Lycopene mitigates initiation of N-nitrosodiethylamine induced Hepatocellular carcinoma: A Radiometric and Biochemical study

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Abstract

The present study was designed to evaluate the chemopreventive potential of lycopene enriched tomato extract (LycT) on hepatocellular carcinoma during its initiation by screening the functional status of liver through non-invasive techniques using 99mTc-mebrofenin. Moreover, various hematological, inflammatory and oxidative stress markers were also assessed. Female Balb/c mice were segregated into four groups: Control, NDEA (cumulative dose of 200mg NDEA/kg b.wt in 8 weeks), LycT (5mg/kg b.wt thrice a week) and LycT + NDEA (co-administration of LycT and NDEA). NDEA administration was started after two weeks of LycT treatment. NDEA treatment induced several histopathological alterations in liver tissue and also caused increased levels of inflammatory markers i.e. TNF-α, IL-6 and IL-β in serum. NDEA exposure also exhibited decreased hemoglobin, red blood cells, platelets and lymphocyte counts while total leucocyte and neutrophil counts were increased. An increase in plasma lipid peroxidation (LPO) levels, superoxide dismutase (SOD) and catalase (CAT) activities with a subsequent decrease in reduced glutathione (GSH) levels were also observed upon NDEA exposure. In addition, NDEA also induced functional alterations in the liver tissue as evident by slow hepatic excretion of 99mTc-mebrofenin. LycT administration to NDEA mice showed improved functional status of liver by enhancing the excretion of 99mTc-mebrofenin. The modulation of these parameters by LycT, demonstrated that LycT administration provides protection against NDEA induced insults which may have significant implications in preventing HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cause of morbidity and mortality worldwide. NDEA induced hepatic cancer model has been used to elucidate the molecular mechanisms behind cancer pro- gression.

99mTc-mebrofenin hepatobiliary test allows earlier HCC detection along with the exceptional insights into the molecular pathogenesis as molecular perturbations precede anatomical alterations.

Reactive oxygen species (ROS), generated during NDEA metabolism causes genetic damage to DNA and other cellular macromolecules, stimulating tumor initiation. NDEA is also known to induce various hematological and inflammatory responses.

Lycopene extracted from red tomatoes has found its widespread use in natural medicine because of its highest antioxidant and radical scavenging activity.
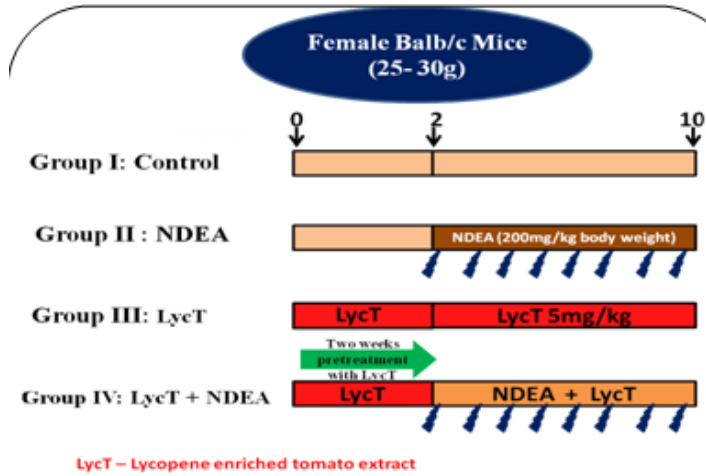
Thus the present piece of work was designed to study HCC by analyzing various hematological, inflammatory and blood antioxidant markers that are linked to early stages of hepatocarcinogenesis and their amelioration by LycT.

In addition the functional status of liver was also assessed using 99mTc-mebrofenin hepatobiliary functional test.

