Khan and Moselhy Afr J Tradit Complement Altern Med. (2016) 13(1):169-175
http://dx.doi.org/10.4314/ajtcam.v13i1.22

SELENIUM ALONE OR IN COMBINATION WITH LYCOPENE MODIFIES LIVER METABOLIZING ENZYMES AGAINST GALACTOSAMINE – A TIME BOUND STUDY

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Abstract

Background: Hepatitis is one of the major public health problems worldwide. This study was designed to evaluate the potential hepatoprotective effects of lycopene (Lyco) and selenium (Se) against galactosamine (Gala) induced hepatitis in rats.
Materials and Methods: Seventy five (75) male albino rats were grouped into five of fifteen rats each. GP 1: Control. Animals in Groups (II-V) were injected i.p with Gala (300 mg/kg b.w daily) for 5 days. GP III: Rats were orally pretreated with Lyco (15 mg/kg b.w). GP IV: rats were pretreated orally sodium selenite (0.1 mg/kg b.w) by gavages. GP V: Rats treated with both Lyco and Se. The treatment was continuous for 30 days.
Results: The levels of serum inflammatory markers interleukine-6 (IL-6), tumor necrosis factor TNF- α, nitric oxide (NO) and malondialdhyde (MDA) were markedly elevated in rats injected with Gala compared with control group. Administration of Lyco combined with Se reversed these effects and significantly reduced the levels of these markers ($p<0.001$) compared with Gala. In addition, combined treatment resulted in a significant improvement in antioxidant activities as superoxide dismutase (SOD) and catalase compared with untreated. Non-significant changes were recorded in the activities of UDP-glucuronyltransferase and sulphotransferase in rats injected with Gala but the activity of glutathione S-transferase was significantly elevated ($p<0.001$).
Conclusion: The combined effect of Lyco + Se showed a significant hepatoprotective action against Gala induced hepatitis in rats through inhibition of release of inflammatory mediators and enhancement of antioxidant capacity.

Key words: Galactosamine- Lycopene- selenium- hepatitis- rats.

Introduction

Hepatitis is a serious inflammation of the liver that may be caused by virus overactive, immune suppression, alcoholism, chemicals and environmental toxins (Ostapowiczand Lee 2000). Hepatitis is one of the major public health problems worldwide and is known to be responsible for considerable morbidity and mortality (Ames etal.,1995). Galactosamiene (Gala) induced rat's hepatitis has been implicated in pharmacological and therapeutic tools (Zhang etal.,2003 and Gupta etal.,2003). The mechanism of Gala induced hepatotoxicity was proceeded via ATP depletion, which finally produce necrosis of hepatocytes. It provokes a state of oxidative stress by releasing free radicals and reduction of antioxidant status (Cohen, 2002).

The development of a safe and non-toxic phytochemical agent for management of hepatitis is the main rationale of recent researches. Functional foods that act as antioxidants exert a potential chemo-preventive agent against oxidative stress in carcinogenesis and pathogenesis liver diseases (Clinton,1996).

Lycopene (Lyco) is the principle pigment that is responsible for red color of tomato fruits and its products. It was found that Lyco is a potent antioxidant compound that act as scavenger of oxygen radicals which caused damage of cell membrane, structure proteins, DNA and RNA. Previous study revealed that, Lyco reversed carcinogenesis compared with other carotenoids (Lim etal.,2000).

Selenium (Se) is an essential micronutrient which plays a vital role in a number of biological functions in humans and many other forms of life. Previous studies have demonstrated that the deficiency of Se induces some pathological disorder as (coronary heart disease, liver necrosis) and was found as a risk factor in the etiology of these diseases (Galanos etal.,1979). Biological and medical advances in the area of Se provide interest in Se for both its antioxidant properties through seleno-enzyme incorporation and its direct pro-oxidant toxic effect through seleno-compounds (Kondo etal.,1994).Se is important for glutathione peroxidase (GSH-Px), thioredoxin reductase and selenoprotein, which contains Se as selenocysteine (Hecht etal.,2001).
The goals of this study are firstly to evaluate the potential antioxidant activities of both Lyco and Se against hepatitis in rats induced by Gala; Secondly to determine the hepatic detoxifying enzymes involved in phase I as CYP1A1 (ethoxyresorufin O-deethylase), CYP1A2 (methoxyresorufin O-demethylase), CYP2E1 (p-nitrophenol hydroxylase) and phase II including UDP-glucuronosyltransferase, GlutathioneS-transferase and sulphotransferase .

