PUBLICATIONS
INHIBITION OF TNFα PRODUCTION AND BLOCKING OF MITOGEN-ACTIVATED PROTEIN KINASE/NFκB ACTIVATION IN LIPOPOLYSACCHARIDE-INDUCED THP-1 HUMAN MONOCYTES BY 3-O-ACETYL-11-KETO-β-BOSWELLIC ACID

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ABSTRACT

Boswellia serrata resin is regarded as a potent anti-inflammatory agent in traditional and herbal medicine in the Indian subcontinent. The compound 3-O-acetyl-11-keto-β-boswellic acid (AKBA) is the most effective boswellic acid and mostly responsible for B. serrata’s anti-inflammatory properties. Here, we reexamined the anti-inflammatory potential of a product selectively enriched with 30% AKBA (BE-30, also known as 5-Loxin®) and evaluated its underlying possible molecular mechanism of action. BE-30 was 42.96% more effective than regular Boswellia extract (BE-3) in inhibiting 5-lipoxygenase activity. In lipopolysaccharide (LPS)-induced THP-1 human monocytes, BE-30 showed a strong anti-TNFα activity (half maximal inhibitory concen-
tration 4.61 ± 0.87 μg/mL), which provides 71.14% (P < 0.001) better efficacy than BE-3. Our investigations suggest that BE-30 inhibits the LPS-induced activation of serine/threonine kinases of mitogen-activated protein kinase family, which are the key players responsible for a variety of cellular responses, including inflammation. Additionally, we also show that BE-30 blocks the LPS-induced NFκB activation by inhibiting IκBα phosphorylation and p65 translocation to the nuclear compartment of THP-1 monocytes. Collectively, these findings provide molecular basis for the anti-inflammatory properties of BE-30.

PRACTICAL APPLICATIONS

This article describes the underlying molecular mechanisms for the anti-inflammatory activities of an enriched formulation containing up to 30% 3-O-acetyl-11-keto-β-boswellic acid (AKBA), the active principle that is mainly responsible for Boswellia serrata’s anti-inflammatory properties. This AKBA-enriched formulation (BE-30), known as 5-Loxin, is commercially available in the United States and is being used as a key ingredient of several formulations for improvement of joint health. This work explains anti-TNFα properties of BE-30 in a cellular inflammation model in vitro and its inhibitory action on MAPK pathways in inflammation and, in addition, its anti-NFκB activities. The findings will provide a further comprehensive mechanism of anti-inflammatory action of 5-Loxin at the cellular and molecular level.

INTRODUCTION

Boswellic acids are pentacyclic triterpenic acids, present in the gum resin of Boswellia serrata, which are known for their anti-inflammatory properties (Reddy et al. 1989; Safayhi et al. 1992). Suppression of leukotriene synthesis via inhibiting 5-lipoxygenase (5-LOX) is considered the key mechanism for their anti-inflammatory effect. Boswellic acids have been regarded as specific, non-redox inhibitors of 5-LOX; they do not interrupt 12-lipoxygenase and cyclooxygenase activities (Safayhi et al. 1992, 1995; Ammon et al. 1993). In addition, boswellic acids also inhibit leukocyte elastase, which may also contribute to their anti-inflammatory properties (Kapil and Moza 1992; Safayhi et al. 1997). Among known boswellic acids, AKBA possesses the most potent inhibitory activity on 5-LOX (Safayhi et al. 1992; Sailer et al. 1996).

A significant number of studies support that Boswellia extract (BE) is beneficial in patients suffering from various diseases such as bronchial asthma,
Crohn’s disease and arthritis (Gupta et al. 1998, 2001; Kimmatkar et al. 2003). AKBA is a minor constituent of the *Boswellia* extract and it typically varies from 2 to 3% in the higher grade commercial material. It is interesting to assume that enhancement of anti-inflammatory potential of BE might be achieved by increasing the concentration of the active ingredient, i.e., AKBA. Therefore, with an intention to generate more efficacious anti-inflammatory agent, we have developed a novel *Boswellia* compound comprising 30% AKBA (BE-30) (Gokaraju et al. 2004).

Previously, in a human genome screen study we have shown that BE-30 downregulates 113 genes in TNFα-induced human microvascular endothelial cells (HMECs). These genes are directly related to inflammation, cell adhesion and proteolysis (Roy et al. 2005). In another study, BE-30 showed almost complete abrogation of matrix metalloproteinase-3, -10 and -12 expression and activities in TNFα-induced HMECs (Roy et al. 2006). The *in vitro* anti-inflammatory properties of BE-30 were further substantiated in carrageenan and Freund’s complete adjuvant-induced rat paw inflammation models. Furthermore, in comparison with the regular *Boswellia* extract, BE-30 exhibited significantly better anti-inflammatory efficacy in Freund’s adjuvant-induced arthritis model of rats (Roy et al. 2005, 2006). However, the molecular basis for the anti-inflammatory properties of BE-30 has not been clearly elucidated. Therefore, in the present study we evaluated possible mechanisms underlying the anti-inflammatory efficacy of BE-30. BE-30 was shown to inhibit the production of pro-inflammatory cytokine TNFα in LPS-induced THP-1 human monocytes; and it downregulates key modulatory proteins of 5-LOX-arachidonic acid cascade such as 5-LOX activating protein (FLAP) and 5-LOX in LPS-induced THP-1 human monocytes. To explore further, we also show that BE-30 downregulates mitogen-activated protein kinase (MAPK)/NFκb activation in LPS-induced THP-1 monocytes.

AKBA formulation enriched to 30%, i.e., BE-30 has been available in the United States since 2004 as a nutraceutical supplement for the management of inflammatory disorders, such as arthritis and osteoarthritis. This present study will further elucidate the molecular basis of anti-inflammatory properties of BE-30 and strengthen the argument in favor of an effective food supplement for inflammatory disorders like arthritis, psoriasis, asthma etc.

**MATERIALS AND METHODS**

**Reagents and Chemicals**

Aprotinin, Dulbecco’s modified Eagles medium (DMEM), phenylmethylsulfonyl fluoride (PMSF), leupeptin, pepstatin, linoleic acid, 5-LOX and
anti-actin monoclonal antibody were purchased from Sigma Chemical Co. (St. Louis, MO). Human TNFα enzyme-linked immunosorbent assay (ELISA) kit was procured from R&D Systems Inc. (Minneapolis, MN). Super Signal West Pico Chemiluminescent substrate for Western immunoblot assay was purchased from Pierce Biotechnology Inc. (Rockford, IL).

