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METABOLIC CONVERSION OF GUGGULSTERONE: A HYPOLIPIDEMIC AGENT

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ABSTRACT
Guggulsterone is an active constituent of guggulipid, an ayurvedic drug, and is reported to have hypolipidaemic activity. Since Guggulipid is a mixture of E- and Z- isomer, it is necessary to understand the biophase behaviour of individual isomer of guggulsterone. The initiation of the pharmacokinetic study was, therefore, made with Z-guggulsterone. High Performance Liquid Chromatographic analysis of treated rat serum and bile samples at post dose administration of Z- guggulsterone, showed the presence of isomerized product namely E- guggulsterone. A large variety of E- and Z- isomerisation reactions catalyzed by aconitate isomerase (aconitase) is reported in the literature. Isomerization reaction of guggulsterone in the presence of the quoted enzyme was, therefore, studied. This study was expected to assess the *in-vitro* biotransformation of Z- to E- guggulsterone.

KEYWORDS: Hypolipidemic agent, Aconitate isomerase, Guggulipid, Pharmacokinetic, Biotransformation, Isomerization.

INTRODUCTION
Guggulipid has been marketed in India under the trade name "Guggulip" as hypolipidemic agent.[1] It is derived from the plant Commiphora mukul[2] and contains several plant sterols, diterpenes, steroids, esters and higher alcohols.[3] The main ingredient of the drug is guggulsterone (Fig. 1) which possess marked cholesterol and lipid lowering activity.[4-6]

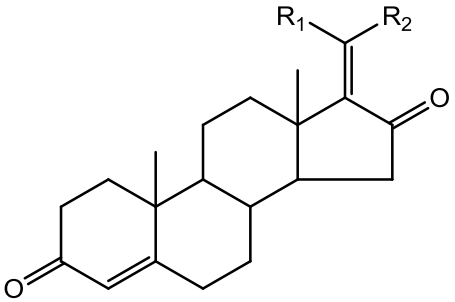

Fig. 1. (1)

R_1 = H, R_2 = CH_3; Z- Guggulsterone

R_1 = CH_3, R_2 = H; E- Guggulsterone

Pharmacokinetic behaviour of guggulsterone was studied in rats after single oral and intravenous doses of Z-guggulsterone in rats.[7] During pharmacokinetic studies it was observed that Z- guggulsterone was isomerized to E- isomer after oral and intravenous dose administration of Z- guggulsterone. This result prompted us to study *in-vitro* conversion of the mentioned isomeric biotransformation.

METABOLIC STUDIES
Earlier studies indicated that there was no such isomerisation in spiked serum samples.[8] But blood, when spiked with Z-guggulsterone, showed a peak of E-isomer on HPLC analysis. This indicated that blood itself is the media for the observed geometric isomerisation. In order to confirm this observation, red blood corpuscles were spiked with Z- guggulsterone and after extraction with hexane the extract was analysed by HPLC. The presence of the peak for the E- isomer was conspicuous. Biochemical composition of erythrocytes reveals the presence of an enzyme, aconitate isomerase (aconitase), which has been claimed in literature to be responsible for E- and Z- isomerisation. Thus for in-vitro studies, the enzyme aconitate isomerase was isolated freshly from pig heart. The study was conducted in spiked serum samples after addition of enzyme. Prepared sample on HPLC analysis showed decreased concentration of Z-isomer and a broad peak in the near polar region, which might be expected due to formation of some polar complexes. Slightly basic pH (7.4-7.7) of blood prompted to create the basic environment and was obtained by the addition of 2N, NaOH. When NaOH was added to spiked serum sample before incubation, HPLC analysis showed a prominent peak for E- isomer in the

polar region. Presence of adenine base in erythrocytes, gave a clue that it provides a basic environment. Thus for final confirmation of the isomerisation process NaOH in spiked serum sample was replaced by adenine and HPLC analysis after extraction showed the peak for both Z- and its isomerized product E- isomer (Fig 2).