Objectives

- Histopathological Analysis of hepatic tissue using H & E staining

- Assessment of alterations in functional status of liver using 99mTc-mebrofenin Hepatobiliary functional test

  Assessment of serum inflammatory markers (TNF-α, IL-6 and IL-β) using ELISA

- Assessment of hematological parameters (Hb, TLC, RBC, Platelets, Lymphocytes, Neutrophils)

- Analysis of lipid peroxidation (TBARS assay) and antioxidant defense system in liver/liver tumors

Work Plan

Control animals showed normal Histoarchitecture

#NDEA animals showed the formation of high grade dysplastic nodule, increased nuclear to cytoplasmic ratio, nuclear atypia, hyperplasia and increased eosinophilic staining

LycT animals showed normal histoarchitecture

LycT + NDEA animals showed near normal histo-architecture with inflammatory lymphocyte infiltration

Central vein (CV), hepatocytes (H), kupffer cells (KC), sinusoids (S)

Results
Histopathological Analysis

Hepatic and heart T_{peak} and $T_{1/2\ peak}$ of ^{99m}Tc-Mebrofenin
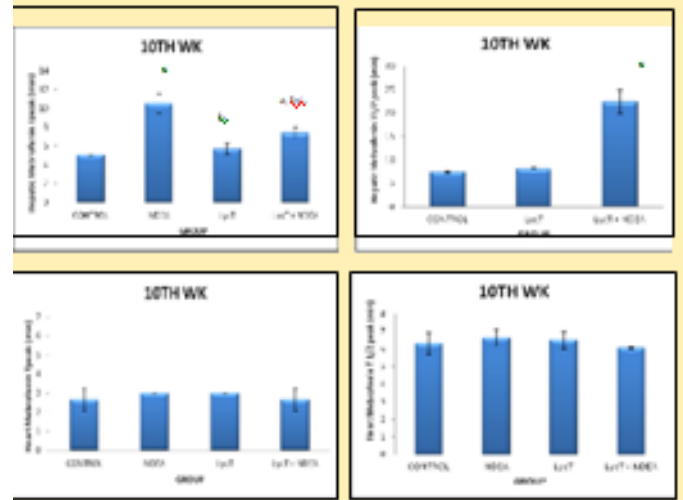
Values are expressed as mean $\pm$ SD (n=5) and analyzed by one way- ANOVA followed by post-hoc test. a and a_1 (p$\leq$0.001 and p$\leq$0.01 respectively) represent significant difference wrt Control group; b (p$\leq$0.001) represents significant difference wrt NDEA group; c_2 (p$\leq$0.05) represents significant difference wrt LycT group.
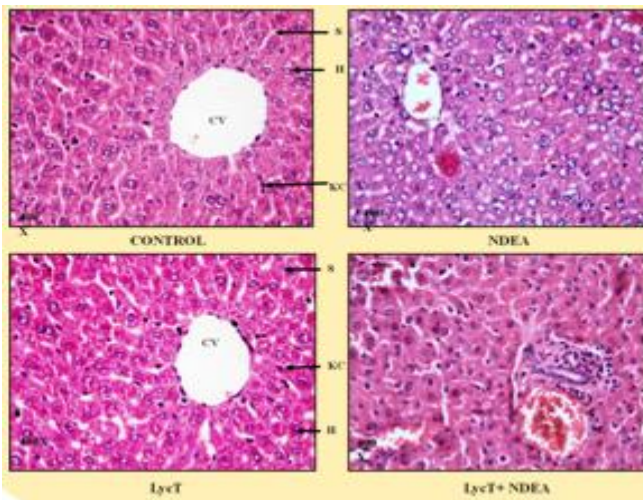
Hematological parameters