BE-30 was acquired from Laila Nutraceuticals (Vijayawada, India). It is a *B. serrata* gum resin extract containing 30% AKBA. It is produced by selectively enriching the extract in AKBA content by converting less active boswellic acids into the most potent AKBA.

**5-LOX Assay**

5-LOX enzyme inhibitory activity was measured using the method described earlier (Reddanna *et al.* 1990). Briefly, the assay mixture contained 80 µM of linoleic acid and a sufficient amount of potato 5-LOX in 50 mM phosphate buffer (pH 6.3). The reaction was initiated by addition of enzyme buffer mix to linoleic acid and the enzyme activity was monitored as the increase in absorbance at 234 nm, which is the absorption maximum of the major reaction product, 9(S)-10E, 12Z-hydroperoxy octadecadienoic acid (9[S]-HPODE) (εm = 23,000/M/cm). The reaction was monitored for 2 min and the inhibitory potential of the test substances was measured by incubating various concentrations of test substances two minutes before addition of linoleic acid. Each evaluation was performed in triplicate. Percentage inhibition of enzyme activity was calculated by comparing the slope of test substances with that of control.

**Cell Culture and Treatments**

Human monocytes derived THP-1 cells (procured from National Center for Cell Science, Pune, India) were cultured in DMEM with L-glutamine, containing 100 IU/mL penicillin, 10 µg/mL streptomycin and 10% (v/v) heat-inactivated fetal bovine serum (HyClone, Logan, UT) and maintained at 37°C in an atmosphere containing 95% air and 5% CO2. BE-30 was dissolved in DMSO as 1 mg/mL stock solutions and stored at −20°C. The cells were harvested, washed and re-suspended with phenol red free and serum free DMEM supplemented with 0.2% fetal bovine serum. Equal number of cells was plated into each well of a 96-well cell culture plate, keeping the outside wells blank. Inflammatory response was induced by 100 ng/mL of LPS in cells pretreated with BE-30 for 1 h. Medium containing 0.1% DMSO was used as vehicle control. Culture supernatants were collected for pro-inflammatory cytokine ELISA.

For immunoblot analysis experiments, cells were cultured in DMEM containing 10% FBS and plated in 35-cm culture dishes. THP-1 cells were
pretreated for 1 h with 10 μg/mL of BE-30 and then stimulated with 100 ng/mL of LPS for either 1 h or for different time periods. The cells were washed two times with chilled phosphate buffered saline and cell pellets were stored at −80°C until further use.

**TNFα ELISA**

Pro-inflammatory cytokine, TNFα was quantitatively measured in the cell culture supernatants by human TNFα ELISA development kit (R&D Systems Inc., Minneapolis, MN). Briefly, 96-well microtiter plates (Corning, MA) were coated with anti-TNFα capture antibody and following blocking the non-specific binding sites, the wells were reacted with cell-free culture supernatants (clarified at 10,000× g for 10 min, 4°C). Specifically bound cytokines were probed with TNFα detection antibody and followed by streptavidin-HRP conjugate. The color reaction was developed by 3,3′,5,5′-tetramethylbenzidine in the presence of H2O2. Finally, the absorbance was read at 450 nm in an ELISA reader (BioRad, Hercules, CA). Standard wells contained known concentrations of recombinant human TNFα. Standard curves were drawn by plotting the OD at respective concentration of the standard.

**Preparation of Nuclear Extracts**

Nuclear extracts were prepared following the protocol described earlier (Aikawa et al. 2002). Briefly, the cells (10⁶/mL) were washed with ice-cold phosphate-buffered saline (PBS), suspended in 200 μL of lysis buffer (10 mM N-(2-hydroxyl)piperazine-N-(2-ethenesulfonic acid [HEPES]; pH 7.9), 10 mM potassium chloride (KCl), 0.1 mM EDTA, 0.1 mM (ethyleneglycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), 1 mM DL-DTT, and were allowed to swell on ice for 15 min, and followed by addition of 12.5 μL of 10% Nonidet P-40 (Igepal CA630, Sigma Chemical Co., St. Louis, MO). The cell suspension was mixed thoroughly for 15 s and spun at 15,000× g at 4°C for 8 min. The supernatant was saved as the cytoplasmic fraction and the nuclear pellet was re-suspended in 25 μL of ice-cold nuclear extraction buffer (20 mM HEPES [pH 7.9], 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT) and kept on ice for 15 min with occasional vortexing. The nuclear extracts were clarified at 14,000× g for 5 min at 4°C, and were stored at −80°C in aliquots.

**Western Immunoblot Analysis**

Immunoblots analyses were carried out following the procedure described earlier (Sengupta et al. 2006), with some modifications. To prepare the whole cell lysates, the cells were lysed in lysis buffer (50 mM Tris, pH 7.5 containing
150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 1 mM PMSF, 1 µM pepstatin, 1 µg/mL aprotinin and 1 µg/mL leupeptin). Cell lysates were clarified at 14,000×g for 10 min at 4°C and the protein concentrations were estimated with Bradford reagent. Equal amount of proteins was run in either 7.5 or 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The resolved proteins were electroblotted onto nitrocellulose membranes and blocked with Superblock (Pierce) and incubated with the primary antibodies overnight at 4°C. MAPK and phospho-MAPK family antibody sampler kits were purchased from Cell Signaling Technology (Beverly, MA). Anti-FLAP, anti-5 lipoxygenase, anti-NFκB p65 and anti-IκB alpha antibodies were purchased from Abcam, Cambridge, UK. Immunoreactive bands were developed with Super Signal West Pico Chemiluminescent substrate (Pierce Biotechnology Inc.) and the band intensities were analyzed using Molecular Imaging Software, version 4.0 (Eastman Kodak Company, Rochester, NY).

