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HEPATOTOXICITY OF EUGENOL

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ABSTRACT: *EUGENOL a widely used pharmaceutical agent proceeds toxicity on inhalation in rats. Two different doses 20 & 30 µg/100g body weight/ day of eugenol were given intramuscularly to male albino rats for 10 days and the liver function was assessed by measuring the specific enzyme activities, and total and differential bilirubin concentration. There was an appreciable increase in total as well as differential bilirubin fractions and a dose dependent increase was noticed in the activities of alkaline phosphatase, transminases, lactate dehyrogenase with a decrease in the activity of glytamyl transferase activity, and suggesting eugenol to have a toxic effect on liver.*

INTRODUCTION

Plant principles used for medicinal purposes should be administrated in proper concentration so as to avoid any toxicity due to their accumulation. Sometimes, even the therapeutic doses given for a particular ailment may be toxic to other systems. Therefore, the study of any plant principle on various systems is mandatory.

Eugenol, a phenolic compound is a major constituent of the essential oil extracted from cloves (*Eugenia aromatica)* and can be synthesized from anethold. Nutmeg *(Myristica fragrans)* and betelvine *(Piper betle L)* are also good sources of eugenol[1]. It is an ingredient of dentifrices, gargles, chewing gums and also used for scenting soaps and toilet waters in perfumery. This oil is employed as a local analgestic for hypertensive dentistries and carious cavities[2], externally used as a rubefacient and counterirrigant, and internally as a carminative and antispasmodie agent[3]. The antipyretic activity of eugenol has been demonstrated in rabbits[4].

Eugenol being very widely used in dentistry in a Zincoxide-eugenol combination and also as an antibiotic motivated the toxicity study of this compound on liver tissue, the sole organ responsible for the detoxification of drugs.

MATERIALS AND METHODS

Adult male albino rats of wistar strain weighing about 200 – 250 gm body weight were maintained under uniform laboratory conditions and were provided with standard rat pellet diet (M/s. Hindustan Lever Ltd., Bangalore) and water ad libitum. They were divided into 3 groups, each comprising of 5 animals. Animals in Group I received 1% Sodium hydroxide solution and served as controls. Group II and Group III received eugenol of 20 µg and 30 µg respectively per

100 gm. Body weight per day intramuscularly for 10 days.

24 Hours after the last injection, the animals were sacrificed by decapitation. The liver was removed and cleaned in 0.9% saline. Required amount of tissue was homogenized and centrifuged in appropriate media and speed respectively and the supernatant was used for the estimation of total protein[5], alkaline phosphatase[6], aspirate and alanine transaminases[7], lactate dehydrogenase[8], Y Glutamyl transferase[9], estimation of total and conjugated bilirubin[10]. The data were analysed statistically, using students't' used.

RESULTS AND DISCUSSION

Table: 1 depicts the effect of eugenol at two different doses on total protein concentration, bilirubin levels and a few enzymes in liver. Eugenol administration in both the doses caused a significant decrease in total protein concentration. The total as well as the conjugated and un-conjugated bilirubin levels showed a significant increase with the high dose treatment. However, the low dose group did not show any significant change. On treating the animals with a low and high dose of eugenol, there was significant increase observed in the alkaline phosphatase, alanine and asparatate transminases and lactate dehydrogenase in the liver tissue when compared with the control but the r-glutamyl transferase showed a significant decrease.

Liver is the vital organ concerned with the drug metabolism. It is essential to study the effect of any pharmaceutical agent on these organs, if it is intended to be used for human welfare in any form. This is important because liver damages, if any, will alter the physiology and structure of the vital organs and have a serious effect on the overall metabolism. Clark[11] found eugenol to produce temporary distress like irregular breathing, weight loss and reduced food and water intake. The observed decrease in liver protein concentration in the present study substantiates this report. As liver is the chief organ of protein synthesis, even mild lesions in this organ may alter its normal function.

