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Pharmacodynamic and Pharmacokinetic Evaluation of Soy Lecithin Based Tablet of Boswellic Acid

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ABSTRACT
Background: The oleo-gum resin of *Boswellia serrata* contains pentacyclic triterpenic acids known as Boswellic acids (BAs) that are responsible for the anti-inflammatory and anti-arthritic activities by inhibition of 5-lipoxygenase.
Methods: Soylecithin based tablet formulation of an enriched extract of BAs were formulated and evaluated comparatively with the unformulated extract for bioavailability on rabbits and therapeutic efficacy against arthritis on rats targeting two primary constituent 11-keto β-boswellic acid (KBA) and 3-O-acetyl 11-keto β-boswellic acid (AKBA). Total BAs content in the enriched fraction was measured and characterized by HPLC analysis. Soy lecithin based tablet of BAs enriched extract was prepared and evaluated for different parameters. Tablets at 160 and 320 mg/kg, and unformulated extract 160 mg/kg was assessed on CFA-induced arthritic rat model and bioavailability was evaluated on the rabbit.
Results: Tablet formulation showed two times higher efficacy in increasing hot plate reaction time, reduction in paw volume, and TNF-α levels compared to unformulated extract signifying enhanced systemic absorption and availability of the BAs at the site of action. The tablet at 320 mg/kg dose showed repair of articular surfaces with small areas of erosion and irregularities in the connective tissue. Plasma samples of rabbit showed identified peaks only for KBA.
Conclusion: The soy lecithin based tablet of BAs enriched extract at both doses showed higher peak plasma concentration and AUC compared to unformulated enriched extract. The results of the study substantiated higher efficacy and bioavailability of *B. serrata* gum resin enriched extract in the form of lecithin based tablet formulation.

Key-words: Anti-arthritic, Boswellic acid, *Boswellia serrata*, Pharmacokinetic, Soy lecithin

INTRODUCTION
Arthritis affects over 180 million people in India, with a prevalence rate higher than diabetes, AIDS and cancer. Arthritis is reported with a significantly higher age-adjusted prevalence in women than in men. The most prominent symptom of rheumatic arthritis (RA) is pain and stiffness in joints and the musculoskeletal system. RA cannot be cured permanently, and the patient has to take medications throughout their life [1]. People with RA may continue to have symptoms despite the use of conventional treatments and have difficulties coping with side effects.

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Many patients use complementary and alternative medicine together with or in place of conventional medicine. With the increasing awareness regarding the associated adverse effects of modern therapy, the popularity of herbal therapy is increasing day by day. Increasing demand for herbal therapy for a disease like RA is alerting standardization of herbal drugs for active content, safety, pharmacokinetic and pharmacodynamic parameters to be acceptable in global standards [1].
Varieties of *Boswellia* (*B. serrata* and *B. carterii*) produces resin that has shown promising anti-inflammatory and immunosuppression properties. *B. serrata* (Family: Burseraceae) is being used for the treatment of conditions like inflammation, arthritis, diarrhea in India, China and South Africa. *B. serrata* is a medium to large-sized deciduous balsamiferous tree commonly found in the dry forests of India [2,3]. This tree on injury exudes an oleo gum resin known as Indian olibanum or salai-guggul. The oleo-gum-resin secreted

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from the cortex is fragrant, sticky, golden-yellow sap that solidifies to brownish yellow drops and crusts. Various species of *Boswellia* are known to possess anti-inflammatory action by inhibiting 2-lipoxygenase (LOX_2) enzyme responsible for the synthesis of potent inflammatory mediator leukotrienes. Ayurvedic healers of India are using *Boswellia* for centuries to treat arthritis and rheumatism [4].

The gum fraction of *B. serrata* is composed of arabinose, xylose, and galactose sugar. The resin contains four major pentacyclic triterpenic acids such as α/β boswellic acid (BA), α/β 3-O-acetyl β-boswellic acid (ABA), 11-keto β-boswellic acid (KBA) and 3-O-acetyl 11-keto β-boswellic acid (AKBA) [5]. These are the major component responsible for anti-inflammatory and anti-arthritic activities of *B. serrate* by 5-LOX_5 inhibition [6]. Two to three hours after an oral dose of 1.2 gm dry boswellia gum resin extract, plasma mostly contains 10-32 µg/L of KBA and 18-20 µg/L of AKBA [7]. Reports of pharmacokinetic studies of BAs on human and animals revealed its very low plasma concentrations in vivo that is insufficient for producing pharmacological activity while administered at a very high dose of 3000 mg/day limiting its use in clinical practice [8]. BAs belong to the class of pentacyclic triterpenoids with poor water solubility and strong tendency of self-aggregation. Lecithin formulation of BAs showed significantly improved absorption accompanied with enhanced tissue penetration in rats leading to plasma concentrations effective for anti-inflammatory activity [9,10]. Huesch *et al.* [9] reported seven times higher bioavailability of KBA from soy lecithin formulation (phytosome) compared to *B. serrata* gum resin extract on rat. Riva *et al.* [10] reported quicker and higher absorption of BAs in healthy volunteers when administered with a lecithin-formulated Boswellia extract capsule dosage form.

This literature review has prompted us to formulate and evaluate soy lecithin based tablet formulation of BAs enriched from *B. serrata* extract and to compare the bioavailability as well as the therapeutic efficacy against complete Freund's adjuvant (CFA) induced arthritis on rat corresponding to the unformulated BAs enriched extract. The BAs enriched extract was standardized for two significant constituents, KBA and AKBA *in vivo* on rabbit plasma. The enriched extract contains 26% BAs. Thus 160 mg of extract formulated with lecithin was compared with non-formulated extract. Quantification of total boswellic acid, identification of major bioactive components KBA and AKBA with the establishment of pharmacodynamics and pharmacokinetic parameters of prepared soy lecithin based tablet formulation was carried out compared to boswellic acid enriched extract.