**NFκB p65 Localization**

The effect of BE-30 on LPS-induced nuclear translocation of NFκB p65 was examined using immunofluorescence method as described previously (Sengupta *et al.* 2001). Briefly, cells were pretreated for 30 min with 10 µg/mL of BE-30 and then incubated further with 100 ng/mL of LPS for 30 min. Vehicle control and LPS control cultures received only 0.1% DMSO in place of BE-30. Washed cells were fixed with 4% paraformaldehyde for 15 min. After washing, the cells were permeabilized with 0.1% Triton X-100 for 5 min and followed by blocking with 1% BSA. The washed cells were incubated with 4% paraformaldehyde for 15 min. After washing, the cells were permeabilized with 0.1% Triton X-100 for 5 min and followed by blocking with 1% BSA. The washed cells were incubated with 1:500 dilution of rabbit monoclonal NFκB p65 antibody in PBS containing 0.5% Tritonx-100 and 1% BSA. After overnight incubation, the washed cells were incubated with goat anti-rabbit IgG-FITC (1:200) for 30 min. Finally, the washed cells were mounted on glass slides and analyzed under an epifluorescence microscope (TS-100F, Nikon, Tokyo, Japan).

**Statistical Analyses**

Results are expressed as mean ± standard deviation. The significance of data was evaluated using Student’s *t*-test for comparison between two groups. The values of *P* < 0.05 were considered as statistically significant.

**RESULTS**

**Anti-TNFα Property of BE-30**

In response to inflammatory stimulation, production and secretion of pro-inflammatory cytokines from macrophages or monocytes are essential
phenomena. Among pro-inflammatory cytokines, TNFα holds the key role in the inflammatory process. Hence, we assessed the ability of BE-30 and regular commercial *Boswellia* extract (BE-3) to inhibit the TNFα production in LPS-induced THP-1 human monocytes in a comparative manner. In LPS-induced THP-1 cells, BE-30 and BE-3 showed inhibition of TNFα production in a dose dependent manner (Fig. 1A,B). Interestingly, in comparison with BE-3, BE-30 is 71.14% (*P* < 0.001) more potent in inhibiting the TNFα production in the inflamed cells. The half maximal inhibitory concentrations (IC₅₀) of BE-30 and BE-3 for TNFα production are 4.61 ± 0.87 and 7.89 ± 1.13 μg/mL, respectively (Fig. 1C).

*Boswellia* extract is known for its 5-LOX inhibitory activity. We further analyzed the comparative ability for inhibition of 5-LOX activity of BE-3 and BE-30. Interestingly, *in vitro* enzymatic assay shows that BE-30 possesses 42.96% more effectiveness in inhibiting 5-LOX activity in comparison with BE-3. The IC₅₀ for 5-LOX activity of BE-30 and BE-3 were 57.8 and 82.63 μg/mL, respectively (Fig. 1C).

<table>
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<tr>
<th>Compounds</th>
<th>Activities (IC₅₀) (μg/ml)</th>
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<tr>
<td></td>
<td>5-lipoxygenase</td>
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<tr>
<td>BE-30 (BE-30)</td>
<td>57.8</td>
</tr>
<tr>
<td>Boswellia</td>
<td>82.63</td>
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<td>Extract (BE-3)</td>
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**FIG. 1. COMPARATIVE ANALYSIS OF ANTI-INFLAMMATORY PROPERTIES OF BE-30 AND REGULAR BOSWELLIA EXTRACTS (BE-3)**

(A,B) Bar diagrams depict the dose-dependent effect of BE-30 and BE-3 on TNFα production in LPS-stimulated THP-1 human monocytes. THP-1 cells were pretreated with various concentrations of BE-30 and BE-3 for 1 h and treated with 100 ng/mL of LPS for 4 h. Secreted TNFα in cell-free culture supernatants were quantitatively measured by enzyme-linked immunosorbert assay. The values are the means ± standard deviations of three independent experiments. 0.1% DMSO-treated cultures were treated as the vehicle controls. (C) Comparative abilities of inhibiting the 5-lipoxygenase activity (half maximal inhibitory concentration [IC₅₀]) by BE-30 and BE-3 in enzymatic assays; and IC₅₀ values for TNFα inhibition derived from the experiments shown in panels A and B, respectively.
BE-30 Inhibits 5-LOX and FLAP Expressions in LPS-Induced THP-1 Cells

The enzyme 5-LOX and FLAP are required for the synthesis of leukotrienes in intact cells, which lead to inflammatory response via arachidonate pathway (Dixon et al. 1990). We evaluated whether BE-30 can downregulate the expression of these two regulatory proteins of 5-LOX-arachidonic acid inflammatory pathway in LPS-induced THP-1 cells. As expected, the immunoblots show significant increase in 5-LOX and FLAP protein expressions in LPS-induced THP-1 monocytes in 24 h; and BE-30 is able to abrogate completely the overexpressed 5-LOX and FLAP in LPS-induced THP-1 cells. Interestingly, BE-30 alone does not exhibit significant modulation of these protein expressions in vehicle-treated cells (Fig. 2).

BE-30 Inhibits Activation of MAPK Pathway in LPS-Induced THP-1 Monocytes

The MAPK pathways transduce a large variety of external signals, leading to a wide range of cellular responses, including inflammation, growth, differentiation and apoptosis (Qi and Elion 2005). In inflammation, production of pro-inflammatory cytokines including TNFα is increased via activated
MAPK pathway in monocytes and lymphocytes. To elucidate whether BE-30 is able to downregulate the activated kinases in MAPK pathway, the effect of BE-30 on MAPK activities in LPS-induced THP-1 monocytes was analyzed. THP-1 cells were either pretreated with 10 μg/mL of BE-30 or treated with 0.1% DMSO and then stimulated with 100 ng/mL of LPS for 0, 15, 30, 60, 120 and 240 min. In immunoblot assay, the activated MAPK proteins expressions were assessed in the cell lysates of LPS-induced cells treated with or without BE-30. In the LPS-treated cells, significant induction of phosphorylation of all the three members of MAPK were observed, showing the peaks at 60, 120 and 30 min for phospho-ERK, phospho-JNK and phospho-P38 proteins, respectively (Fig. 3). Interestingly, at these time points, BE-30-treated cells exhibited considerable reductions in phosphorylated forms of all kinases. Also, BE-30-treated cells did not show any considerable increase in phosphorylated forms of ERK and JNK. These data clearly suggest that BE-30 can inhibit the LPS-induced activation of MAPK family kinases in THP-1 monocytes.