	CONTROL	NDEA	LycT	LycT + NDEA
Hb	14.0 ± 0.15	10.6 ± 0.42^a	14.1 ± 0.21^b	13.5 ± 0.20^{a},^{bc}
TLC	7.53 ± 0.29	9.51 ± 0.27^a	7.40 ± 0.36^b	8.45 ± 0.30^{a},^{b},^{c}
RBC	7.90 ± 0.10	6.53 ± 0.35^a	7.97 ± 0.25^b	7.17 ± 0.15^{a},^{b},^{c}
PLATELETS	4.43 ± 0.29	3.29 ± 0.11^a	4.69 ± 0.18^b	4.07 ± 0.12^{a},^{bc}
NEUTROPHILS	29.7 ± 1.53	38.7 ± 3.21^a	27.3 ± 2.52^b	33.7 ± 1.52^b^{c}
LYMPHOCYTES	58.7 ± 1.53	49.7 ± 2.08^a	58.3 ± 2.52^b	55.7 ± 2.51^{b}

Values are expressed as mean $\pm$ SD (n=5) and analyzed by one way-ANOVA followed by post- hoc test. a, a_1 and a_2 (p$\leq$0.001, p$\leq$0.01 and p$\leq$0.05 respectively) represent significant difference wrt control group; b, b_1 and b_2 (p$\leq$0.001, p$\leq$0.01 and p$\leq$0.05) represent significant difference wrt NDEA group; c_1 and c_2 (p$\leq$0.01 and p$\leq$0.05 respectively) represent significant difference wrt LycT group. **Units:** Hb levels were expressed as g/dl, TLC expressed as counts X 10^3/mm^3, RBC expressed as

counts X 10^6/mm^3, platelets expressed as counts X 10^5/mm^3, neutrophils and lymphocytes counts were expressed as percentage (%).
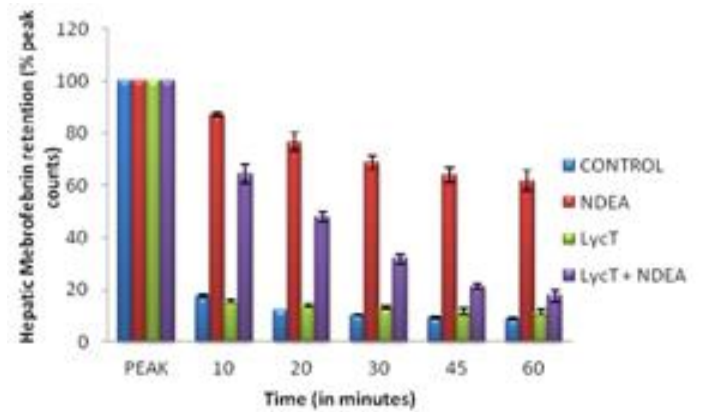
Lipid Peroxidation
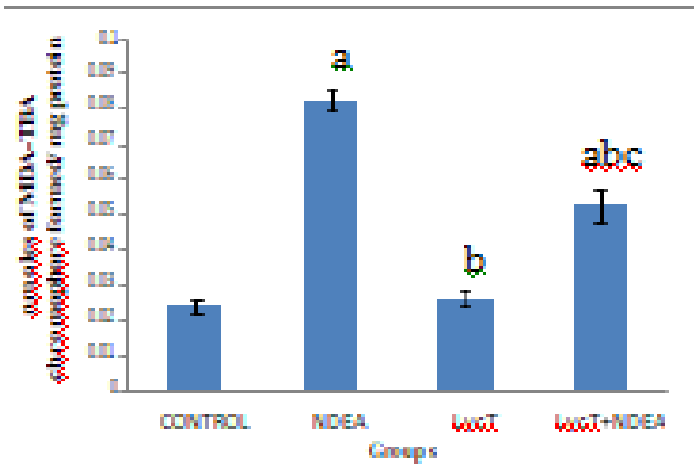
Data is expressed as mean ± SD and analyzed by One-way ANO- VA followed by post hoc test. 'a' (p≤0.001) represents signifi- cant difference wrt control group; 'b' (p ≤ 0.001) represents signifi- cant difference wrt NDEA group; 'c' (p ≤ 0.001) represents signifi- cant difference wrt to LycT group.
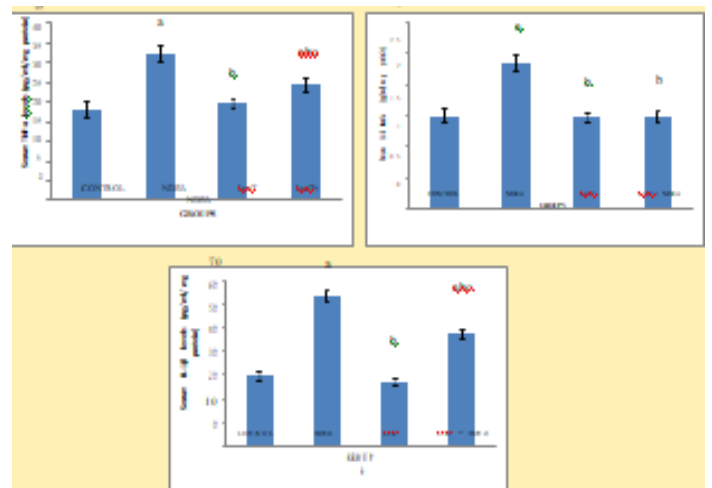
Hepatic and blood pool time-activity curves of ^{99m}Tc-mebrofenin
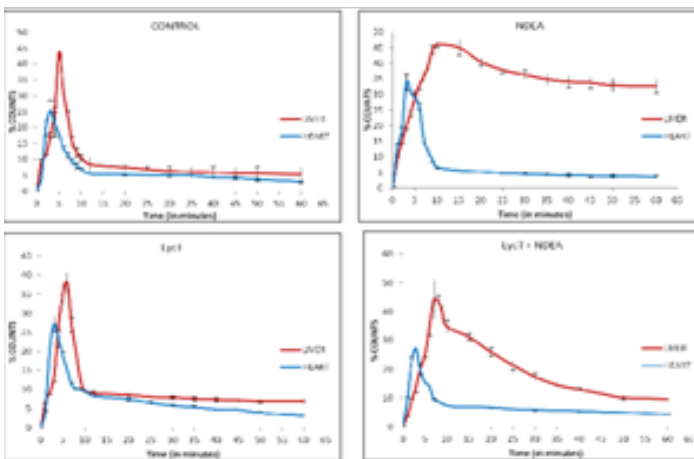
Hepatic ^{99m}Tc-Mebrofenin retention