MATERIALS AND METHODS

Plant Material- Alcoholic extract of *B. serrata* (Batch no. KIL/65BS1021) was procured from Kuber Impex Pvt. Ltd., Indore. This study was performed in the department of Pharmacology, Radharaman College of Pharmacy, Ratibad, Bhopal, India. Enrichment and authentication of boswellic acid were first attempted in 2013 and detailed animal studies were performed in June-July 2017.

Enrichment of boswellic acid in the procured extract- The supplied ethanolic extract of *B. Serrata* contains 65% of boswellic acid. Four different methods have been tried for the enrichment of BAs in *B. serrata* ethanolic extract. In method A, *B. serrata* ethanolic extract was further extracted with petroleum ether followed by fractionation with water following Rajpal [11]. In method B boswellic acid was precipitated as barium salt, mixed anhydride was prepared with acetic acid, refluxed with methanol while dissolved in chloroform and the boswellic acid acetate thus formed was hydrolyzed [11]. Aqueous fraction of basified *B. serrata* ethanolic extract was extracted with n-hexane and ethyl acetate, aqueous part was separated, acidified and the precipitate of total organic acids was re-basified in method C [12]. *B. serrata* ethanolic extract was refluxed with pyridine and acetic acid, treated with chromium trioxide, and solid part was separated by mixing with ice water, heated with hydrochloric acid, cooled and filtered as per Gokarajuet al. in method D [13]. The percentage yield was 2.50% (sample A), 10.35% (sample B), 43.22% (sample C) and 58.65% (sample D), respectively for method A, B, C and D.

Determination of boswellic acid content by titrimetric analysis- As the yield for sample A was found to be very low, it was rejected, and further studies were performed on sample B, C and D. Ethanolic extract of *B. serrata*, sample B, sample C, and sample D were titrated to estimate the amount of total BAs present using 0.1M NaOH and phenolphthalein solution as indicator. Each ml of 0.1N NaOH was equivalent to 45.36 mg of boswellic acid [14]. The amount of BA was found to be 8.68, 13.70,

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14.24 and 26.52 mg/100 mg, respectively in ethanolic extract of *B. serrata,* sample B, C, and D.

Thin Layer Chromatography (TLC)- Ethanolic extract, sample B, C, and D were subjected to TLC employing ethyl acetate: n-hexane (30:70) as mobile phase and observed for the number of spots [13]. Ethanolic extract, sample B, C,and D respectively showed 7, 5, 4 and 4 spots with Rf value similar for 3 spots as off ranged from 0.14-0.16, 0.33-0.35 and 0.86-0.087 in all four samples.

Column Chromatography- The column chromatography using Silica gel G (60-200 mesh) was performed on the sample obtained by method D to separate pure fractions as it showed maximum content of BAs. A mixture comprising of n-hexane and ethyl acetate was used in variable ratio with increasing polarity as a mobile phase [15]. TLC profile of elutes obtained from the column was performed which showed two spots with Rf value 0.34 and 0.16 in fractions 16-20.

High Performance Liquid Chromatography (HPLC)- Fractions 16-20 were mixed to perform HPLC. Shimadzu SPD-M20A system (Prominence Diode ARR, Japan) having photodiode array detector (PDA 100), quaternary pump 680, autosampler ASI-100, injector with a 200 µl loop, column oven and data system LC solution was used for HPLC analysis. HPLC analysis conditions were maintained according to the procedure of Tawab *et al.* [16]. Two solvent system was used as solvent A (water; methanol; orthophosphoric acid in 55: 40: 0.5 ratio) and solvent B (methanol: acetonitrile: water; orthophospheric acid in 55: 40: 4.5: 0.5 ratio). For first 11 minutes, the composition of the mobile phase was kept constant at 10% A and 90% B then changed to 100% B in 4 min. The column was reequilibrated for 10 min until the next sample was injected. The flow rate was kept at 1 ml/sec with a sample injection volume of 20 µl. Sample detection was carried out at 254 nm wavelength, and data analysis was performed by software LC solution.

Dose selection and preparation- Forty mg/kg equivalent of total BAs was selected as a maiden dose. The dose was calculated from the amount of BAs estimated by titration. Sample D is considered as BA enriched extract for further studies which contains 40 mg of BAs per 160 mg. The BA enriched extract 160 mg and soy lecithin in a ratio of 1:1 was added with half of the microcrystalline cellulose (45 mg) [17]. This physical mixture was further mixed with the excipients like microcrystalline cellulose 45 mg, carbopol 30 mg, 30 mg of citric acid anhydrous, dibasic calcium phosphate 20 mg, PEG 400 10 mg and methylparaben 0.1%, and processed for wet granulation.

Pre-formulation study- The granules obtained was tested for the angle of repose, loose bulk density, tapped bulk density, Carr's Index and Hausner's ratio.

Evaluation of Tablets- The tablets were compressed at the specified weight of 500 mg. The flow of granules from the hopper was satisfactory with no capping or sticking. The prepared tablet formulation was evaluated as per the Pharmacopoeia of India for weight variation, thickness, hardness, friability and disintegration time [18].

Animals- The *in vivo* studies were performed on male Wistar rats (150-200 gm) and albino New Zealand rabbits (1.5-2.2 kg). Institutional Animal Ethical Committee approved the project and all procedures were performed following the guidelines of CPCSEA, New Delhi. Rat was housed with paddy husk bedding and rabbits in rabbit cages at the temperature of 25±2°C, relative humidity of 65±5%, and 12:12 hr light: dark cycle was maintained. Animals were allowed free access to potable water and standard palette diet (Hindustan Lever, Mumbai).