**BE-30 Prevents NFκB Activation in LPS-Induced THP-1 Cells**

The nuclear transcription factor NFκB is an important modulator of immune and inflammatory responses (Tak and Firestein 2001). During inflammation, the activation process of NFκB in the cells includes: overexpression of p65 subunit of NFκB, deactivation of an inhibitory protein, i.e., IκB through phosphorylation and its subsequent degradation, and finally translocation of activated NFκB p65 to the nucleus. To assess the effect of BE-30 on NFκB activation in LPS-induced THP-1 monocytes, first we analyzed the expression of NFκB p65 subunit protein in cell lysates. Immunoblot assay demonstrates gradual increase in p65 protein expression in LPS-induced THP-1 cells in a time-dependent manner; whereas, in comparison, the overexpressed p65 protein significantly reduced in the LPS-induced cells co-treated with BE-30 (Fig. 4A). Densitometry analyses reveal that BE-30 exhibited 53.7% reduction in overexpressed p65 protein in LPS-induced THP-1 cells.

Next, we were interested to evaluate the modulatory effect of BE-30 on IκBα phosphorylation in LPS-induced monocytes. Immunoblot assay revealed that BE-30 treatment caused gradual and time-dependent increase in phosphorylation of IκB in LPS-induced THP-1 cells, whereas phospho-IκB expression was virtually unchanged in the LPS-induced cells during the time course of the experiment (Fig. 4B).

NFκB p65 protein translocation to the nucleus is the crucial step for NFκB activation in inflammation. In the present study, we assessed whether BE-30 can prevent p65 protein translocation to the nucleus of LPS-induced THP-1 cells. Immunofluorescence assay reveals that p65 protein is localized in the cytoplasmic compartment of the vehicle-treated THP-1 cells (Fig. 5A),
whereas LPS-induced cells showed significantly bright fluorescence staining in the nucleus (Fig. 5B). On the contrary, p65 staining was observed mainly in the cytoplasmic compartments of the BE-30-treated cells, virtually no immunofluorescence was seen in the nuclear compartments (Fig. 5C). These observations clearly indicate that BE-30 prevents p65 protein translocation to the nuclear compartment in LPS-stimulated THP-1 cells.

To substantiate this observation, we further analyzed the p65 protein in subcellular fractions in BE-30-treated cells followed by LPS induction. Den-
sitometry of the immunoblotted p65 protein revealed that nuclear fraction of the LPS-stimulated cells accumulated >3-fold p65 protein in comparison with the vehicle-treated cells, whereas only 1.2-fold accumulation of nuclear p65 protein was observed in the BE-30 treated cells (Fig. 5D). These data together provide strong evidence in favor of anti-inflammatory potential of BE-30 by deactivating NF\(\kappa\)B in LPS-induced THP-1 human monocytes.

**DISCUSSION**

A large number of studies have revealed the therapeutic benefits of *Boswellia* extracts in chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease and asthma (Gupta *et al.* 1997, 2001). Classically, the anti-inflammatory mechanism of action of *Boswellia* extracts and, especially, of AKBA was found to involve inhibition of arachidonic acid metabolism via the 5-LOX pathway (Safayhi *et al.* 1995). The ordinary *Boswellia* extract (BE-3) contains only 2–3% AKBA, which is the active component responsible for its anti-inflammatory properties. The AKBA
content in the extracts varies a lot from batch to batch depending upon the nature and raw materials and process. To have a consistent Boswellia product and improved AKBA concentration, we produced a novel Boswellia extract called BE-30 by selectively enriching the AKBA concentration to a minimum of 30%. In agreement with our hypothesis, 42.96% improvement of 5-LOX inhibition was achieved in BE-30 when compared with BE-3. Similarly, BE-30

FIG. 5. BE-30 BLOCKS THE TRANSLOCATION NFκB (p65) PROTEIN IN LPS-STIMULATED THP-1 CELLS

Immunofluorescence staining assays show the subcellular localization of p65 protein in 0.1% DMSO-treated vehicle control (A), 100 ng/mL LPS-stimulated (B) and BE-30 pretreated, LPS-stimulated THP-1 cells (C). “N” indicates the location of nucleus. (D) Representative immunoblot shows modulation of expression of p65 protein in cytoplasmic (C) and nuclear (N) compartments of LPS-stimulated cells pretreated with either BE-30 or 0.1% DMSO. Bar diagram shows normalized p65 protein expression in nuclear and cytoplasmic compartments.
showed 71.14% ($P < 0.001$) improvement in TNF$\alpha$ inhibition in LPS-induced monocytes model of inflammation. These findings indicate that BE-30 has a better anti-inflammatory potential than the ordinary Boswellia extract and this observation led us to further investigate the possible mechanisms underlying its anti-inflammatory properties.

Upon stimulation, granulocytes, monocytes/macrophages and mast cells produce leukotrienes, which modulate inflammatory and allergic reactions (Samuelsson et al. 1987). 5-LOX catalyzes leukotriene biosynthesis from arachidonic acid (Samuelsson 1983) and membrane-bound FLAP serves as an activator by providing arachidonic acid-binding capabilities (Mancini et al. 1993) and functions as an arachidonic acid transfer protein for 5-LOX (Abramovitz et al. 1993). At the downstream of the inflammatory pathway, leukotrienes enhance production of TNF$\alpha$ at the early phase, and overexpressed TNF$\alpha$ modulate production of other pro-inflammatory cytokines, and serves as a key regulating factor of inflammatory diseases such as rheumatoid arthritis, Crohn’s disease, psoriasis, asthma and atherosclerosis, among others. Thus, 5-LOX inhibition-based therapeutic strategy has become a focus of attention for regulating various inflammatory diseases (Evans et al. 2008). In this study, immunoblot analyses demonstrated that BE-30 is able to inhibit the expression of 5-LOX in LPS-induced THP-1 monocytes and in addition, it also inhibits the protein expression of FLAP in experimentally produced inflammatory reactions in monocytes. Therefore, our observations indicate that BE-30 acts as a double-edged sword against 5-LOX enzyme, by inhibiting its production and downregulating its activating protein, i.e., FLAP’s expression in cellular inflammatory conditions.