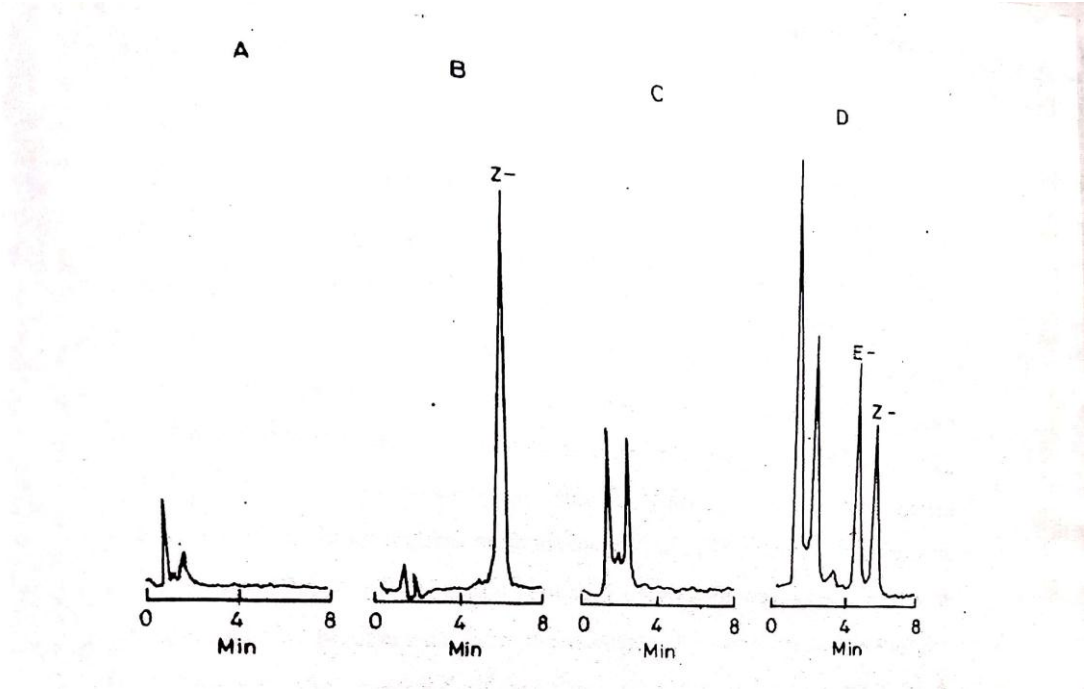

Fig. 2. Chromatograms showing
(A) Drug free serum
(B) Serum spiked with 500 ng/ml of Z- guggulsterone
(C) Serum spiked with enzyme and adenine base
(D) Serum spiked with 500 ng/ml of Z- guggulsterone in presence of enzyme and adenine base

DISCUSSION

After dosing the rats with Z-guggulsterone, the presence of E- guggulsterone in serum from the first sampling time point throughout the study period indicated a possible presystemic first pass metabolic isomerisation either in gut or liver or both. However, similar observations from 5 min i.v. dose indicated more specifically that the site of metabolic conversion might be blood itself. This was confirmed by *in-vitro* metabolic studies. Earlier studies of development and validation of bioanalytical methods had indicated that there is no metabolic conversion of Z- to E- guggulsterone in spiked serum. However, blood when spiked with Z-guggulsterone showed the presence of both the isomers. Biochemical composition of blood reveals the presence of aconitate isomerase which is reported to participate in cis-trans isomerization.[9] In the presence of aconitate isomersase, isolated from pig heart, at basic pH maintained by the addition of adenine, serum samples spiked with Z- guggulsterone also showed the presence of E- guggulsterone, confirming that the Z- isomer gets isomerized into E- isomer in the blood compartment.