Anti-arthritic activity on Rats- Rats were divided into five groups each containing 6 animals as; group 1 vehicle control, group 2 methotrexate 2.5 mg/kg, group 3 BA enriched extract 160 mg/kg (≅ 40 mg/kg of BAs) and group 4 and 5 tablet formulation 500 mg/kg (≅ 40 mg/kg of BAs) and 1000 mg/kg (≅ 80 mg/kg of BAs) given per orally. Each tablet of 500 mg contains 160 mg of BA enriched extract that is equivalent to 40 mg of BAs. Tablet doses were administered according to the body weight of animals; the equivalent weight of the tablet was weighed and suspended in distilled water with 2% CMC. Standard drug methotrexate was also suspended according to the dose requirement in 2% CMC. Rheumatoid was induced by subcutaneous injection of 0.1 ml of Freund's Complete Adjuvant (CFA) sub planter at right hind paw and the base of the tail. The vehicle, BA enriched extract and both the doses of tablet formulations were administered continuously up to the 28th day starting from day zero. Standard drug methotrexate was administered every fourth day. On the 7th day of treatment, the CFA was injected again in to

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each group. The rats were observed for the paw volume on zero day before treatment followed by on 1st, 7th, 14th and 28th day using mercury Plethysmometer. Animals were individually placed gently on Eddy's hot plate at 55±1°C for not more than 15 sec. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate was determined on 0, 1, 7, 14, 21 and 28th day [19]. Arthritis index was measured as inflammation intensity on right hind paw by grading all rats on the 28th day based on the extent of erythema and edema of the tissue on a scale of 0-4. The observation parameters for arthritis index is: no inflammation=0, unequivocal inflammation of one joint of the paw= 1, unequivocal inflammation of at least two joints or moderate inflammation of one joint of the paw=2, severe inflammation of one or more joint= 3 and maximum inflammation of one or more joints in the paw=4 [20].

On the 28th day, all animals were sacrificed, and their blood sample was collected by heart puncture. Blood samples were kept at room temperature for 30 min, centrifuged at 1500 rpm for 10 min; separated plasma was analyzed for TNF-α by using rat TNF-α ELIZA kit (Ray Biotech Inc, Norcross G A) and EIZA plate reader [21]. The right hind paw was amputated above the knee joint and fixed in 70% formaldehyde solution. The paw was then decalcified, embedded in paraffin and sectioned in a mid-sagittal plane with a microtome (5 µm). Histological slides were stained with hematoxylin and eosin and examined under the 40× lens for parameters like an articular cavity, joint inflammation, synovial pannus, mononuclear cells, cartilage erosion and bone destruction [22].

Pharmacokinetic study- Overnight fasted healthy male New-Zealand rabbits of 2-2.5 kg were divided into 3 groups as; group 1 BA enriched extract 160 mg/kg, group 2 tablet 500 mg/kg and group 3 tablet 1000 mg/kg. All the drugs were given orally and blood samples were collected through marginal ear vein puncture after 30, 60, 90, 120, 180, 240, 300 and 420 minutes in EDTA tubes [21]. After 30 min at room temperature blood samples were centrifuged at 1500 rpm for 10 min, separated plasma was stored at -20°C till analysis. BAs was extracted from 100 µl of plasma and analyzed by HPLC for concentration determination. The plasma was taken in a stoppered test tube and added with an equal volume of 1N hydrochloric acid and vortex for 1 min. Plasma samples was further added with five volume of diethyl ether: n-hexane (2:1), vortex for 1 min and centrifuged at 400 rpm for 5 min. The upper layer was separated and evaporated till dryness at 40°C. BAs was extracted with the addition of 70% methanol pH adjusted to 10.70 with liquor ammonia. The sample was vortex for 30 seconds and centrifuged at 3000 rpm for 1 min. The organic layer was separated and evaporated under nitrogen at room temperature. The residue was reconstituted in n-hexane, filtered through 0.45 µm filter and 20 µl was injected into the set conditions for HPLC analysis [23]. Using the response factor determined for HPLC analysis, the concentration of the analyte in all the plasma samples was calculated.

Concentration of BAs in the sample= Area % of the peak/ Response factor. Response factor is the ratio between a signal produced by an analyte and the quantity of analyte, which produces the signal. Response factors compensate for the irreproducibility of manual injections into the column. The plasma concentration vs time profile was plotted for all three samples. The area under the concentration-time curve (AUC) was calculated by the trapezoidal method with extrapolation to infinity by the addition of the last observed concentration divided by the terminal elimination rate constant, and the pharmacokinetic parameters were calculated [24].

Statistical Analysis- All the results were expressed as mean±standard error mean (SEM). Data were analyzed using one way ANOVA followed by turkey's multiple comparison tests, *p<0.001* was considered as statistically significant. The analysis was carried out using Graph pad software of version 4.

RESULTS

Enrichment and determination of boswellic acid- The procured ethanolic extract of *B. serrata* was subjected to enrichment of BAs content following four different methods. The method reported by Gokaraju et al. showed a maximum yield of 26.52 mg of total BAs per 100 mg extract, named as sample D. TLC of sample D showed four spots and further column chromatography resulted in a fraction with two well separated spots having Rf value of 0.34 and 0.16. HPLC analysis of these particular fractions showed well separated peaks for KBA and AKBA (Fig. 1).

