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AN IN VIVO EVALUATION OF THE PHARMACOKINETICS AND BIOAVAILABILITY STUDIES OF *BOSWELLIA SERRATA* EXTRACT (BOSWEGEX®) IN MALE WISTAR RATS

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ABSTRACT
In comparison to NSAIDs, boswellic acids (BAs) exhibit elevated tolerance and fewer side effects, all while retaining their effectiveness in addressing inflammation, immunosuppression, and tumors. Sourced from the oleo gum resin of *Boswellia serrata* Roxb. ex Colebr., these compounds hold promise. Despite their efficacy, BAs' limited solubility results in suboptimal oral absorption. To address this, our study aimed to assess Boswegex®'s pharmacokinetic profile post a single oral dose using LC-MS/MS analysis of serum at varying time points. Our findings revealed a Cmax and Tmax of 10548.19 ng/ml at 6 hours for Boswegex®. Furthermore, the AUC0-24 reached 324403.5 ng.h/ml. Notably, these results underscore Boswegex®'s potential as an innovative treatment avenue, defying concerns over pharmacokinetic interactions. Its heightened absorption and enhanced bioavailability distinguish it, making it a viable alternative for conventional therapeutic applications. Recent developments in Boswegex® highlight its superior absorptive qualities, reinforcing its potential significance as a new standard of care in pain management.

KEYWORDS: Boswegex®, Oral bioavailability, Wistar rats, Tmax and Cmax.

INTRODUCTION
The process of transforming the preclinical potential of many botanical extracts into therapeutic water solubility and strong self-aggregation is significantly hampered by bioavailability. This is applicable to several triterpenoid acids and polyphenols. Because oral administration has a high patient compliance rate and a relatively simple and affordable production process, it has traditionally been the most prevalent and efficient technique for drug delivery. The pharmacokinetic and pharmacodynamic properties of the medicine are usually taken seriously when developing a new form of administration .[1] When consumed orally, certain drugs that are not highly bioavailable remain sub therapeutic. This decreased oral absorption of a drug may be attributed to P-glycoprotein (P-gp) efflux from the drug's site of action and metabolism by the drug's corresponding enzymes, like CYP450. Unless and until very large doses are administered, the majority of a given dose may never reach the systemic circulation in order to exert its pharmacological actions. The oral bioavailability of such drugs can be substantially enhanced while reducing the dose and frequency of administration. The most common application of plant-based compounds is as bioavailability boosters in combinations of medications.[2] Following the administration of natural bioenhancers, the medicine's dose may be reduced, in addition to the possibility of drug resistance and dose-dependent toxicity.

B. serrata extract is a gum resin derived from *B. serrata* Roxb., a tree native to India and the Punjab region and known as the frankincense tree (Family: Burseraceae; Genus: Boswellia). In its conventional applications,[3] it is used as a herbal remedy to treat rheumatoid arthritis and cardiovascular diseases. The primary active components of Boswellia are a group of pentacyclic triterpenes known as boswellic acids.[4,5] Examples of compounds that have been found to inhibit 5-lipoxygenase, microsomal prostaglandin E synthase-1, or the serine protease cathepsin G include 11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA), and β-boswellic acid (BBA).[6] The extremely effective anti-inflammatory properties of boswellic acids (BAs) include 3-acetyl-11-keto-boswellic acid (AKBA) and 11-keto-boswellic acid (KBA), which inhibit 5-lipoxygenase as well as having anti-inflammatory effects.[7]

Because of their poor water solubility and high lipophilicity, KBA and AKBA particularly showed poor absorption in preliminary pharmacokinetic studies of *B.*

serrata extract (BSE) after oral administration.[8,9] Studies using Caco-2 cells demonstrated that Boswellic acids possess poor to moderate permeation capacities, with KBA having poor permeability through Caco-2 cell lines and AKBA showing moderate permeability. In order to provide the necessary therapeutic benefit, boswellic acids need to be improved due to the fact that they have poor absorption and are extensively metabolised (particularly KBA).

Pharmacokinetic investigations have shown that Boswellic acids, particularly KBA and AKBA, have a relatively low systemic absorption in both animals and humans [10], which justifies efforts to improve their oral bioavailability. The excellent improvement in absorption in this situation is essential. However, the plasma concentration of boswellic acid was determined at various time intervals.

Our Boswegex® is standardised and contains 65% of boswellic acids. This product is more bioavailable because of its quick dissolving and long-lasting solubility. The primary benefits of Boswegex® are better pharmacokinetics, increased bioavailability, and greater absorption.

MATERIALS AND METHODS

Preparation of Boswegex® with Regular boswellic acids

An aqueous ethanolic extract of the gum resin of *Boswellia serrata* was used for manufacturing Boswegex®, which contains Regular boswellic acids. Boswegex® with Regular boswellic acids were standardised to contain 65% by HPLC, in order to maintain quality and batch-to-batch consistency.