Materials and Methods

Animals

Seventy five (75) male albino rats (weighing 180-250g) were used throughout this study. They were housed under standard condition and allowed free water and standard pellet diet *ad libitum*. The experiment was carried out according to ethical committee for animal care and

handling. The rats were grouped into five of fifteen animals each (table 1). GP 1: Control. Animals in Groups (II-V) were injected *i.p* with Gala (300 mg/kg b.w) for induction of hepatitis (Combs and Gray,1998). GP III: rats were orally pretreated with Lyco (15 mg/kg b.w) for one week before Gala injection. GP IV: rats were pretreated orally with selenium as sodium selenite for one week (0.1 mg/kg b.w. per day) *via* gavage. The selected doses of selenium and Lyco were given according to (Moselhy and Mesalmi , 2008). GP V; rats were pretreated with combination of Lyco and selinate for one week. The treatment was continuous for 30 days. At the end of the experiment, animals were sacrificed after anesthesia with thiopental. Blood samples were collected and sera were separated for biochemical assays of alanine transaminases, aspartate transaminases, malondialdhyde, catalase and SOD using commercial diagnostic kits from bioline (England). Serum Nitric oxide, IL-6 and tumor necrosis factor were assayed using an available ELISA kit from R&D (R&D, Berlin,Germany) according to the manufacturer's instructions.

Table1: timetable of the doses injected to different groups.

Days Groups	1st -----7th	8th	9th -39th
G I: Control	--------	-------	-------
GII: Galactosamine	-------	✓	-------
GIII: Lycopene (15 mg/kg b.w)	✓	✓	✓
GIV: Selenium (0.1 mg/kg b.w)	✓	✓	✓
GV: Lycopene+ Selenium	✓	✓	✓

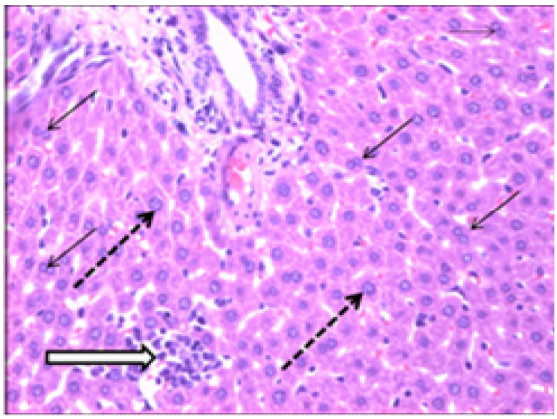

Figure 1: H&E stain of rat liver of control group.

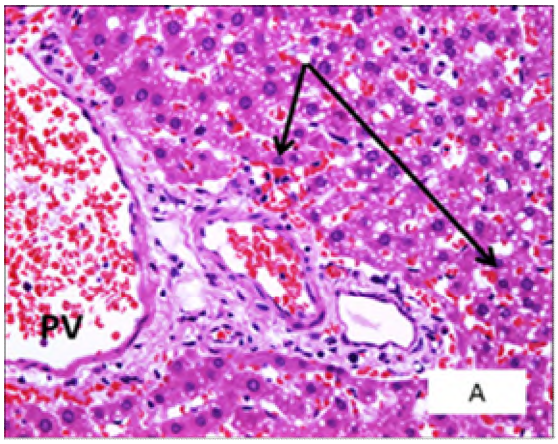

Figure 2: H&E stain of rat liver injected with Gala.

Preparation of Liver Microsomes

All sample preparation steps were carried out at - 4 ºC using cold sterile solutions and equipment. Liver (two grams) was manually homogenized in 5 ml phosphate buffer saline (pH =7.5) using a glass vessel and a Teflon plunger. The homogenate was adjusted to 25% (w/v) with ice-cold 1.15 % (w/v) KCl. The liver homogenate was centrifuged at 47,000 g for 15 min at 4ºC.The microsomal fractions freshly prepared were used for the enzymes assay. The concentration of protein content of liver tissue was determined according to the method Lowry et al., (1951). A standard curve was constructed using a serial dilution of bovine serum albumin (500 µg/ml) for protein content assessment.