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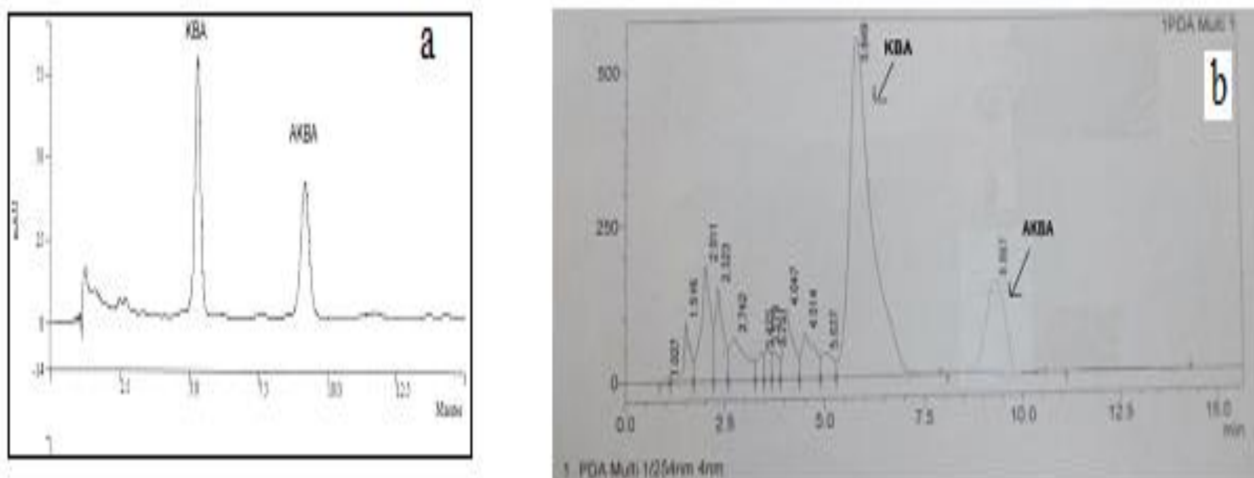

Fig. 1: HPLC chromatogram showing standard peak of AKBA and KBA (a) and column fraction separated from sample D with KBA Rt 5.84 and AKBA Rt 8.96 (b)

Preparation and standardization of soy lecithin tablet formulation- BAs equivalent to 40 mg/kg was selected as a maiden dose that is equal to 160 mg/kg dose for sample D designated as BA enriched extract. Tablet formulation was developed containing 160 mg of BA enriched extract in 500 mg tablet with soy lecithin in 1:1 ratio. The granules obtained were satisfactory with the angle of repose 31.74±1.03°, loose bulk density 0.44±0.005 gm/ml, tapped bulk density 0.48±0.004 gm/ml, Carr's Index 16.32 and Hausner's ratio 1.17. The weight variation of the tablets was 1.30±0.014%, hardness was in the range of 3.25±0.48 kg/cm², and friability was not more than 0.79±0.008%. The thickness of the tablets was in the range of 4.5–4.7 mm². The tablets showed a disintegration time limit within 12.30±1.22 min.

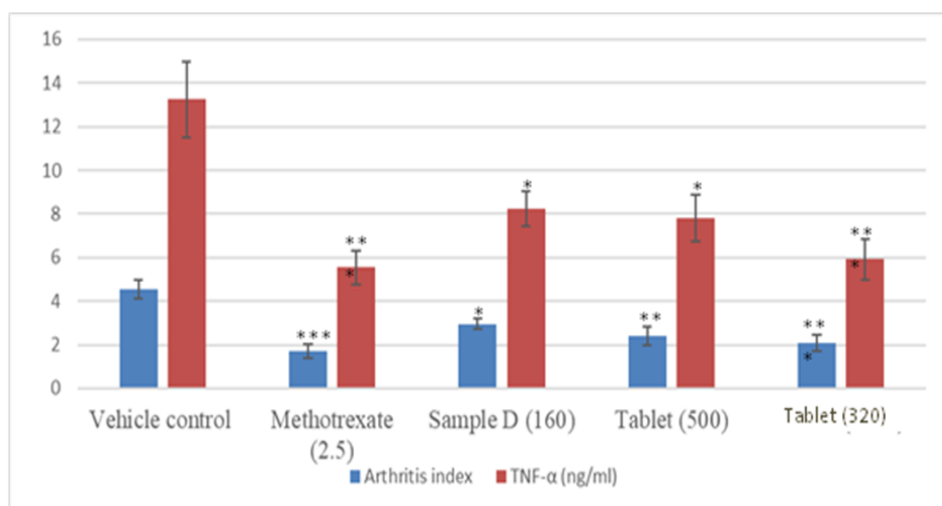

Fig. 2: Effect of boswellic acid-enriched extracts and formulated tablet on arthritis index and TNF-α of Freund's Complete Adjuvant induced arthritic rats.

All the data expressed as M±SEM, n=6 per group. **p<0.05, **p<0.01, ***p<0.001* and ns=not significant, when compared to vehicle control group.

Anti-arthritic activity- BA tablets at both doses showed extremely significant (*p<0.05-0.001*) reduction in paw volume 14th day onward whereas the BA enriched extract significantly (*p<0.05-0.01*) reduced paw volume 21st day onward. Tablet at 320 mg/kg dose showed 45.33% inhibition of paw volume compared to 49.33% of methotrexate (Table 1). BAs enriched unformulated extract and tablets at 160, and 320 mg/kg dose showed significant (*p<0.01-0.001*) increase in hot plate reaction time 21st day onward with 18.58, 39.10 and 53.45% corresponding increase on the 28th day (Table 2). The tablet formulation at 160 and 320 mg/kg dose showed

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significant reduction of arthritis index (*p<0.05-0.01*) and TNF-α level (*p<0.01-0.001*) in rat plasma on the 28th day of treatment compared to unformulated extract (Fig. 2). Tablet formulation at 160 mg/kg and 320 mg/kg dose showed minimal joint inflammation, cartilage erosion and bone destruction whereas BA enriched extract showed the presence of bone destruction, cartilage erosion and pannus formation. Tablet at 320 mg/kg dose showed articular surfaces damage reversal, small areas of erosion and irregular connective tissue (Fig. 3).

