 ± 0.002

t½ - Elimination half life; C_{max} - Maximum concentration; t_{max} – Time to reach maximum concentration; F – Absolute bioavailability; Vd_{ss} – Volume of distribution at steady state; AUC – Area under the curve; CL_{tot} – Total clearance.

The pharmacokinetic parameters obtained in the present study seem to correlate well with the gastrointestinal permeability data of sesamol. The t_{max} of sesamol was found to be 2h when administered orally. It may indirectly suggest that sesamol is absorbed better from proximal part of GIT. The absorption rate constant (Ka) of sesamol was 5.15 h^{-1} indicating its rapid absorption.

The only earlier study on sesamol pharmacokinetics, conducted by Jan et al.[22], reported C_{max}, $t_{1/2}$ and AUC as 1.4±0.7 and 2.3±0.6 μg/mL, 563.7±36.9 and 29.2±6.1min, and 501.3± 00.8 and 141.4±9.0 min·μg/mL, respectively for oral (50mg/Kg) and i.v. (5mg/Kg) administration respectively. The results showed that the concentrations of sesamol declined rapidly and the elimination half-lives did not relate to the dosage ranges. However, oral bioavailability of sesamol was reported at 35.5±8.5% by these workers, while we presently observe a much higher value of 95.6%. This marked difference in bioavailability may partly be attributed to the different extraction procedure followed in both the studies. We have used methanol (widely used for protein denaturation) for extraction of sesamol in plasma, while, Jan et al.[22] have used pH 5.0 sodium acetate buffer for the same. However, a major reason for the difference in observed bioavailability may be attributed to dose dependent kinetics followed by sesamol. Further to it, a lower bioavailability observed at a much higher dose may be explained in terms of the saturation of absorption beyond a certain limiting dose.

Howsoever variable dose kinetic studies are indicated to establish this fact. Observation of high permeability from stomach and other regions of GIT also indicate that a detailed data on absorption of different doses may be explored in future studies to explain the difference in bioavailability of various doses.

3.4. Single dose tissue distribution of sesamol after i.v. and oral administration

Levels of sesamol (4mg/Kg) obtained in various tissues at different times are shown in Fig. 5. The highest level of sesamol was found in kidneys followed by brain when administered as i.v. solution while in case of oral administration highest concentration though again found in kidney was followed by lungs. The pharmacokinetic parameters of sesamol in various organ tissues are also listed (table 4).Tissue distribution studies are an important component of the non-clinical kinetics program. For most compounds, it is expected that single dose tissue distribution studies with sufficient sensitivity and specificity will provide an adequate assessment of tissue distribution and the potential for accumulation. On i.v. administration sesamol bypasses first pass metabolism. Also

being lipophilic (log P= 1.29) in nature it seems to easily cross the blood brain barrier resulting in its high concentration in brain (Fig. 5).

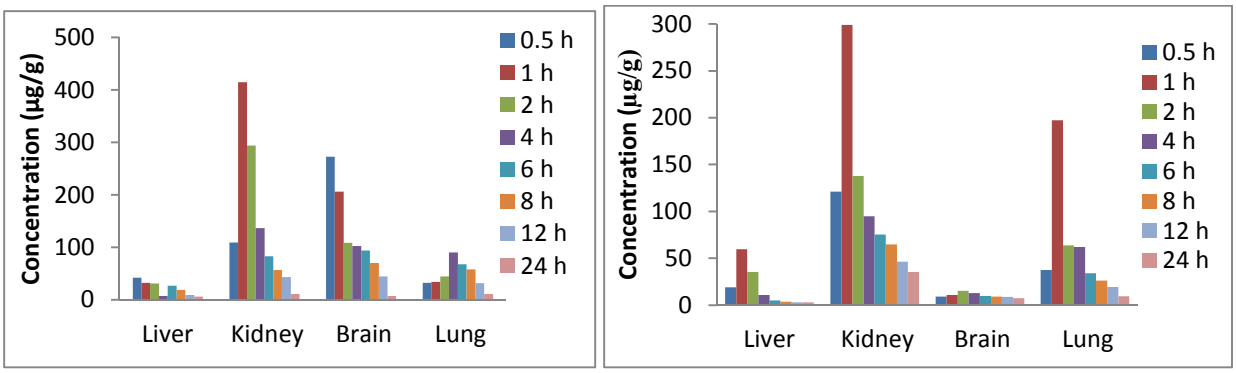

Fig.5 Tissue concentration-time profile of sesamol when administered through i.v. (A), oral (B) route.

Table 4. Pharmacokinetic parameters of tissue distribution of sesamol, when administered as a solution by i.v. and oral route and as different formulations of sesamol

Organs	Formulations	Kel (h^{-1})	t½ (h)	C_{max} (µg/ml)	t_{max} (h)	AUC [(µg/ml).h]
Liver	Intravenous	0.1727	4.01	41.96	0.5	362.00
	Oral	0.0733	9.44	59.48	1	229.19
Kidney	Intravenous	0.0652	10.61	414.45	1	1717.88
	Oral	0.0832	8.32	299.1	1	2034.88
Brain	Intravenous	0.1494	4.63	272.45	0.5	1505.41
	Oral	0.0871	7.95	15.34	2	308.64
Lungs	Intravenous	0.2633	2.63	90.54	4	934.50
	Oral	0.1811	3.82	197.41	1	728.38

Presence of high sesamol concentration in lungs on its peroral administration can however be explored for its lung targeting. There is a report on the repeated dose tissue distribution of sesamol in rats[22] wherein sesamol dissolved in normal saline was administered to rats via gastric gavage, for 4 days in three daily doses of 100 mg/kg of sesamol (300mg/kg/day). Highest sesamol concentration (AUC) was observed by these workers in the intestine followed by lung, plasma, brain, kidneys and liver. According to the authors, sesamol may, at first be, incorporated into the liver and then transported to the other tissues (lung, kidney, and brain). According to ICH[38],

repeated dose tissue distribution studies are not required normally for most compounds and should only be conducted when apparent half-life of the test compound in organs or tissues significantly exceeds the apparent half-life of the elimination phase in plasma and is also more than twice the dosing interval in the toxicity studies. However, as observed presently half-life of sesamol in various tissues was either less or similar to that observed in the plasma hence we did not perform any repeat dose tissue distribution studies for sesamol.

4. CONCLUSION

The antioxidant activity of sesamol has received a great deal of attention and thus information on the bioavailability and disposition of sesamol is important for not only understanding its biological effects but also the expected clinical application.

Present study for the first time explores this promising molecule for its physicochemical characteristics, gastrointestinal permeability and single dose pharmacokinetics and tissue distribution. Results show that sesamol has good aqueous solubility (~38.8 mg/mL), with a pKa of 9.79 coupled with a favourable lipophilicity (log P 1.29). Sesamol is well absorbed throughout the GIT, including stomach (~85% absorption). Pharmacokinetic and tissue distribution data clearly highlight the fact that sesamol, being a small molecule with favorable biphasic (lipid and aqueous) solubility, is rapidly absorbed and readily distributed throughout the body. In order to harness the therapeutic potential of this molecule, it needs to be restrained in a delivery system, which controls its release, ensures prolonged circulation and thus avoids fast clearance. Some studies from our group do validate this claim.

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