Enzymes Assays

The following enzymes were assayed using isolated liver microsomes: Phase I detoxifying enzymes including CYP1A1 (ethoxyresorufin O-deethylase) (Guo etal.,2013), CYP1A2 (methoxyresorufin O-demethylase) (Habig etal.,1974), CYP2E1 (p-nitrophenol hydroxylase) (Jemai et al.,2008) and phase II including UDP-glucuronosyltransferase, (Caffrey and Frenkel, 2003), GlutathioneS-transferase (Chen and Berry, 2003).

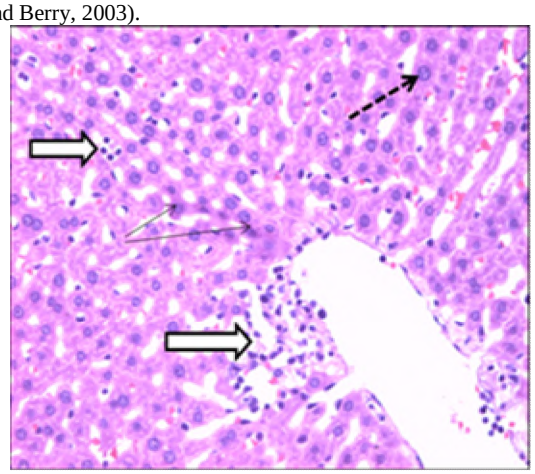

Figure 3: H&E stain of rat liver pretreated with Lyco.

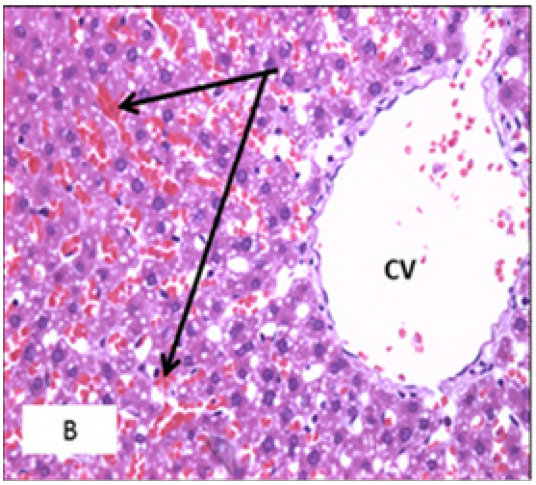

Figure 4: H&E stain of rat liver pretreated with Se.

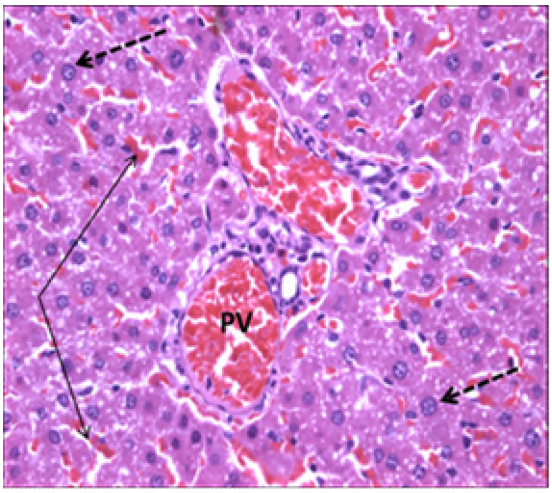

Figure 5: H&E stain of rat liver pretreated with Lyco and Se

Histopathological Examination

Liver tissues were washed by cold normal saline 3 times; then fixed in formalin solution 10%, processed, and embedded in a paraffin film. Sections of 5-µm thick were prepared. The sections were stained with H&E.