Table 1: Effect of boswellic acid-enriched extract and formulated tablet on paw volume of Freund's Complete Adjuvant induced arthritic rats

Treatment (mg/kg, p.o)	Paw volume (ml±SEM)						Increase in paw volume on 28th day (%)
	0 day	1st day	7th day	14th day	21st day	28th day	
Vehicle control (2% CMC)	0.22±0.032	0.40±0.016	0.57±0.016	0.88±0.033	0.78±0.013	0.75±0.013	240.90
Methotrexate (2.5)	0.25±0.028	0.37±0.023	0.56±0.041	0.66±0.023***	0.52±0.021***	0.38±0.022***	52.00
BA enriched extract (160)	0.19±0.018	0.32±0.031	0.52±0.027	0.75±0.033ns	0.64±0.022*	0.55±0.034**	189.48
Tablet (160)	0.20±0360	0.34±0.02	0.58±0.031	0.73± 0.037*	0.66±0.033*	0.49±0.043***	145.00
Tablet (320)	0.23±0.029	0.40±0.029	0.50±0.032	0.69±0.036**	0.62±0.043**	0.41±0.036***	78.26

*All the data expressed as M±SEM, n=6 per group. *P<0.05, **P<0.01, ***P<0.001 and ns=not significant when compared to vehicle control group.*

Table 2: Effect of boswellic acid-enriched extract and formulated tablet on paw withdrawal reaction time of Freund's Complete Adjuvant induced arthritic rats

Treatment (mg/kg, p.o)	Reaction time (Sec±SEM)						Change in reaction time on 28th day (%)
	0 day	1st day	7th day	14th day	21st day	28th day	
Vehicle control (2% CMC)	6.25±0.65	4.36±0.45	4.86±0.34	4.91±0.42	4.30±0.35	4.11±0.58	– 34.24
Methotrexate (2.5)	5.33±0.87	4.66±0.34	6.22±0.56	7.82±0.68*	8.44±0.47***	9.68±0.92***	+ 81.61
BA enriched extract (160)	6.24±0.67	4.52±0.64	6.83±0.83	6.80±0.62ns	7.56±0.80**	7.40±0.63**	+ 18.58
Tablet (160)	6.24±1.067	4.37±0.45	7.35±0.67	7.26±0.66ns	7.35±0.53**	8.68±0.33***	+ 39.10
Tablet (320)	5.50±1.03	4.21±0.67	6.54±0.37	6.94±0.80ns	7.60±0.66**	8.44±0.54***	+ 53.45

*All the data expressed as M±SEM, n=6 per group. *P<0.05, **P<0.01, ***P<0.001 and ns=not significant when compared to vehicle control group.*

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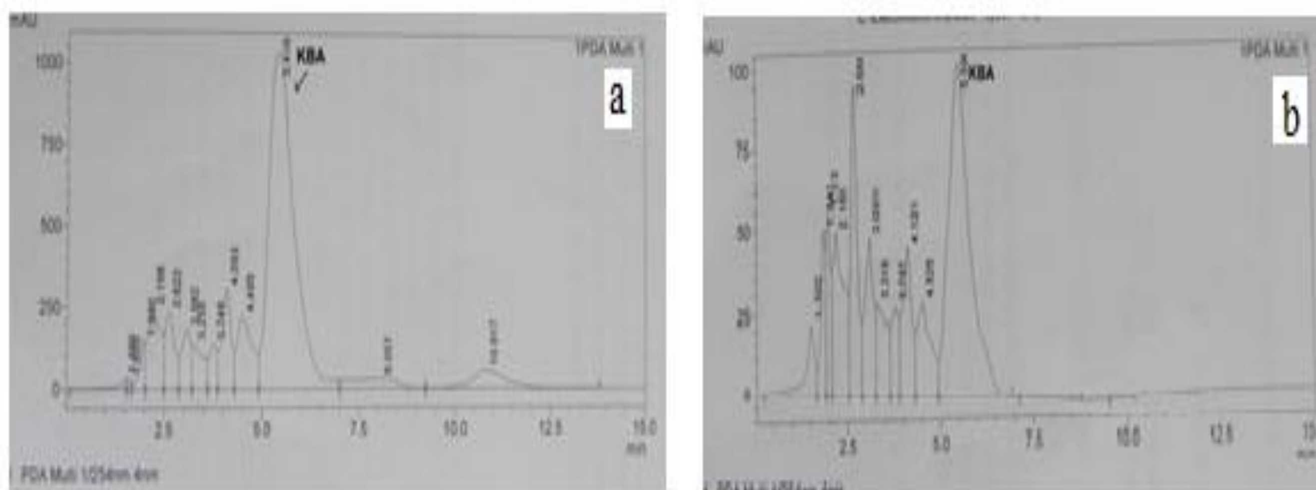

Fig. 3: Photomicrograph showing histopathological changes in Freund's Complete Adjuvant induced arthritic rats to knee joint treated with boswellic acid-enriched extract and formulated tablet.
a) CFA treated vehicle control rat, (b) Methotrexate treated, (c) Sample D (160 mg/kg, (d) Tablet 160 mg/kg and (e) Tablet 320 mg/kg.

