Journal of Animal Research: v.6 n.4, p. 593-596. August 2016

DOI: 10.5958/2277-940X.2016.00068.1

Ameliorative Effect of Ginger Extract on Serum Biochemical Alterations in Diethylnitrosamine Treated Rats

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Received: 25 February, 2016 **Accepted**: 08 May 2016

ABSTRACT

Present work was designed to study ameliorative effect of ginger extract (GE) on serum biochemical alterations in diethylnitrosamine (DEN) treated rats. Fifty one male Wistar albino rats were randomly allotted to four groups. DEN (0.01%) was given in drinking water *ad libitum* and ginger extract (50 mg/kg BW) was administered in olive oil *per os* either alone or in combination for 90 days. Hypoproteinaemia, hypoalbuminaemia, hypoglycaemia, elevated serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gammaglutamyl transferase (GGT) and total cholesterol levels were observed in the DEN treated rats. Concurrent administration of ginger extract with DEN significantly ($P<0.05$) alleviated the effects of DEN on serum enzyme level and other parameters. To conclude, present study demonstrated the ameliorative effect of ginger extract in partial to complete reversal in serum biochemical alterations.

Keywords: DEN, ginger extract, serum biochemistry, rats

Hepatocellular carcinoma (HCC) is an aggressive and most predominant form of primary liver malignancy. It accounts for more than 90% of all primary liver malignancies with increased incidence and mortality rate (Niu *et al.,* 2015). Being the fifth most common malignancy and third leading causes of cancer-related deaths globally, it represents a major health concern in developed and developing countries (Imamoto *et al.,* 2014). The risk of developing HCC has been attributed to damage and scarring of the hepatic tissue due to hepatitis B and C virus (HBV and HCV) infection, aflatoxicosis, cirrhosis, alcoholism, exposure environmental carcinogens, toxic industrial chemicals, air and water pollutants (Al-Rejaie *et al.,* 2009).

Diethylnitrosamine (DEN) is a potent hepatotoxin and an important emerging organic pollutant which is widespread in nature such as in various processed foods (viz. milk and meat products), alcoholic beverages, water, tobacco products, cosmetics and agricultural chemicals (ATSDR 1989). It exerted carcinogenicity after bioactivated by cytochrome P450 (CYP) enzymes in the liver resulting in DNA-adducts through an alkylation mechanism (Verna *et al.,* 1996). Diethylnitrosamine induced hepatocellular carcinoma model in animals simulated pathological process of occurrence and development of human liver cancer and served as a standard model to study the beneficial effects of many drugs for treatments of HCC (Xiao *et al.,* 2014).

Chemoprevention is a novel approach for prevention and management of malignancies. So, most of the recent cancer studies are focused on identifying the promising chemopreventive agents in dietary components and particularly phytochemicals because of their efficacy in different cancer models (Shen *et al.,* 2014).

Ginger (*Zingiber officinale*, Zingiberacae) is an important medicinal plant cultivated in various countries like India, China, South East Asia, West Indies, Mexico and other parts of the world. Rhizome of ginger is widely used as spice, food-flavouring agent and common condiment

for a variety of compounded foods and beverages. It has been used in Chinese, Ayurvedic and Tibb-Unani herbal medicines all over the world since ancient times for treating common human aliments. Rhizomes of ginger contain active principles like gingerols, 6-dehydrogingerols, shogaols, paradols, gingerdiols, gingerdiones, zingerone, amaldehyde, zerumbone etc (Ghosh *et al.*, 2011). These ingredients exhibited excellent pharmacological properties including antioxidant, anti-inflammatory and anti-angiogenic properties contributing to anti-mutagenic and anticancer activity. Gingerols are identified as principal pungent component of ginger mainly responsible for chemopreventive and chemotherapeutic effects of ginger (Ramakrishnan, 2013). So, the present study was undertaken to evaluate the ameliorative effect of treatment of ginger extract on serum biochemical changes in diethylnitrosamine (DEN) treated rats.

MATERIALS AND METHODS

Chemicals

Diethylnitrosamine or N-Nitrosodiethylamine (Isopac, 1g, Product No. 0258) was obtained from M/s Sigma Chemical Co. USA. The certified Ginger Soft SCF Extract 35% containing high concentration (35%) of gingerols was obtained from M/s Sami Labs Limited, Peenya Industrial Area, Bengaluru-560058. Serum biochemical kits were purchased from M/s Agappe Diagnostics Pvt. Ltd., Cochin.

Animals

Study was approved by Institutional Animal Ethics Committee (Approval Letter Number. 1614/DFBS/B/2014 dated 16.06.2014) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). For the study, male Wistar albino rats of 7-9 weeks age weighing around 272g were obtained from Department of Laboratory Animal Medicine, Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai-600051. The animals were housed in polypropylene cages at 27±2ºC with relative humidity 55±5%. Animals were acclimatized for two weeks, with 12hr light and dark cycle prior to study. The standard commercial pellet laboratory animal diet (procured from M/s. Tetragon Chemie Pvt. Ltd., Bengaluru, India) and water were provided *ad libitum.*

Experimental protocol

Fifty one male Wistar albino rats were weighed and randomly allotted to four groups. The normal control (first) group consisted of six rats and maintained on commercial rat pellet diet and water. All the treated (second, third and fourth) groups consisted of fifteen rats each. Second group received DEN (0.01% v/v) in drinking water *ad libitum* for 90 days. Third group received DEN (0.01% v/v) in drinking water *ad libitum* plus ginger extract (50 mg/kg BW) in olive oil *per os* for 90 days. Fourth group received ginger extract (50 mg/kg BW) in olive oil *per os* for 90 days. The experiment was terminated on 90th day and before sacrifice, blood samples were collected for biochemical analysis and were allowed to clot and centrifuged at 1500 rpm for 30 min to separate the sera. Serum total protein and albumin were estimated by modified Biuret and Duma's method, ALT, AST and ALP by IFCC (International Federation of Clinical Chemistry) method, GGT by Szasz kinetic method, total cholesterol and glucose by CHOD-PAP and GOD-PAP method respectively using semi-automatic biochemical analyzer.