LPS is regarded as a potent activator for inflammatory response in monocytes (O’Connell et al. 1998). LPS activates the MAP kinase family of serine/threonine kinases (p42/p44 MAP kinase, JNK and p38) and plays a major role in cellular activation in a variety of cell types (Brunet and Pouyssegur 1997; Minden and Karin 1997; Ono and Han 2000; Roux and Blenis 2004). In neutrophils, the activated p38 MAP kinase in response to LPS regulates the production of cytokines, adhesion and migration (Nick et al. 1996, 1999, 2000; Detmers et al. 1998; Zu et al. 1998). Previously, Gayathri et al. (2007) showed that a methanolic extract of B. serrata inhibited MAPK enzyme activation in human peripheral blood mononuclear cells. Having seen that BE-30 inhibited LPS-induced TNF$\alpha$ in THP-1 monocytes, we assessed whether BE-30 can inhibit the enzyme activation of the MAPK pathways. Our observations suggest that BE-30 significantly blocks all three MAPK enzymes (p42/p44 MAP kinase, JNK and p38) activations in LPS-induced monocytes. Consistent with the previous findings, we have found that LPS increased the MAPK enzyme activities that led to enhanced production of TNF$\alpha$ pro-inflammatory cytokine in THP-1 monocytes. Interestingly, when the mono-
cytes were pretreated with BE-30, the MAPK enzyme activities were blocked in LPS-activated monocytes. Therefore, our observations suggest that BE-30 might inhibit TNFα production in LPS-induced inflammatory condition by blocking the MAPK activities in human monocytes.

Transcription factor NFκB plays the central role in immune response and chronic inflammation (Karin 2005; Perkins 2007). It targets the genes encoding proinflammatory cytokines, adhesion molecules, chemokines, growth factors and inducible enzymes (Lawrence et al. 2001). NFκB is normally localized in the cytoplasm of unstimulated cells and is translocated into the nucleus for its function. This translocation process is tightly regulated by IκB proteins, a family of inhibitory proteins that bind NFκB and prevent its entry into nucleus. Specific extracellular stimuli lead to the rapid phosphorylation of IκB, which frees NFκB and enables translocation into the nucleus, where it regulates gene transcription (Karin and Ben-Neriah 2000; Perkins 2007). Transfection experiments with dominant-negative mutants showed that LPS stimulation induced NFκB activation through phosphorylation of IκBα and IκBβ in THP-1 human monocytes (O’Connell et al. 1998; Hawiger et al. 1999).

Several plant products have been shown to interfere with the NFκB pathway (Bharti et al. 2003; Estrov et al. 2003; Shishodia et al. 2003; Takada and Aggarwal 2003). Previously, authors showed incensole acetate isolated from Boswellia resin inhibits NFκB activation through impairing IκBα phosphorylation in TNFα-induced macrophage cells (Moussaieff et al. 2007). In another study, Syrovets et al. (2005) demonstrated that acetyl boswellic acids blocked LPS-mediated TNFα induction in monocytes via inhibiting IKβ kinases. To characterize further, Takada et al. (2006) reported AKBA blocks TNFα-induced NFκB activation and downregulates the NFκB dependent gene products related to cell proliferation, anti-apoptosis and migration by inhibiting the gene transcription in human myeloid cells. However, we revisited and, consistent with the previous findings, we found that BE-30 treatment is able to inhibit IκBa phosphorylation, which is regarded as a critical factor for NFκB activation and also blocks the over-production of NFκB p65 protein in LPS-induced monocytes. In addition, immunoblot assay and subcellular localization showed BE-30 treatment caused significant inhibition of translocation of p65 protein to the nuclear compartment in LPS-stimulated cells.

In summary, according to the current literature available so far, this is the first report that provides evidence that an enriched composition of AKBA of Boswellia resin inhibits TNFα production in human monocytes and its anti-TNFα potential might be offered by blocking NFκB activation and via inhibition MAPK pathways. However, further validation of its anti-TNFα properties in in vivo animal models is warranted.
CONCLUSION

The results of this work have demonstrated that BE-30 is a powerful anti-inflammatory compound and possesses higher anti-inflammatory potential than commercially available regular *Boswellia* extracts. This AKBA enriched formulation exerts the anti-inflammatory activity by: (1) impairing TNFα over-production; (2) blocking activated MAPK enzyme pathways; and (3) inhibiting the NFκB activation in LPS-stimulated THP-1 human monocytes. BE-30 is marketed as a food supplement for the treatment of arthritis and joint disorder in the United States and Europe. This study provides significant insight into the molecular mechanism of anti-inflammatory properties of BE-30 and strengthens the basis for use of BE-30 as a nutritional supplement for the treatment of inflammatory diseases.

NOMENCLATURE

5-LOX  5-lipoxygenase  
AKBA  3-O-acetyl-11-keto-β-boswellic acid  
DMSO  Dimethyl sulfoxide  
DTT  Dithiothreitol  
EDTA  Ethylenediaminetetraacetic acid  
ERK  Extracellular receptor kinase  
FLAP  5-lipoxygenase activating protein  
FITC  Fluorescein isothiocyanate  
HEPES  4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid  
JNK  c-Jun N-terminal kinase  
LPS  Lipopolysaccharide  
MAPK  Mitogen activated protein kinase  
NFκB  Nuclear factor kappa B  
TNFα  Tumor necrosis factor alpha

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Synthesis of Coenzyme Q<sub>10</sub>

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Abstract: Problem statement: CoQ<sub>10</sub> is a key compound in ATP synthesis having wide number of health application especially for treating humans suffering from pathophysiological condition. The CoQ<sub>10</sub> presently available in the market is solely derived from fermentation process. A commercially viable synthetic process is yet to be realized. Approach: The researchers described a new synthetic route for the preparation of CoQ<sub>10</sub> (1). This new process utilized inexpensive isoprenol as a precursor for the synthesis of an early intermediate with a single isoprene unit. Another key step was the selective oxidation of trans methyl of isoprene unit as a prelude to the expansion of the side chain to decaprenyl group using solanesol. Results: Prenylation of 2, 3-dimethoxy-5-methylhydroquinone using isoprenol in presence of a Lewis acid, followed by selective oxidation of trans methyl group of isoprenyl side chain and subsequent allylic bromination yielded a bromide precursor (7). The p-toluenesulfination of the bromide followed by coupling with solanesyl bromide and de-p-toluuenesulfination yielded dimethyl derivative of the CoQ<sub>10</sub>-quinol. Finally CAN oxidation of dimethyl quinol followed by purification yielded CoQ<sub>10</sub> in 13% overall yield. Conclusion: The present process achieved CoQ<sub>10</sub> starting from a relatively inexpensive precursor. Further improvement in the coupling reaction between 8 and solanesyl bromide may lead to a better and viable synthetic process.