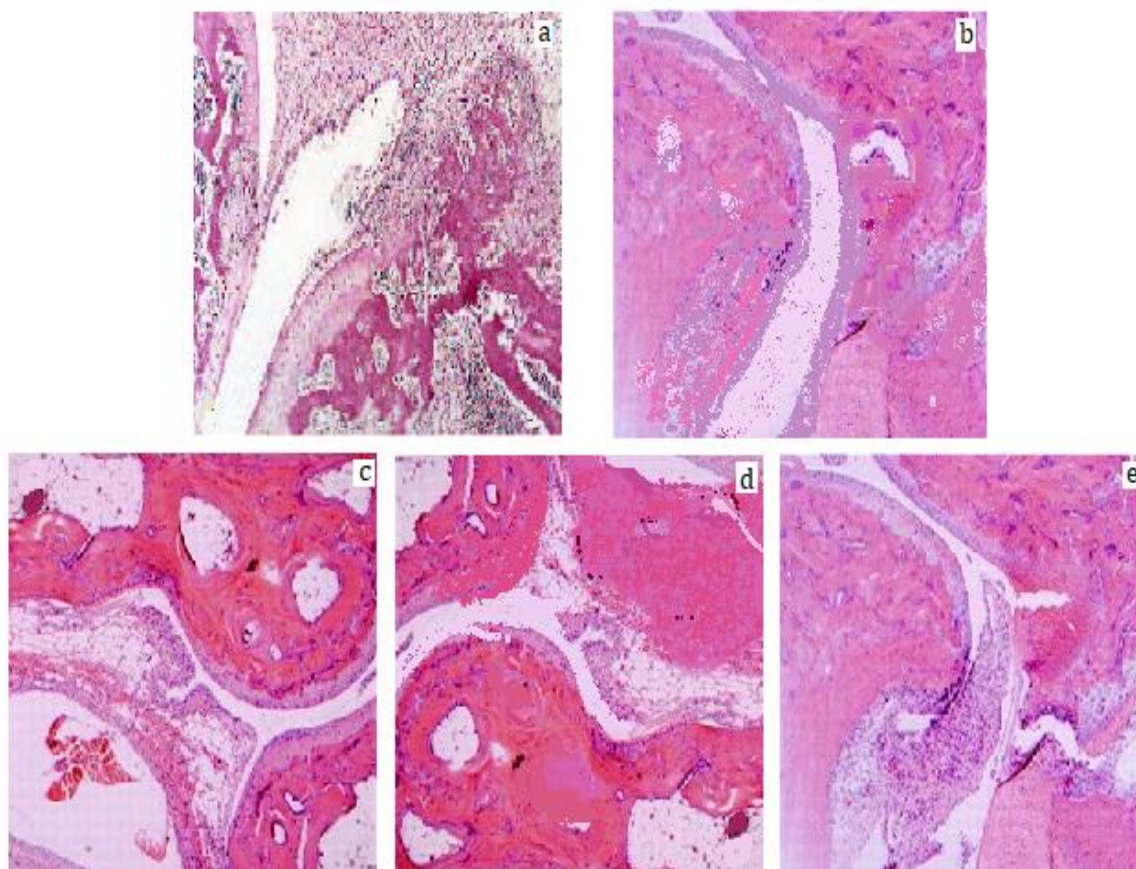

Fig. 4: HPLC chromatogram of BAs separated from rabbit plasma treated with boswellic acid enriched extract (a) and soy lecithin based formulated tablet (b)

Pharmacokinetics- The HPLC analysis of BAs extracted from the plasma sample of rabbits treated with both BAs enriched unformulated extract and tablet showed an identical single peak at RT 5.81 attributed to KBA along with other peaks (Fig. 4). Fig. 5 represented plasma concentration *vs* time curve of boswellic acid enriched extract and formulated tablet on rabbit. The soy lecithin based tablet of BAs enriched extract at 320 mg/kg showed significantly (*$p<0.01$-0.001*) higher C_{max} (980.20±15.45 ng/ml) and AUC (23.78×10^4±2.15 ng.hr/ml

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and lower T_{max} (3 hr) and elimination rate K_e 37.80 ±1.74 min^{-1} as compared to 160 mg/kg dose of tablet (C_{max} 575.13±12.98 ng/ml, AUC 14.52×10^4±1.15 ng.hr/ml, T_{max} 4 hr and K_e 48.61±2.65 min^{-1}) and unformulated BAs enriched extract (C_{max} 487.55±11.73 ng/ml, AUC 12.68×10^4±1.60 ng.hr/ml, T_{max} 5 hr and K_e 67.83±3.04 min^{-1}). The volume of distribution V_d and half life ($T_{1/2}$) were 331.60 ± 9.71 L and 66.52±2.82 min with tablet at 320 mg/kg compared to 152.34±8.32L and 51.68±2.08 min for 160 mg/kg, 179.20±7.63 L and 36.16±1.04 min in case of unformulated extract (Table 3).