Statistical Analysis

The values were expressed as mean ± SD. SPSS for windows was performed to examine whether there were any significant differences between different treated groups. Statistical significance of the results was evaluated by using one-way ANOVA (analysis of variance) test and post-comparison was carried out with Student's *t*-test.A probability value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

In the current study, we examined the extent of liver injury after Gala administration by elevation of serum ALT and AST activities (Table 2). The activities of these enzymes were reduced in rats pretreated with Lyco or Se or combined. Liver MDA level was significantly elevated ($p<0.001$) in Gala injected rats and a significant reduction of SOD ($p <0.001$) and catalase ($p <0.05$) activities compared with control. Pretreatment with Lyco or Se or combined, reversed these actions. However, the protective effect of the combined was more potential than individually treatment ($p<0.01$).

Data in table (3) showed that the serum levels of IL-6, NO and TNF-α were significantly increased in Gala injected rats. Administration of combined Lyco and Se significantly reduced the elevated levels of these inflammatory markers compared with Gala untreated. The combined treatment showed a significant improvement than individual treatment.

Results obtained in table (4) showed that rats injected with Gala (GP III) caused a significant elevation in the phase I enzymes activities CYP1A1 (ethoxyresorufin O-deethylase) and CYP2E1 (p-nitrophenol hydroxylase) as compared with control group. However, rats treated with Lyco or Se or combined attenuated this elevation of enzyme activities but don't return to the normal values. The potential of combine is more significant than individual ($P < 0.001$). The impact of Lyco + Se administrated on hepatic phase II conjugating enzymes was shown in Table (5). UDP-glucuronoyltransferase and sulphotransferase activities were not significantly modulated following Gala injection or Gala treated with Lyco or Se compared with control group. Gala injection cause a significant elevation in the activity of glutathione S-transferase compared with control. Oral administration of Lyco + Se reduced the activity of glutathione S-transferase as compared with untreated ($P < 0.001$). Histological examination of liver control rats (fig1A) showed normal hepatic cells with well-preserved cytoplasm prominent nucleus. The liver of Gala-intoxicated rats (fig 1B) showed massive fatty changes, necrosis, and broad infiltration of the lymphocytes and deposition of collagen. The histological architecture of liver sections of the rats treated with Lyco (fig 1C) or Se (fig 1D) or combination (fig 1E) showed more or less normal pattern with disappearance of septal collagen mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the control group.

Table 2: Serum aminotransferase enzymes (ALT and AST) and liver lipid peroxide product (Malendialdlyde), superoxide dismutase (SOD), catalase, and of all studied groups (Mean±SD).

Animal groups / Parameters	Control	Gala-	Gala+Lyco	Gala+Se	Gala+Lyco+Se
Serum ALT (IU/ml) Mean±SD	28.4±4.56	64.0±7.86[a]	47.9±7.1[a,b]	37.6±5.08[a,c]	35.6±5.1[a,b,c]
Serum AST (IU/ml) Mean±SD	32.9±3.1	74.0±6.2 [a]	36.8±5 [a,b]	34.0±3.1 [a,c]	31.0±2.2 [a,b,c]
MDA (mmol/mg/protein) Mean±SD	3.31± 0.14	8.1± 0.57 [a]	4.9±0.27[a,b]	3.30±0.3 [a,c]	3.90± 0.42 [a,b,c]
SOD (MU/mg protein) Mean±SD	316.8± 13.8	209± 34 [a]	312± 25 [a,b]	289.3± 23[a,c]	260.3±23.2 [a,c]
Catalase (nmol/min/mg protein) Mean±SD	8899±667	3192±146[a]	7582±482[a,b]	5277±505[a,c]	6781.3± 435 [a,b,c]

a,b,c letters refer to significant p<0.0; a- comparison with normal control; b- combined versus individual;
c- comparism with Gala intoxicated group

Table 3: Serum IL6, TNF- α and Nitric oxide (NO) content in the different studied groups (Mean ±SD)