Dried gum was ground into a coarse powder and then extracted using aqueous ethanol. The extraction process was repeated two to three times. The mixed extracts were then filtered under vacuum, and the filtrate was then heated in a rotary evaporator to between 50-60oC to create a viscous extract. The resinous portion of the extract was then removed by washing, and after that, the organic acids were selectively precipitated by treating with alkaline water and then acidification. A semi-dried cake was then produced by decolorizing, filtering, and washing in water with a neutral pH. To make powder, this was further dried at 50–60°C in an oven with a vacuum. By using HPLC, this product was further standardised to 65% of boswellic acids.

Characterization of boswellic acids (Boswegex®) by HPLC

Extraction and purification processes were standardized for Boswegex®. The analytical method was standardized based on HPLC. Briefly, HPLC analysis was performed using a Phenomenex Luna C18, 250 × 4.6 mm, 5 µl column with a flow rate of 1 ml/min in a Shimadzu liquid chromatography equipped with a UV/Vis Detector at a wavelength of 210nm and 254nm to detect the peaks. The sample was eluted by injecting 20 µl of mobile phase (Acetonitrile: Water: Acetic acid (90:10:0.1)) with a run time of 35 min. The peaks 1 to 6 represented 11-keto-β-boswellic acid (KBA), 3-O-acetyl-11-keto-β-boswellic acid, α- boswellic acid, β- boswellic acid, 3-O-acetyl- α- boswellic acid, and 3-O-acetyl-β-boswellic acid, respectively (Figure 2).

Chemicals and Reagents

EDTA (2522110) was bought from Fisher Scientific, India. HI Media in India delivered picric acid (0000175497) and isoflurane anaesthesia (IF-17020), which were procured from Raman & Weil Pvt. Ltd.

Experimental design

Six male Wistar albino rats (250±20 gms) were used in this experiment. They were collected from the animal house of Radiant Research Services Pvt. Ltd., Karnataka, India. Animal experiments were conducted in accordance with the guidelines of the committee for the purpose of controlling and supervising experiments on animals (CPCSEA Registration Number: 803/PO/RcBi/S/2015/CPCSEA). Each animal was marked with picric acid, and numbering was given individually to each animal. The animal was housed in a standard stainless steel cage with facilities for normal chow diet and drinking water in bottles. Aqua guard on line water was provided *ad libitum*. Animals had continuous access to fresh, potable, uncontaminated drinking water. They were kept in these cages under conventional laboratory settings, which included a temperature of 22± 3^{o}C, relative humidity of 30–70%, and a 12-hour light and 12-hour dark cycle. Every procedure involving animals was carried out in an ethical way, under the supervision of competent professionals. The research protocol was reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of Radiant Research Services Pvt. Ltd. before the study could begin.

In vivo bioavailability study in male Wistar rats

The animal study protocol has been reviewed and approved by Radiant Research Services Pvt. Ltd.'s institutional animal ethical committee (IAEC). Wistar male rats weighing 250±20 g were used for the purpose of the study. The rats fasted for 10 hours before being given unlimited access to water and food the night before the dose. Boswegex® was administered to animals by oral gavage at a dose of 50mg/kg.bw in a volume of 10 ml/kg.bw. Using isoflurane anaesthesia, 1000µL of blood samples were collected in EDTA-containing tubes from the retroorbital route of the rats at various time intervals: 0, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after dosing. Within 20 minutes after blood collection, plasma was separated by centrifuging blood samples at 2500 rpm for 10 minutes. The plasma was then separated and stored at -20°C until LC-MS/MS studies for the pharmacokinetic profile were performed.

Statistical analysis

The values were expressed in Mean ± SEM. The significance of *in vivo* data was analyzed by one-way analysis of Variance.

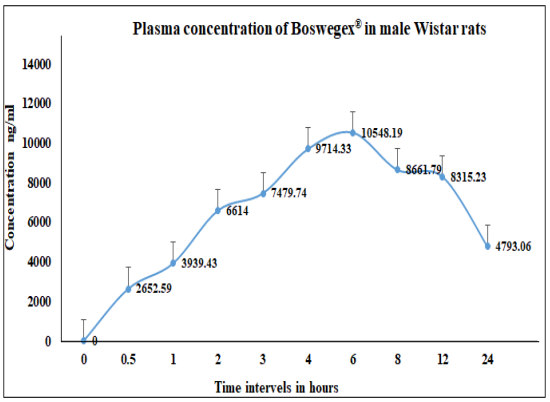
RESULTS AND DISCUSSION

pharmacokinetic data of Boswegex®

The pharmacokinetic data of Boswellic acids (Boswegex®) in male Wistar rats are shown in Table 1 and Figure 1. In the present study, the pharmacokinetic profile of Boswellic acids (Boswegex®) in rat plasma after single oral administration was examined by LC-MS/MS analysis at different time intervals. The Cmax and Tmax of Boswellic acids (Boswegex®) were shown to be 10548.19 ng/ml at 6 hours. The AUC0-24 of the Boswellic acids (Boswegex®) was 324403.5 ng.h/ml hours (Table 2).