Key words: Coenzyme Q<sub>10</sub>, isoprenol, sodium-p-toluenesulfinate

INTRODUCTION

Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>), a prominent member of the ubiquinone family, is an essential component of the mitochondrial electron transfer chain, which is required for ATP synthesis and functions as an antioxidant in cell membranes and lipoproteins<sup>[1]</sup>. CoQ<sub>10</sub> is also a powerful antioxidant not only within the mitochondria but also in other organelle membranes containing CoQ<sub>9</sub>. CoQ<sub>10</sub> is ubiquitously present in the mammalian tissues, especially in the heart. The fact that the levels of endogenous CoQ<sub>10</sub> in the heart decreases during ischemic heart disease including heart failure prompted clinical trials with CoQ<sub>10</sub> in patients that suffered from heart failure.<sup>[3]</sup> Randomized, double blind, placebo-controlled trials of oral administration of CoQ<sub>10</sub> have confirmed the effectiveness of CoQ<sub>10</sub> in improving angina episodes, arrhythmias and left ventricular function in patients with acute myocardial infarction.<sup>[4]</sup>

CoQ<sub>9</sub> is found in rodents like mice and rats, while CoQ<sub>6</sub>, CoQ<sub>7</sub> and CoQ<sub>8</sub> are found in yeast and bacteria.<sup>[5,6]</sup> The majority of CoQ<sub>9</sub> in rat liver is present in its reduced form (ubiquinol), which exerts its antioxidative function.<sup>[7]</sup> Similar to CoQ<sub>10</sub>, CoQ<sub>9</sub> is not merely a compound responsible for energy transduction in mitochondrial membrane in rat heart; it also serves as a functional element in the cells and possesses ability for redox cycling. The CoQ<sub>9</sub> differs from CoQ<sub>10</sub> with respect to the number of isoprenoid units in the tail; CoQ<sub>9</sub> has nine units in contrast to the presence of 10 units in CoQ<sub>10</sub>.

Most of the CoQ<sub>10</sub> is found in mammalian hearts including human myocardium.<sup>[7]</sup> CoQ<sub>10</sub> is not an essential nutrient, because it can be synthesized in the body. High amounts of CoQ<sub>10</sub> can also be found in several food products, including meat, fish, peanuts and broccoli.<sup>[8]</sup> Dietary intake of CoQ<sub>10</sub> is about 2-5 mg day<sup>-1</sup>, which is inadequate for the body under pathophysiological conditions.<sup>[2]</sup> A number of methods have been developed for the synthesis of CoQ<sub>10</sub> since the first industrial approach by Hideki Fukawa at Nisshin in 1974.<sup>[27]</sup>
The researcher describe herein a novel process for the synthesis of Coenzyme Q\(_{10}\) as shown in Fig. 1.

![Chemical Structure](image)

**Fig. 1:** (i): CCl\(_4\), 2-methyl-3-buten-2-ol, BF\(_3\), rt, 96.1%; (ii): NaOH, DMS; 90°C, 78% (iii): SeO\(_2\), EtOH; (iv): EtOH, NaBH\(_4\), rt, 65%; (v): EtOH, NaBH\(_4\), rt, 83%; (vi): CH\(_2\)Cl\(_2\), TEA, Sodium p-toluenesulphinate, rt, 83%; (vii): t-BuOK, THF, Solanesylbromide, -20-30°C, 74%; (viii): THF, EtOH, Na, rt, 79%; (ix): CH\(_3\)CN, CH\(_2\)Cl\(_2\), CAN, 0°C, 72%

**MATERIALS AND METHODS**

2, 3-dimethoxy-5-methyl-p-benzoquinone and isoprenol were purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Sodium dithionate, dimethylsulphate, sodiumborohydride, phosphorous tribromide, sodium metal, cericammoniumnitrate were purchased from SD Fine chemicals, TV Industrial estate, Mumbai, India. Selinium dioxide was purchased from Molychem, Souri building, Mumbai, India. All the above chemicals were used as received. Solanesyl bromide was prepared from solanesol, which was isolated from tobacco waste using a known procedure. IR spectra were recorded on a Perkin-Elmer (model spectrum BX) FT-IR instrument in chloroform. \(^1\)H NMR spectra were recorded on Bruker Avance AV 400 MHz Spectrometer and \(^13\)C NMR spectra were recorded on Bruker Avance AV 100MHz Spectrometer. Mass studies were performed on LC-MS system equipped with Agilent 1100 series, LC/MSD detector and 1100 series Agilent HPLC pump. Normal phase silica gel (ACME, 100-200 mesh) was used for column chromatography. Silica gel pre-coated plates (Alugram Sil G/UV\(_{254}\)) were used for thin layer chromatography using the solvent system CHCl\(_3\)/MeOH (9:1) and visualized by immersing the plate in vanillin sulfuric acid reagent followed by heating at 110°C.

**2, 3-Dimethoxy-5-methyl hydroquinone (2):** 2, 3-dimethoxy-5-methylquinone (6 g, 0.0329 mol) and 48 mL of acetonitrile, 12 mL of water and sodium dithionate (8.6 g, 0.0494 mol) were taken in a round bottom flask. Reaction mixture was stirred at room temperature for 2 h. After completion, the reaction mixture was poured in to ice cold water and extracted with EtOAc. The organic layer was washed with brine, dried over Na\(_2\)SO\(_4\) and concentrated under vacuum at 40°C temperature to yield 2 (5.5 g, 90.7%).

**2, 3-Dimethoxy-5-methyl-6-prenylquinol (3):** A mixture of 2, 3-Dimethoxy-5-methylhydroquinone (2, 5.5 g, 0.0298 mol), 28 mL of carbon tetrachloride and 2-methyl-3-buten-2-ol (4.1 g, 0.0476 mol) was taken in a round bottom flask. Under vigorous stirring, BF\(_3\) etherate (0.85 g, 0.00597 mol) was added drop wise to reaction mixture at 0-5°C. Then reaction mixture was allowed to warm up to room temperature and continued the stirring. After 3 h, the reaction mixture was poured into ice cold water and the mixture extracted with EtOAc. The organic layer was washed with brine, dried over Na\(_2\)SO\(_4\) and evaporated under vacuum at 40°C to obtain 3 (8 g, 96.1%).