Animal groups	Control	Gala	Gala+Lyco	Gala+Se	Gala+Lyco+ Se
IL-6 (ng/dl) Mean±SD	202± 38	1102±106 [a]	687±75[a,b]	471±23[a,c]	390±30[a,,b,c]
TNF- α (ng/dl) Mean±SD	0.13±0.01	2.54±0.12[a]	0.92± 0.05[a,b]	0.82±0.05[a, c]	0.79±0.04 [a,,b,c]
NO (µg/dl) Mean±SD	73± 1.6	304± 16 [a]	202±15[a,b]	132± 9[a,c]	105± 6[a,b,c]

a,b,c letters refer a significant p<0.05; a-comparison to normal control; b- comparison to Gala intoxicated group c- Combined versus treated

Table 4: The activities of phase I enzymes CYP1A1 (ethoxyresorufin O-deethylase), CYP1A2 (methoxyresorufin O-demethylase) and CYP2E1 (p-nitrophenol hydroxylase) in livers of rats of different groups (Mean± SD).

Animal groups	Control	Gala	Gala+Lyco	Gala+Se	Gala+Lyco+ Se
EthoxyOdeethylae (IU/mg protein) Mean±SD	32.5± 0.1	95± 6 [a]	56±12 [a,b]	65.5±5[a,c]	45.5±7 [a,c]
(Methoxydemethy las(IU/mg protein) Mean±SD	68± 13.2	70±2.8[a]	75± 3	70± 5	66± 8
PNPhydroxylase(I U/mg protein) Mean±SD	19± 1.3	45± 5 [a]	33± 1.5[a,b]	40±5 [a,c]	29±3 [a,c]

a,b,c letters reefer a significant p<0.05; a- comparism with normal control; b- comparism with Gala intoxicated Group; c- combined versus treated

Table 5: The activities of phase II enzymes, UDP-glucuronosyltransferase, glutathione S-transferase, and sulphotransferase in livers of rats of different groups (Mean± SD).

Animal groups / Parameters	Control	Gala	Gala+Lyo	Gala+Se	Gala+Lyco+Se
UDPGlucuronyltransferase Mean±SD	122.5± 13	126± 20	135± 15	129± 0.5	129± 0.5
Glutathione s transferase Mean±SD	68± 3.3	157± 2.8 [a]	95± 8 [a,b]	88±12 [a,b]	72±11 [a,b,c]
Sulphotranferase Mean±SD	39±1.3	44± 3.5	36± 2	38± 2.0	42± 4.0

a,b,c letters reefer a significant p<0.05; a- comparison with normal control; b- combined versus individual; c- comparison with Gala intoxicated group

Discussion

D-Galactosamine (Gala) is known to induce acute hepatitis in rats. Induction of hepatitis in rats have been mediated by peroxidation of endogenous lipid and loss of plasma lipid membrane integrity (Zhang etal.,2003).The toxic effect of Gala is due to insufficiency of UDP-glucose and UDP-Galactose and the loss of intracellular calcium homeostasis. After Gala injection, the proteoglycans function was changed in the liver (Agay etal.,2005). Gala also inhibits the oxidative phosphorylation of hepatocytes and block energy production (kim and Combs 1993). Gala affect the enzymes involved in the transport of substrates to the mitochondria and modify the phospholipids composition of membranes (Dilsiz etal.,1999).

In this study, a significant elevation in the serum activities of AST, ALT in Gala injected rats compared with control (Stahl and Sies, 1996). Because the levels of these enzymes are proportional to the extent of liver damage, the activity of these enzymes can be used as indicators of prognosis of the disease. The elevation of serum levels of AST and ALT has been attributed to the damaged structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage .Lyco combined with Se seems to preserve the structural integrity of the hepatocellular membrane as evident from the significant reduction in these enzymes in rats injected with Gala.

Antioxidant enzymes, mainly SOD, CAT and GPx are the first line of defense against free radical induced oxidative stress. SOD is responsible for catalytic dis-mutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide (Levy, 1995). CAT is

responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water (Levy, 1995). In the present study, Gala caused a significant elevation of MDA (p <0.001) and a significant reduction in activities of SOD, CAT in rat liver compared with control.

This is in accordance with the study that stated that Se significantly reduces the depletion of GSH concentration and antioxidant defense enzyme activity in the kidney of rat treated with cispalatine. The protective effects of Se seem to be primarily associated with its presence in the GSH-Px, which is known to protect DNA and other cellular components from damage by ROS. Selenoenzymes are also known to play roles in carcinogen metabolism, in the control of cell division, oxygen metabolism, detoxification processes, apoptosis induction, and the functioning of the immune system (Houglum, 1990).