After production in the peripheral tissues, bilirubin is transported to the liver in the association with albumin[12]. Inside the hepatocytes, bilirubin is rapidly conjugated with glucuronic acid to produced bilirubin monoglucuronide and bilirubin diglucuronide, which are then exerted in bile[13]. There may be a reduction in the number of functioning liver cells as in chronic hepatitis, so that all liver functions are impaired. Conditions such as infective or toxic jaundice in which there is extensive damage to liver cells, but also a considerable degree of intrahepatic obstruction resulting in appreciable decrease in the exertion of conjugated bilirubin[14]. The present study with administration of eugenol reveals that the liver function is affected which is supported by the observation made by an appreciable increase in the total bilirubin and conjugated bilirubin fraction suggesting liver toxicity.

Serum alkaline phosphatase measurements are of particular interest in the investigation of two groups of conditions: Hepatobiliary disease and bone disease associated with osteoblastic activity. Recent studies suggest that the response of liver to any form of biliary tree obstruction is to synthesize more alkaline phosphatase, i.e., the effect is one the enzyme induction. The main site of new enzyme synthesis is the hepatocyctes adjacent to the biliary canaliculi. Some of the newly formed enzymes enter the circulation to raise the enzyme level in

serum. The elevation tends to be more marked (more than 3 fold) in extrahepatic obstruction than in intrahepatic obstruction and is greater, the more complete is the obstruction. Intrahepatic obstruction of bile flow by drugs such as chlorpromazine that affect the biliary tree also raised alkaline phosphatase, but usually to lesser extent. In the present study, the treatment of eugenol led to a dose dependent increase in ALP activity, probably suggesting an intrahepatic obstruction. In infectious hepatitis and other inflammatory conditions affecting the liver, alkaline transaminase is characteristically as high as or higher than aspirate transaminase[15]. Slight or moderate elevations of both transminase activities may be observed after intake of alchol, in delirium tremens and after administration of a variety of drugs, such as opiates, salicylates or ampicillin[16]. Although serum levels of both alanine and aspirate transminases become elevated whenever disease processes, affect liver cell integrity, alanine transminase is the more liver specific enzyme[17].

Lactate dehydrogenase activity may be elevated in almost all causes of hemolysis and liver disease but not as great as the increase seen in amino-transferase activity[18]. Administration of eugenol induced in increase in transaminase and dehydrogenase levels in liver supports the hepatotoxicity of the drug as shown by Manake[19].

All forms of liver disease lead to an increase in membrane bound gamma glutamyl transferase (r-GGT) in serum. This elevation of serum activity may be due to a release of cell membrane fragments into the circulation, a process that may be a result of membrane fragmentation by bile acids, particularly in cholestatic disease, the stasis and prolonged contact of bile acids (which are detergents) with canlicular and bile duct epithelia could solubilzie and release r-glutamyl transferase20. The observed decrease in r-GGT activity in liver may be due to loss of liver cell membrane fragments into the circulation.

Thus, it is evident that eugenol administration exerts as adverse effect on the liver as indicated by the responses of the biochemical parameters studied.

EFFECT OF EUGENOL ON LIVER OF ADULT RATS

PARAMETER	CONTROL	LOW DOSE	HIGH DOSE
Protein (mg/g wet tissue)	98.54 ± 11.0	95.32 ± 9.51	79.12 ± 4.7*
Total Bilirubin	20.05 ± 1.06	18.93 ± 2.12	24.89 ± 2.13*
Conjugated Bilirubin	2.50 ± 0.02	1.95 ± 0.01*	5.46 ± 1.01*
Unconjugated Bilirubin (mg/g Wet tissue)	17.34 ± 0.75	16.51 ± 0.68*	19.23 ± 1.81*
Alkaline Phosphatase	0.41 ± 0.06	0.50 ± 0.03*	0.70 ± 0.01*
Alanine Transaminase	1.72 ± 0.21	2.11 ± 0.14*	3.43 ± 0.09*
Asparate Transaminase	1.23 ± 0.02	1.91 ± 0.01*	2.36 ± 0.02*
Lactate dehydrogenase	1.65 ± 0.01	2.15 ± 0.05*	2.93 ± 0.02*
r-glutamyl transferase	0.56 ± 0.02	0.42 ± 0.01*	0.37 ± 0.01*

VALUES ARE EXPRESSED AS MEAN ± S.D. OF 6 SAMPLES
STATISTICAL SIGNIFICANCE AT $P < 0.05$ IS REPRESENTED BY A*

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