Inflammatory markers

Values are expressed as mean ± SD (n=5) and analyzed by one way- ANOVA followed by post-hoc test. a (p≤0.001) represents significant difference wrt Control group; b (p≤0.001) represents significant difference wrt NDEA group; c(p≤0.001) represents significant difference wrt LycT group.

Antioxidant Defense System

	CONTROL	NDEA	LycT.	LycT.+NDEA
GSH	7.82 ± 0.81	4.11 ± 0.45	7.87 ± 0.50	$6.40 \pm 0.80_{1}$
SOD	0.09 ± 0.012	0.18 ± 0.010^{a}	0.11 ± 0.009^{b}	0.14 ± 0.013^{abc}
CAT	0.61 ± 0.01	1.15 ± 0.19^{a}	0.58 ± 0.03^{b}	$0.84 \pm 0.02^{a\,bc}$

Values are expressed as mean $\pm$ SD (n=5) and analyzed by one way-ANOVA followed by post-hoc test. a and a_1 ($p \leq 0.001$, $p \leq 0.01$ respectively) represent significant difference wrt Control group; b ($p \leq 0.001$) represents significant wrt NDEA group; c and c_1 ($p \leq 0.001$, $p \leq 0.01$ respectively) represent significant difference wrt LycT group. Units: Reduced glutathione (GSH) expressed as nmoles of GSH/ mg protein, SOD activity expressed as IU/mg protein, CAT activity expressed as µmoles/min/mg protein

Conclusion

The present study provides evidence that LycT administration provides protection against NDEA induced insults which may have significant implications in delaying HCC initiation.

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