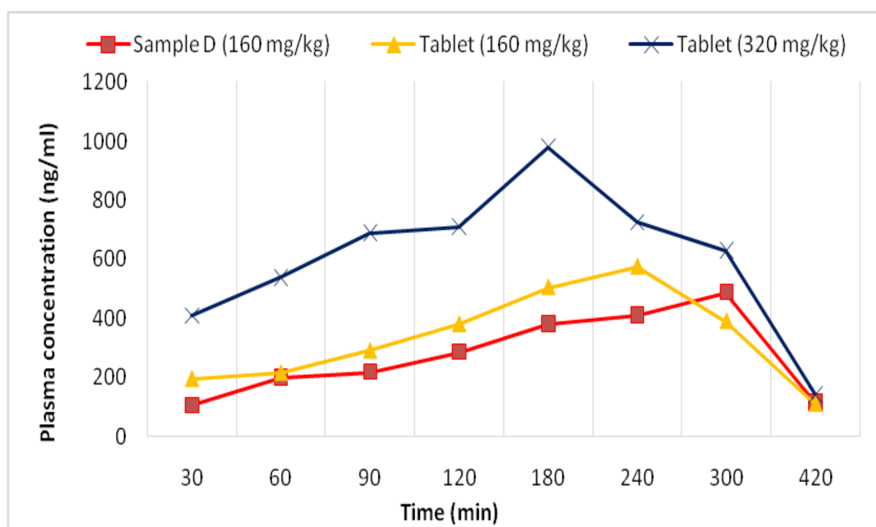

Fig. 5: Mean plasma concentration *vs* time curve of boswellic acid enriched extract and formulated tablet administered on rabbit.

Table 3: Pharmacokinetic parameters of boswellic acid enriched extract and formulated tablet in rabbit plasma

Pharmacokinetic parameters	BA enriched extract (160 mg/kg)	Tablet (160 mg/kg)	Tablet (320 mg/kg)
C_{max} (ng/ml)	487.55±11.73	575.13±12.98**	980.20±15.45***
T_{max} (min)	300	240	180
AUC_{0-24} (ng.hr/ml)	12.68×10^4±1.60	14.52×10^4±1.15^ns	23.78×10^4±2.15**
K_e (min^{-1})	67.83±3.04	48.61±2.65***	37.80±1.74***
V_d (L)	179.20±7.63	152.34±8.32^ns	331.60±9.71***
Cl (ml/min)	202.22±7.11	123.24±5.25***	208.91±8.50^ns
$T_{1/2}$ (min)	36.16±1.04	51.68±2.08*	66.52±2.82**
$AUMC_{0-24}$ (mg.hr₂/L)	328.40×10^7±20.19	368.33×10^7±21.36^ns	573.25×10^7±22.42***
MRT (min)	2600.80±126.23	2530.90±141.22^ns	2480.45±121.73^ns

*All the data expressed as M±SEM, n=6 per group. **p<0.01, ***p<0.001* and *ns=not significant when compared to the vehicle control group.*
C_{max}: Mean maximal plasma concentration, T_{max}: Meantime required to C_{max}, AUC: Area under the plasma concentration versus time curve, Ke: Elimination rate constant, V_d: Volume of distribution, Cl: Clearance, $T_{1/2}$: biological half life, AUMC: Area under first momentum curve, MRT: Mean residual time.

DISCUSSION

The ethanolic extract of *B. serrata* was subjected to the enrichment of BAs content following four different methods. The product of the method reported by Gokaraju et al. showed to have maximum yield, and HPLC analysis showed well-separated peaks for KBA and AKBA [13,20]. Sample D obtained a higher amount of AKBA may be because this patented method involves acetylation and allylic oxidation steps. This process also efficiently utilizes the un-reacted pyridine and acetic acid from the acetylation step to form a highly active

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oxidizing system such as CrO_3/pyridine and CrO_3/acetic acid. This process has also eliminated the presence of possible chromium impurities in AKBA enriched products by acid-base treatment [13]. All the significant pharmacological actions attributed to the plant *B. serrata* are correlated to KBA and AKBA. Tablet formulation containing 160 mg of BA enriched extract in 500 mg and evaluated for weight variation, hardness, friability, thickness and disintegration time found to be within IP limit.

The enriched extract dose was administered at 160 mg/kg containing 40 mg equivalent of BAs. Soy lecithin based formulated tablet also contained 160 mg of extract in a 500 mg tablet. The dose of the tablets evaluated was 500 mg/kg and 1000 mg/kg thus administering equivalent BAs at 40 mg/kg and 80 mg/kg dose. The CFA-induced arthritic rat model is routinely used for screening compounds having anti-arthritic potential. Intraplantar injection of CFA causes peripheral tissue injury resulting in increased sensitivity to noxious stimulus like heat, mechanical and tactile stimulation. CFA was injected in the right hind footpad to study the acute inflammatory reaction and also in the tail base on the 7th day to study the effect of drugs on delayed immunological reactions that develop approximately 9 days later [25]. The study showed that administration of soy lecithin based BA tablet was more efficacious towards the reduction in paw volume compared to BAs enriched unformulated extract. Tablets at both doses showed an extremely significant reduction in joint inflammation and paw volume starting from 14th day signifying higher and earlier effectiveness of the BAs. Tablet formulation at 160 mg/kg dose showed more than the double increment in hot plate reaction time compared to unformulated extract signifying enhanced systemic absorption and availability of the BAs at the site of action.

The mechanism underlying the anti-inflammatory effect of boswellic acids is inhibition of 5-lipoxygenase, the key enzyme in leukotriene biosynthesis, which also accounts for their anti-inflammatory effectiveness [26]. Acetyl-11-keto-beta-boswellic acid acts directly on the 5-lipoxygenase enzyme at a selective site for pentacyclic triterpenes [27]. Verhoff et al. reported that BAs could inhibit microsomal prostaglandin E2 synthase that is also a molecular basis supporting its analgesic effect along with anti-inflammatory actions [28]. *B. serrata* gum resin extracts, AKBA and KBA also exhibit variable actions in the immune system. A higher dose can reduce primary antibody titers while lower doses showed enhanced secondary antibody titers. BAs induced suppression of the classic way of the complement system is due to inhibition of the conversion of C3 into C3a and C3b. Also, BAs can affect production or release of cytokines with an increase in phagocytic activity of macrophages [29]. These effects explain the effectiveness of BAs in suppressing the late phase deleterious effects of CFA induced adjuvant arthritis.