Table 1: Plasma concentration vs. time profile of Boswellic acids (Boswegex®) in male Wistar rats.

Sl. no.	Time (Hours)	Averageplasma concentration of Boswellic acids (Boswegex®) (ng/ml)
1	0	0.00±0.00
2	0.5	2652.59±66.03
3	1	3939.43±618.43
4	2	6614.00±143.22
5	3	7479.74±908.11
6	4	9714.33±908.11
7	6	**10548.19±252.61**
8	8	8661.79±187.11
9	12	8315.23±659.07
10	24	4793.06±338.44



Values were expressed as Mean ± SEM

Fig. 1: Plasma concentration vs. time profile of Boswellic acids (Boswegex®) in male Wistar rats. Values were expressed as Mean ± SEM.

Table 2: Pharmacokinetic (PK) profile of Boswellic acids (Boswegex®) in male Wistar rats.

Sl. no.	Time (Hours)	Average concentration of the pharmacokinetic profile of Boswellic acids (Boswegex®) (ng.h/ml)
1	0	0.00±0.00
2	0.5	12980.67±142.95
3	1	16344.67±3452.33
4	2	34590.17±771.60
5	3	39254.00±4892.09
6	4	51292.33±1360.87
7	6	**55784.17±1519.42**
8	8	45621.83±1008.09
9	12	43755.00±3550.53
10	24	24780.67±1823.25

AUC0-t = Area under the concentration-time curve from dosing (time 0) to the time of the last measured concentration (t=24) peak plasma concentration

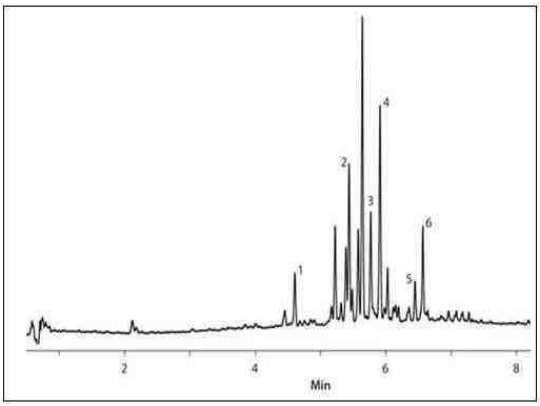


Fig. 2: A representative high-performance liquid chromatography image shows the elution profile of the major boswellic acids in Boswegex® at 210 nm. The peaks 1 to 6 represent 11-keto-β-boswellic acid, 3-O- acetyl-11-keto-β-boswellic acid, α-boswellic acid, β-boswellic acid, 3-O-acetyl-α-boswellic acid, and 3-O- acetyl-β-boswellic acid, respectively.

Whenever a number of medications or herbs are combined into a single formulation for oral, dermal, parenteral, or rectal administration, the bioavailability frequently rises noticeably. This results in improved pharmacokinetic and pharmacodynamics properties, a reduction in unfavourable drug effects, and a minimum quantity of hypersensitivity reactions. This establishes a novel and effective pharmaceutical product for preventative and therapeutic use in both animals and humans. These combination formulations may also reduce total risk factors. From the standpoint of diagnostics, treatments, and prophylactics in both humans and animals, there are not many successful combinations of medicines.

Reduced metabolism, which may also be caused by the combined effects of inhibiting metabolising enzymes from the CYP group and inhibiting the efflux transporter P-gp, is the cause of the greater bioavailability. Several studies have already reported improved absorption. *Boswellia serrata* extract (BSE) pharmacokinetic research conducted *in vivo* is extremely significant. The effectiveness and toxicity of *B. serrata* can be better understood through pharmacokinetic research, so we developed a straightforward, sensitive, and highly investigative LC-MS/MS approach.

The quantity of the drug that gets administered that reaches the bloodstream systemically as an unaltered substance is referred to as bioavailability. By comparing the drug's plasma levels after a certain method of administration with the levels of the drug itself, bioavailability can be determined. When a medicine is taken orally, only a portion of the supplied dose enters the bloodstream. By using LC-MS/MS analysis at various time intervals, the pharmacokinetic profile of Boswegex® in rat plasma following a single consumption was investigated in the current study. At 6 hours, Boswegex®'s Cmax was 10548.19 ng/ml. Boswegex®'s Tmax became evident at 6 hours. The Boswegex® AUC 0–24 was shown to be 324403.5 ng.h/ml in hours.

CONCLUSION

A rapid and precise LC-MS/MS approach was created and validated in this work for the simultaneous measurement of Boswegex® plasma levels in Wistar rats. The technique was successfully utilised to investigate the plasma pharmacokinetics of rats treated with Boswegex®. In conclusion, a pharmacokinetic study was successfully conducted to analyse the bioavailability of Boswegex® using a quick, sensitive, and selective LC-MS/MS approach. This medication's high efficacy, sensitivity, selectivity, and accuracy are assured for a variety of biological activities. The current results could help with our understanding of how Boswegex® is absorbed as well as their potential preclinical value.

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