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Therapeutic efficacy of Green Tea Polyphenols on 4-Nitroquinoline-1-oxide induced hepatotoxicity

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Abstract

Objective: 4-Nitroquinoline-1-oxide (4-NQO), a potent experimental oral carcinogen produces intracellular oxidative stress, which leads to lipid peroxidation following hepatotoxicity. Green tea contains high content of polyphenols, which are potent antioxidants. Thus, green tea can play a protective role in liver damage and hepatotoxicity. In the present investigation it was decided to study the efficacy of Green tea polyphenols(GTP) against 4-NQO induced hepatotoxicity. Materials and methods: Hence, the activity of hepatic markers such as Aspartate Transaminase (AST), Alanine Transaminase (ALT) Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (ALP) in the liver homogenate were assessed and the liver histology were studied. Results: Histology showed cohesive focal necrosis of hepatocytes. A significant increase was observed in all the markers of hepatic function. On administration of GTP for 30 days, a marked decrease in the activity of markers was observed and a lesser degree of necrosis was found in the histology. Conclusion: We conclude that the changes in the activity of markers and histology could be attributed to the therapeutic efficacy of GTP.

Key words: Green tea polyphenols, Hepatoxicity, 4-Nitroquinoline-1-oxide, Oral carcinogen, Hepatic markers.

1. Introduction

Green tea contains a rich source of polyphenols, which are antioxidant in nature [1]. Green tea contained varied assortments of polyphenols, known as catechins, they are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC) and epicatechin-3-gallate (ECG) [2]. GTP can play a protective role in liver injury and hepatotoxicity [3-6]. 4-NQO, a water soluble oral carcinogen produces papilloma and invasive squamous cell carcinoma, resulting in clinical and histological changes which is similar to those observed in neoplasms of humans [7-9]. Thus 4-NQO is used as experimental oral carcinogenesis. 4-NQO is known to cause liver damage in three hours of administration [10]. It causes acute toxicity, mutagenicity or carcinogenicity by inducing a proxidant state *in vivo* [11]. In rat liver 4-NQO is reduced to 4-hydroxyaminoquinoline-1-oxide (4-HAQO) [12]. 4-NQO at the concentration of 0.5% may cause a severe damage to the liver.

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2. Materials and methods

Extraction of green tea polyphenols (GTP 80%) was done by adapting the procedure of Shaowen Lee (Director, Human King long Bioresource Co. Ltd., China).

Male Wistar rats (TANUVAS, Chennai, India) 8 to 10 weeks old, 80 to 120 gms were used for the study. All animals were given a standard rat feed (Hindustan Lever Ltd., Bangalore) and water *ad libitum.* The animals were housed in a controlled temperature and humidity with 12 h light: dark cycles.

Animals were divided into four groups. Group I (n=6) served as control, Group II (n=12) served as induced, applied 4-NQO 0.5% in propylene glycol using No.4 painting brush, thrice a week for 22 weeks. Group III (n=6) served as drug control received GTP 200 mg / kg *b.wt. p.o.,* for 30 days. Group IV served as treated (6 from Group II) received GTP same as Group III.

After the completion of the experimental period, the overnight fasted animals were euthanised under diethyl ether anesthesia. The liver was excised, washed in ice-cold saline. A small portion of the liver was weighed, minced and 10% homogenate was prepared using Tris-HCl buffer pH 7.4, centrifuged, supernatant was used for the enzyme assay.

2.1 Assay as AST and ALT

AST and ALT activity in the liver homogenate was assessed according to Mohur and Cooke [13], using aspartate,α-oxaloglutarate for AST and alanine,α-oxaloglutarate for ALT as substrates. Optical density (OD) at 520 nm in Shimadzu UV spectrophotometer was read. The enzyme activity was expressed as µmol of pyruvate liberate / hr/mg of protein.

2.2 Assay of LDH

LDH activity was assessed according to King [14], using lithium lactate as substrate and sodium pyruvate as external standard. OD at 420 nm read in Shimadzu UV spectrophotometer. The activity of the enzyme was expressed as µ moles of pyruvate formed / min / mg of protein.