The understanding of the mechanism of this biotransformation, which involves the isomerisation of geometrical isomers, requires on understanding of the mechanism of action of aconitase. The enzyme aconitase, a 83,000 dalton polypeptide containing 4Fe-4S cluster is a mitochondrial enzyme.[10] Three of the Fe atoms are ligated by cysteine residues and the fourth Fe is the site of substrate interaction which involves dehydration of citrate to yield the intermediate product namely the cis-aconitate and is followed by rehydration to form (2R,3S) iso-citrate.[11] The biotransformation of Z- guggulsterone also involves the hydration and dehydration of the α,β-unsaturated carbonyl system involving positions 16 and 17 of the steroid. It is also evident that the enzyme aconitase identifies this part of the substrate molecule namely guggulsterone. Protonation of the carbonyl group at the position 16 and the enzyme assisted delivery of OH^- at the β carbon of the α,β-unsaturated system generate an intermediate in which the carbon at position 20 changes from sp^2 hybridisation to sp^3 hybridised state. Abstraction of a proton from the enol of the envisaged intermediate leads to a subsequent dehydration process during which the isomerisation occurs because there is no steric control for the elimination of water molecule. The proposed mechanism is described in Fig 3.

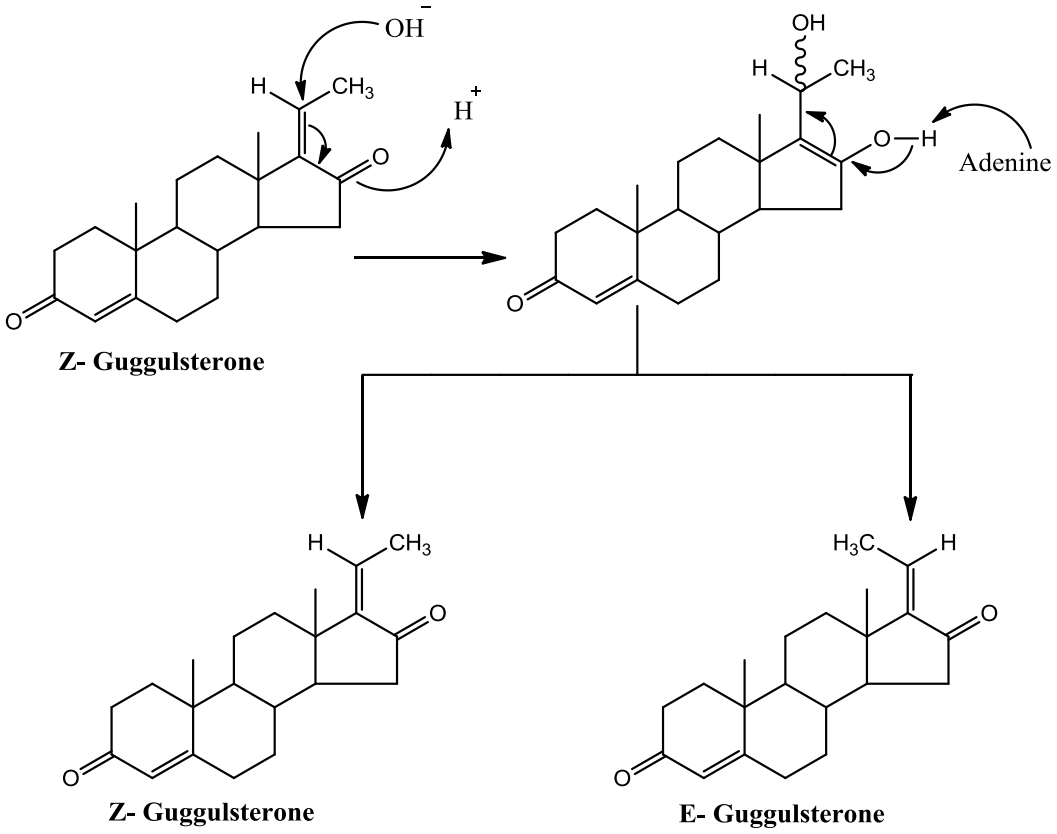

Fig. 3. Proposed mechanistic route for enzymatic biotransformation

CONCLUSION

Repeated analysis of analytical standards and spiked serum samples during the assay validation showed that the Z to E conversion does not take place in the spiked serum samples. Detailed *in-vitro* metabolic studies conclude that the biotransformation of Z- guggulsterone to E- guggulsterone occurs in the blood due to the presence of aconitase enzyme.

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