**2, 3, 4, 5-Tetramethoxy-6-prenyl toluene (4):** 2, 3-dimethoxy-5-prenyl-6-methyl quinol (3, 8 g, 0.03174 mol) and sodium hydroxide (5 g, 0.125 mol) were dissolved in 21 mL of water in a round bottom flask. Dimethyl sulphate (19.9 g, 0.1587 mol) was slowly added to reaction flask at room temperature and the reaction mixture was stirred at 90°C for 2 h. After completion, the reaction mixture was poured in to ice cold water, acidified with 5N H\(_2\)SO\(_4\) and extracted with EtOAc and the organic layer was washed over Na\(_2\)SO\(_4\) and concentrated under vacuum. The residue (9 g) was subjected to column chromatography over silica gel using 2% EtOAc/hexane to yield 4 (6.9 g, 78%).

**2, 3, 4, 5-Tetramethoxy-6-prenyltoluene (5) and 2, 3, 4, 5-tetramethoxy-6-(2-methylbut-2-ene-1-ol-4yl)toluene (6):** To a solution of 2, 3, 4, 5-tetramethoxy-6-prenyl toluene (4, 7.3 g, 0.06576 mol) in ethanol (100 mL) was slowly added
SeO₂ (32.5 g, 0.2959 mol) at room temperature and the reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, the mixture was poured into ice water and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated to obtain a mixture of 5 and 6 (5.5 g, 80%). For identification purpose, a small sample (0.5 g) of the mixture was subjected to column chromatography over silica gel using hexane and ethyl acetate mixtures. The fractions eluted with 15% EtOAc/hexane were monitored, identical fractions combined and evaporated to yield 5 (225 mg). Similarly, the fractions eluted with 30% EtOAc/hexane were monitored and identical fractions combined and evaporated to yield 6 (150 mg).

2, 3, 4, 5-Tetramethoxy-6-(2-methylbut-2-ene-1-al) toluene (5): IR (CHCl₃, 3, 4, 5-Tetramethoxy-6-(2-methylbut-2-ene-1-al) toluene (400 MHz, CDCl₃): 1467, 1407, 1352, 1219, 1104, 1039; ¹H NMR δ (400 MHz, CDCl₃): 9.30 (1H, s), 6.32 (1H, m), 3.84 (3H, s), 3.83 (3H, s), 3.74 (3H, s), 3.72 (3H, s), 3.58 (2H, dd, J = 6.8, 0.8 Hz), 3.07 (3H, s), 1.83 (3H, d, J = 1.2 Hz); ¹³C NMR δ (100 MHz, CDCl₃): 195.06, 152.56, 148.00, 147.87, 145.94, 144.89, 138.92, 125.50, 125.28, 61.03, 60.96, 60.68, 26.99, 11.93, 9.29; LCMS (ESI, positive scan): m/z 295 (M+H)⁺, m/z 317 (M+Na)⁺, m/z 333 (M+K)⁺.

2, 3, 4, 5-Tetramethoxy-6-(2-methylbut-2-ene-1-al) toluene (6): IR (CHCl₃) ν_max cm⁻¹: 2932, 1687, 1467, 1407, 1352, 1219, 1104, 1039; ¹H NMR δ (400 MHz, CDCl₃): 9.30 (1H, s), 6.32 (1H, m), 3.84 (3H, s), 3.83 (3H, s), 3.74 (3H, s), 3.72 (3H, s), 3.58 (2H, dd, J = 6.8, 0.8 Hz), 2.07 (3H, s), 1.83 (3H, d, J = 1.2 Hz); ¹³C NMR δ (100 MHz, CDCl₃): 195.06, 152.56, 148.00, 147.87, 145.94, 144.89, 138.92, 125.50, 125.28, 61.03, 60.96, 60.68, 26.99, 11.93, 9.29; LCMS (ESI, positive scan): m/z 319 (M+Na)⁺, m/z 331 (M+K)⁺.

IR (CHCl₃) ν_max cm⁻¹ : 2932, 1466, 1408, 1351, 1200, 1105, 1073, 1039, 1012, 974; ¹H NMR δ (400 MHz, CDCl₃): 5.44 (1H, t, J = 6.8 Hz), 3.89 (2H, s), 3.83 (3H, s), 3.82 (3H, s), 3.72 (3H, s), 3.71 (3H, s), 3.28 (2H, d, J = 6.8 Hz), 2.06 (3H, s), 1.85 (3H, d, J = 0.8 Hz); ¹³C NMR δ (100 MHz, CDCl₃): 147.93, 147.72, 145.39, 144.78, 131.75, 129.65, 127.36, 125.27, 61.04, 60.12, 60.98, 60.64, 41.48, 26.23, 13.81, 11.77; LCMS (ESI, positive scan): m/z 361 (M+H)⁺, m/z 383 (M+Na)⁺.

2, 3, 4, 5-Tetramethoxy-6-(1-p-toluenesulphinyl-2-methylbut-2-ene-4yl) toluene (7): But-2-ene-2-methyl-1-bromo-4-yl tetramethoxytoluene (1 g, 0.00277 mol) was dissolved in 10 mL of dry dichloromethane treated with triethylamine (0.28 g, 0.00277 mol). Sodium 4-toluene sulphinate (493 mg, 0.00278 mol) was slowly added in portions wise to the reaction mixture and stirred at room temperature for 3 h. Then the reaction mixture was poured in to ice cold saturated sodium bicarbonate solution and extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under vacuum. The residue (2.6 g) was subjected to column chromatography over silica gel using hexane and 2% EtOAc/hexane mixtures. The fractions eluted with 2% EtOAc/hexane mixture were combined and evaporated to obtain 7 (2.2 g, 92%).

J = 1.2 Hz; $^{13}$C NMR δ (100MHz, CDCl$_3$): 147.84, 147.63, 145.32, 144.67, 144.29, 135.59, 134.29, 129.40, 128.45, 127.18, 125.13, 123.50, 66.14, 61.00, 60.94, 60.89, 60.61, 26.22, 21.49, 16.98, 11.64; LCMS (ESI, positive scan): m/z 435 (M+H)$^+$. 

**Synthesis of 9:** 2, 3, 4, 5-Tetramethoxy-6-(1-p-toluenesulphonyl-2-methylbut-2-ene-4yl) toluene (1 g, 0.002304 mol) was dissolved in 10 mL of tetrahydrofuran and solanesol bromide (2.4 g, 0.00345 mol) were dissolved in 10 mL of acetonitrile and added dropwise to the above solution containing 2, 3, 4, 5-tetramethoxy-6-(1-p-toluenesulphonyl-2-methylbut-2-ene-4yl) toluene and solanesol bromide at -20°C. After 30 min, the reaction mixture was allowed to reach ambient temperature and continued the stirring for 2 h. The contents were then poured into ice water and the mixture was acidified to pH 4 with 5N HCl and extracted with EtOAc. The organic layer was washed with brine, dried over Na$_2$SO$_4$ and concentrated under vacuum. The residue was subjected to column chromatography using hexane and 2% EtOAc/hexane mixture were monitored, the fractions eluted with 2% EtOAc/hexane were combined and evaporated to yield C$_2$O$_{10}$ (1, 210 mg, 72%). 