2.3 Assay of ALP

ALP activity was assessed according to King and King [15], using disodium phenyl-phosphate as substrate and recrystallised phenol as external standard. OD was read at 640 nm in Shimadzu UV spectrophotometer. The enzyme activity was expressed as µmol of phenol liberated / min / mg of protein. Protein was estimated according to Lowry *et al* [16].

2.4 Statistical analysis

Results were presented as mean value ± SD. Statistical analysis were performed using SPSS Software Version 10 with Fisher's post hoc least significant difference (PLSD) test. A probability of $p<0.05$ was considered significant.

3. Results

Figure 1 represents the activity of hepatic markers in the liver of control and experimental animals. There was a significant elevation in the activity of hepatic markers in Group II 4-NQO treated animals, when compared to controls. A significant decrease in the activity of hepatic markers was found in Group IV (4-NQO+GTP treated) animals, when compared to 4 NQO treated animals. No significant changes were found when Group I control was compared with Group III drug control.

4. Discussion

4-NQO a potent oral carcinogen has been used in experimental oncology [1]. It also produces intracellular oxidative stress [17]. 4-NQO is known to cause liver damage in three hours of administration [10]. 4-NQO produces acute toxicity, mutagenicity or carcinogenicity by inducing a proxidant state

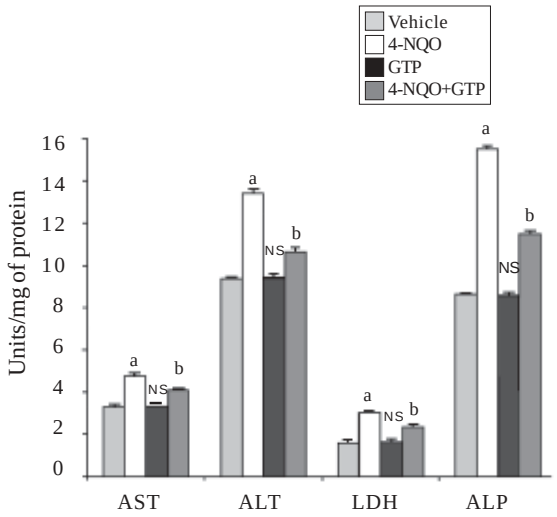

Fig. 1. Activity of AST, ALT, LDH and ALP in the liver of control and experimental rats.

Values are expressed as mean ± SD; Comparisons: a, b are significantly different from Vehicle, 4 NQO treated groups respectively at $p<0.05$ (LSD).
NS - Non significant when compared to vehicle treated group
Units: AST, ALT-micromoles of pyruvate liberated/hr/mg of protein.
LDH-micromoles of pyruvate formed/min/mg of protein.
ALP-micromoles of phenol liberated/min/mg of protein

in vivo [5]. 4-NQO was given at a concentration of 0.5% for the induction of oral cancer in rats, thrice a week for 22 weeks, a part of it was ingested, absorbed and finally reached the liver. 4-NQO is known to produce oxidative stress, proxidant state, toxicity, and liver damage, the concentration of 0.5% 4-NQO may cause severe damage to liver, which leads to hepatotoxicity.

In case of hepatotoxicity, there was a marked elevation in the activity of hepatic markers [18]. GTP is known to suppress the elevated activity of hepatic markers such as AST, ALT, LDH and ALP [9,11,12], which follows a similar trend with the present study. On administration of GTP for 30 days, Group IV rats showed a significant decrease in the activity of hepatic markers, which could be attributed to the therapeutic potency of GTP, which protected the liver from 4-NQO induced damage.

Histological studies showed, 4-NQO administration causes liver damage [4], which shows necrotic hepatocytes, cohesive focal necrosis and single cell necrosis. In case of GTP treated animals the changes were lesser degree, with balloning of hepatocytes, thus GTP protects the liver from damage or injury [12], which correlates with our study.

Hence, GTP nullify the liver damage induced by 4-NQO. From the results presented in the study, it could be summarized that, administration of GTP therapeutically not only possess antitumour activity but also decrease the hepatotoxicity induced by a potent experimental oral carcinogen 4-NQO.

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