The BA enriched extract, and the tablets at both doses showed similar efficacy profile to words reduction of TNF-α level in rat plasma whereas reduction of arthritis index was more prominent with the tablet formulations on the 28th day of treatment. This effect may be attributed to the fact that cytokine TNF-α is an integral part of the acute inflammatory response and are released more rapidly than other proinflammatory cytokines [30]. Significant injury-induced elevation of TNF-α levels was detected up to three days after trauma by Spielmann et al. and after five days of injury, the plasma TNF-α levels gradually returned to normal as reported by Liu and Tang[31,32]. As the level of TNF-α was measured on the 28th day of arthritis induction the level of expression may have come to a basal level. Histological examination of right tibiotarsal joint evaluates and compare the effect of BA enriched extract with soy lecithin based tablet formulation on joint inflammation, articular cavity gap, pannus formation, and bone destruction. CFA treated rats showed extensive bone destruction, cartilage erosion and joint inflammation with pannus formation. The articular cavity was markedly reduced with expanded synovial pannus having densely infiltrated mononuclear cells, cartilage erosion, and massive bone destruction by invading synovium. Methotrexate treatment has reduced joint inflammation and bone destruction but showed cartilage erosion with mononuclear cell infiltration. Tablet formulation at 160 mg/kg and 320 mg/kg dose showed a considerable decrease in joint inflammation, cartilage erosion and bone destruction. BA enriched extract at 160 mg/kg was not efficient enough to check out bone destruction, cartilage erosion, and pannus formation. Maximum amelioration of damaging effect of adjuvant arthritis on the articular surfaces was observed with 320 mg/kg tablet based on soy lecithin, with the repair of articular

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surfaces, small areas of erosion and irregularities in the connective tissue.

The pharmacokinetic study was conducted on the rabbit to study the level of KBA and AKBA in blood up to 8 hours after oral administration at 160 mg/k and 320 mg/kg of soy lecithin based tablets of BAs enriched extract compared to unformulated extract. BAs extracted from the plasma sample of rabbit-treated with both BAs unformulated extract and tablet showed multiple peaks but with an identified single peak at RT 5.81 attributed to KBA. The peak of AKBA was not identifiable in the extracted plasma samples of the rabbits maybe due to the decomposition of the most amount of AKBA in the bloodstream or GI tract. It has been observed that during extraction and working up with *B. Serrata* extract AKBA gets decomposed to 3-O acetyl–9,11-dehydro BA and 9,11-dehydro BA, hence all kinetic data was calculated based on the concentration of KBA [33]. Gerbetha *et al.* [23] reported average steady plasma concentrations of 6.4-247.5 ng/ml for KBA and 0-15.5 ng/ml for AKBA with the administration of a high dose of 4200 mg *B. serrata* gum resin extracts per day on human, which is equivalent to 70 mg/kg dose. Another reason behind lower plasma levels of AKBA might be the higher volume of distribution associated with its greater lipophilicity [34]. The soy lecithin based tablet of BAs enriched extract at 320 mg/kg containing 80 mg equivalent of BAs showed to have the higher peak plasma concentration and AUC in lowest time with lower elimination rate in comparison to 160 mg/kg dose of tablet and unformulated BAs enriched extract. The volume of distribution and half-life was found to be approximately twice with the tablet at 320 mg/kg compared to 160 mg/kg and unformulated extracted.

Soy lecithin based tablet formulation has shown promising activity with higher analgesic and anti-inflammatory activity compared to unformulated extract along with quick and higher bioavailability and increased duration of action. A marked increase in absorption and tissue distribution of BAs is being achieved by using phospholipid lecithin based phytosome and capsules [9,10]. Fatty meals improve absorption of BAs to some extend due to improved solubility in the biliary acids facilitating dispersion in the intestinal fluids [35,36]. Phospholipids like lecithin may act by enhancing the lipophilicity of BAs. Lecithin based formulation of curcuminoids has shown enhanced absorption as reported by Cuomo *et al*. [37]. The phospholipid based formulation strategy can be very beneficial for the other herbal extracts and phytoconstituents that are mostly sticky materials with low lipid solubility.

Anautoimmune disease like RA cannot be cured permanently, and the patients are compelled to take medications throughout their life. Mostly the patients of third world countries rely more on herbal treatment for such incurable and chronic diseases due to less vulnerability to toxicity and cost-cutting. Alternative and occupational therapies are preferred due to lower costs and side effects. The present scenario demands to have an eye on herbal remedies for quality and efficacy for treating chronic and incurable diseases. Beneficial effects *B. serrata* gum resin for arthritis is mentioned in traditional Ayurvedic and Unani texts, though the problem persisted with low bioavailability. Many scientific efforts have been diverted towards the enrichment of the number of active constituents like AKBA and KBA in *B. serrata*, to produce a better effect in low doses reducing bioburden on the patients, and minimizing the risk of side effects and drug interaction. Driven to development of aherbal formulation targeting improved efficacy and bioavailability, and to standardize so that the traditional system of medicine and the natural wealth of India can be acceptable regarding global standards.

CONCLUSIONS

The results of the study substantiated higher efficacy and bioavailability of BAs enriched extract tablet formulation in addition to lecithin. Depending on the results it can be concluded that the BAs enriched fraction is phytochemically and biologically more effective concerning pharmacokinetic and pharmacodynamic parameters when administered in lecithin based tablet form as compared to unformulated extract.

Further studies are required to explain the absorption mechanism and tissue distribution pattern associated with this lecithin based tablet.

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