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Acta Medica Iranica

2009;47(4) : 371-376

"STUDY ON GLUTATHIONE S-TRANSFERASE INHIBITION ASSAY BY TRICLABENDAZOLE ON FASCIOLA spp. "

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Abstract:

Glutathione S-transferase (GST) represents the major class of detoxification enzymes from helminth parasites such as *Fasciola hepatica* and *F. gigantica* and it is a candidate for chemotherapeutic and vaccine design. Therefore, GST enzyme of *Fasciola* spp. could be a target for evaluation of drugs such as triclabendazole (C₁₄H₉Cl₃N₂O₅). For this purpose, GST enzymes were purified from *Fasciola* spp. and sheep liver tissue by glutathione affinity chromatography using a wash-batch method and subsequently their SDS-PAGE pattern was detected. Afterward, GST specific activity levels were assayed in the whole extract and purified solutions spectrophotometrically at 30°C with reduced glutathione (GSH) and 1-chloro-2, 4-dinitrobenzen (CDNB) substrate. Finally, GST inhibition assay was investigated in the solutions by powder and bolus forms of triclabendazole. GST fraction as a 26 kDa (MW) band was obtained on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The level of GST specific activity in purified solutions was detected 18.14 µmol/min/mg proteins for *Fasciola hepatica*, 35.04 for *F. gigantica* and 37.84 µmol/min/mg protein for liver tissue. Comparison of the effect of powder and bolus of triclabendazole in solutions revealed inhibition concentration (IC₅₀) 8.36 and 9.05 µg/ml for *Fasciola hepatica* GSTs and 7.20 and 10.80 for *F. gigantica* GSTs and 8.65 and 9.70 µg/ml for liver tissue GSTs, respectively. These findings suggest the possibility of selective inhibition of *Fasciola* spp. GSTs by triclabendazole in vitro and use of these results for understanding of its effect in vivo and qualification of manufacturing bolus form of drug in comparison with original powder.

TUMS ID: 3280

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