It was reported that sub-chronic treatment with Gala gradually decreased the activities of SOD and CAT in rat liver (Bedossa et al.,1994). In rats pretreated with Lyco and Se, the activities of these antioxidant enzymes were significantly elevated compared with Gala injected rats. In the same respect, the effect of selenium alone (Group IV) was significantly higher than the effect of Lyco alone (Group III). This may be attributed to the role of selenium in the active sites of SOD. Pretreatment with Lyco +Se combination significantly decreased the level of MDA. The protective effect of Lyco and Se on liver resulted from antioxidant activity including stabilization in the intracellular defense systems and reduction in the lipid peroxidation products.

Thus, it may be suggested that Lyco +Se, the parent antioxidants can partially quench the deleterious effects of chronic toxicity of Gala by scavenging the free radicals (Kamimura e tal.,1992). Moreover, the most important metabolic role of Se in mammalian cell occurs due to its function in the active site of selenoenzyme GPx. It also facilitates the action of Lyco in reducing peroxy radicals through permitting higher bioavailability (Wendel and Tieg , 1987).

In the current study, rats injected with Gala caused a significant elevation of inflammatory markers as (IL-6, NO and TNF-α) that cause damage of liver tissue. Treatment with combination of lyco +Se caused a reduction in these mediators and recovery of hepatocyes as indicated by a decrease in transaminases.

Two phases were proceeded in liver to remove toxicants, Phase I (including hydroxylation, oxidation and reduction) and phase II (conjugation) and excreted as conjugates.

Previous study showed that Se and Lyco, individually or concomitantly, had a role in the increase in the activity of antioxidant enzymes in the femoral tissue of rats. This may explain the role of each in reducing the concentration of Cadmium (Cd) in bone tissue. On the other hand, administration of Se and Lyco alone maintained a normal level of CAT activity compared to the other combination group. This may be the reason why these two groups showed more reduction in Cd concentrations in bone tissue compared to the other treated groups. Se maintained the activity of Cu-Zn SOD and GPx by binding directly with Cd and converting it to an inactive complex compound called Cd complex selenide. The function of Se is to protect bone tissue from oxidation by protecting antioxidant enzymes. It also plays an important role in building seleno-proteins like GPx. The role of Se in increasing GPx activity in the bone tissue that protects DNA from oxidation was evident in this study (Gan etal.,2002).

In this study, we investigated the effects of Lyco and Se administration on hepatic phase I and phase II conjugation systems following exposure to Gala. It was found that Gala significantly increased the activities of phase I enzymes ethoxyresorufin and p-nitrophenylhydroxylase compared with control. Treatment with Lyco +Se significantly reduced these activities. It was suggested that treatment of rats with Lyco or Se caused a decrease in hepatic cytochrome P450 proteins, such as CYP2E1 and CYP1A1, the sub-family of cytochromes closely associated with inhibition of bio-activation of Gala. Thus, it can be suggested that the Lyco and Se has the potential role in the inhibition of the CYP2E1 and CYP1A1 enzymes monitored in the present study.

Gala injection cause a significant elevation in phase II enzymes as glutathione S- transferase compared with control. Lyco and Se attenuate this elevation to be normal. The elevation of this enzyme post Gala injection may be due to defense against toxicity of Gala but the combined effects are better than individual treatment. On the one hand, Gala doesn't affect the activities of UDP-glucuronyl transferase or sulphotransferase compared with control.

Our results concluded that Lyco+ Se possess hepatoprotective action against hepatitis induced by Galactosamine through inhibition of release of inflammatory mediators and enhance antioxidant capacity. This will open a window in new protocol for hepatocellular carcinoma induced by viral or chemical.

Conflicting Interest: The authors certify that there is no actual or potential conflict of interest in relation to this article.

Acknowledgments

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant # (557-247-1434).The authors; therefore, acknowledge with thanks DSR technical and financial support.

Authors' contribution: JK participated in the design of the study and performed the statistical analysis. SS conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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