**IR (CHCl$_3$) $v_{\text{max}}$ cm$^{-1}$:** 2925, 2854, 1597, 1465, 1406, 1383, 1314, 1219, 1145, 1105, 1086, 1041; $^1$H NMR δ (400 MHz, CDCl$_3$): 7.57 (2H, d, J = 8.0 Hz), 7.15 (2H, d, J = 8.0 Hz), 5.02 (10H, m) 3.82 (3H, s), 3.79 (3H, s), 3.68 (3H, s), 3.58 (3H, s), 2.32 (3H, s), 1.99 (18H, m), 1.90 (20H, m), 1.85 (3H, s), 1.75 (3H, s), 1.60 (6H, s), 1.51 (15H, s), 1.49 (3H, s); $^{13}$C NMR δ (100MHz, CDCl$_3$): 145.24, 144.67, 138.44, 135.31, 134.93, 134.86, 134.15, 129.52, 129.24, 128.87, 124.44, 124.31, 124.25, 118.79, 73.87, 66.32, 60.94, 60.88, 60.58, 39.74, 26.75, 26.70, 25.62, 21.58, 17.63, 16.28, 15.99, 11.54; LCMS (ESI, positive scan): m/z 1046 (M+H)$^+$. 

**RESULTS**

The synthesis of CoQ$_{10}$ was carried out in nine steps as shown in Fig. 1. 2, 3-Dimethoxy-5-methylquinone was reduced to 2 with sodium dithionate in 90.7% yield. The reaction of 2, 3-dimethoxy-5-methylhydroquinone (2) with isoprenol in presence of
BF₃ etherate in carbon tetrachloride yielded the prenylated hydroquinone (3) in 96% yield. The di-O-methylation of the hydroquinone using dimethylsulphate yielded 2, 3, 4, 5-tetramethoxy-6-prenyltoluene (4) in 78% yield. The oxidation of the terminal methyl in the prenyl side chain with selenium dioxide yielded 5 and 6 in 80% overall yield. The mixture of 5 and 6 was subjected to reduction using potassium tert-butoxide to yield the (2E)-4-[2, 3, 4, 5-tetramethoxy-6-methylphenyl]but-2-en-1-ol (6) in 65% yield. The alcohol 6 was converted to the bromide 7 using PBr₃ in 92% yield and then subjected to p-toluenesulfination using sodium p-toluene sulfinic acid in dry dichloromethane to give p-toluenesulfinate 8 in 83% yield. The p-toluenesulfinate (8) was nonaprenylated using solanesyl bromide in presence of a strong base potassium tert-butoxide to obtain 9 in 74% yield. The reductive de-p-toluenesulfination of 9 using sodium in ethanol yielded 10 in 79% yield. Finally CAN oxidation of 10 in 1:1 mixture of dichloromethane and acetonitrile afforded CoQ_{10} (1) in 72% yield.

**DISCUSSION**

Coenzyme (CoQ₁₀), a member of the ubiquinone family, is an essential component of the mitochondrial electron transfer chain. It is widely being used for cardiac health and also as an antioxidant. It also holds promise as an anticancer agent. The commercial quantities, however, limited as the CoQ₁₀ in the market is solely derived from the fermentation technology and the available synthetic methodologies are not commercially feasible. The present study has been an attempt towards the cost viable synthesis of CoQ₁₀.

A new synthetic route for the preparation of CoQ₁₀ is described. The key steps include prenylation of the substituted quinol moiety with a relatively inexpensive isoprenol and selective oxidation of trans methyl group in the prenylated intermediate to obtain the substituted prenol as an essential precursor for the expansion of the side chain to decaprenyl group. The reaction of 2, 3-dimethoxy-5-methylhydroquinone with isoprenol in presence of BF₃ etherate yielded the prenylated hydroquinone (3). It was then subjected to di-O-methylation, followed by oxidation of terminal methyl with selenium dioxide to yield a mixture of 5 and 6. The mixture of 5 and 6 without further separation was subjected to sodium borohydride reduction to yield the (2E)-4-[2, 3, 4, 5-tetramethoxy-6-methylphenyl]but-2-en-1-ol (6). The alcohol was converted to the bromide (7) using PBr₃ and then subjected to p-toluenesulfination. The p-toluenesulfinate (8) was nonaprenylated using solanesyl bromide in presence of a strong base to obtain 9. This advanced intermediate 9 was de-p-toluenesulfinated using sodium in ethanol to obtain the dimethyl derivative of CoQ₁₀ quinol. Finally CAN oxidation of 10 was afforded CoQ₁₀ (1). The pure CoQ₁₀ was isolated from the crude reaction mixture using column chromatography followed by crystallization in 13% overall yield.

**CONCLUSION**

CoQ₁₀ is a potentially useful compound having wide number of health applications especially those related to cardiovascular diseases. Though the CoQ₁₀ from biotechnology process can able to meet the current demand, no commercially viable synthetic process is available. The present process achieves CoQ₁₀ starting from relatively inexpensive precursor, called isoprenol. It achieves key synthetic intermediate 8, needed for the expansion of the prenyl chain, through a novel viable process. This is more economical than expanding expensive natural nonaprenyl compound called solanesol by an isoprene unit before coupling to the Q₀ precursor. This process can also be used to produce other CoEnzyme Q compounds having different number of isoprene units per side chain.

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Abstract – A naturally occurring nonaprenylsulfate (1) and its synthetic analogue (2) were synthesized from substituted phenolic precursors in three steps with an overall yield of 40-45%. Both compounds exhibited potent anti-inflammatory activity against 5-lipoxygenase, and potent brine shrimp lethality. They also showed moderate antioxidant activity in the super oxide radical scavenging model. Nonaprenylsulfate (1) showed moderate inhibition of paw edema in Freund’s Complete Adjuvant (FCA) induced model of arthritis, thus confirming its anti-inflammatory activity.