Statistical analysis

The data obtained from different parameters of the study were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range test using SPSS (Version 20 for windows) statistical software.

RESULTS AND DISCUSSION

The mean (±SE) values of serum biochemical parameters are presented in Table 1. No significant differences were observed among the normal control and ginger extract treated groups for all serum biochemical parameters. The mean values of all biochemical parameters revealed significant ($P<0.05$) differences between the DEN treated group and other groups. When compared to the normal control group, DEN+ GE treated groups showed significant ($P<0.05$) differences for ALT, AST, ALP, GGT, albumin, glucose and total cholesterol values while no significant difference was observed for total protein values. There was a significant ($P<0.05$) increase in ALT, AST, ALP,

Table 1: Effect of DEN and GE treatment on serum biochemical values in rats of different experimental groups

Serum Parameters	Treatment Groups			
	Control (n=6)	DEN (n=15)	DEN+GE (n=15)	GE (n=15)
Total protein (g/dL)	7.05^{c} ±0.02	4.80^{a} ±0.36	6.39^{bc} ±0.20	7.01^{c} ±0.02
Albumin (g/dL)	3.02^{c} ±0.04	1.85^{a} ±0.06	2.62^{b} ±0.06	3.05^{c} ±0.13
ALT (IU/L)	63.97^{a}±0.29	194.17^{c}±7.69	141.86^{b}±6.41	64.11^{a}±0.21
AST(IU/L)	124.28^{a}±0.38	259.75^{c}±9.39	189.64^{b}±8.48	123.50^{a}±0.24
ALP (IU/L)	146.86^{a}±0.40	287.86^{c}±5.03	212.64^{b}±5.44	145.22^{a}±0.41
GGT(IU/L)	4.64^{a}±0.10	68.03^{c}±4.43	41.78^{b}±5.08	4.94^{a}±0.19
Glucose(mg/dL)	90.11^{c}±0.41	60.64^{a}±2.21	72.56^{b}±1.85	89.19^{c}±0.33
Total Cholesterol (mg/dL)	77.17^{a}±0.96	116.89^{c}±2.58	98.28^{b}±3.21	76.72^{a}±0.48

*Means bearing different superscript (a, b, c) within a row differ significantly (P<0.05)

GGT and total cholesterol values and significant (P<0.05) decrease in the total protein, albumin and glucose values observed in the DEN treated group as compared to the normal control group. When compared to the normal control group, DEN+ GE showed significant (P<0.05) increase in the ALT, AST, ALP, GGT and total cholesterol values and significant (P<0.05) decrease in the albumin and glucose values.

The present study revealed hypoproteinaemia and hypoalbuminemia in the DEN treated group as compared to the normal control group. These results are in consonance with that of Ha *et al*. (2001) who observed such effect on administration of 0.01 percent DEN in drinking water *ad libitum* for 13 weeks in adult male Wistar albino rats. The authors ascribed the changes to affection of protein synthesis due to the development of hepatic lesions as the etiology of this reduction.

In the present study, ginger extract treated group showed reversal of these changes. Though hypoalbuminaemia was observed, the total protein level was not affected in ginger extract treated group. Significant increase in the values of ALT, AST, ALP and GGT were observed in the DEN treated group as compared to the control group. These results are in accordance with Anoopraj *et al*. (2014) who observed significant increase in the ALT, ALP, AST and GGT values in adult male Wistar albino rats treated with 0.01 percent DEN in drinking water *ad libitum* for 120 days. The cause was attributed to hepatocellular damage due to treatment with DEN and subsequent enzyme leakage into the circulation. The DEN- induced lipid peroxidation of membranes of hepatocytes and subsequent hepatocellular damage resulted in leakage of enzymes from liver tissues and their increased activities in serum (Al-Rejaie *et al.*, 2009).

Elevation in serum ALP enzyme level could be ascribed to alteration in flow of bile due to hepatic lesions and increased shedding of ALP by rapidly dividing cells of bile canalicular plasma membrane (Frederiks *et al.*, 1990). Significant increase in the serum GGT levels was reported by Hemalatha *et al.* (1993) which started increasing from 30 days and reached a six fold after 120 days in rats when treated with a weekly dose of DEN (10 mg/kg BW) by gastric intubation. The etiology of elevation of GGT value could be attributed to cholestasis and bile duct necrosis (Bulle *et al.*, 1990).

The DEN treated groups showed hypoglycemia and elevated total serum cholesterol. These findings are in accordance with Afzal *et al.* (2012) who observed that hypoglycemia and altered lipid profile in rats when treated with single carcinogenic dose of DEN (200mg/kg BWT, i.p.). The authors opined that altered lipid metabolism due to liver cancer development had their effect on membrane integrity, fluidity and regulation of cellular processes related to growth and cell survival as the etiology of serum total cholesterol elevation. Hypoglycemia was due to increased utilization of glucose by cancer cells for survival and proliferation.

CONCLUSION

The present study demonstrated ameliorative effect of ginger extract on serum liver function indices through partial to complete reversal of their alterations. The cause might be attributed to protective effect of high concentration of gingerols in ginger extract on DEN induced oxidative cell damage.

ACKNOWLEDGEMENTS

The first author sincerely acknowledged facilities provided by The Dean, Madras Veterinary College, Chennai, for carrying out the research work and The ICAR, New Delhi, for providing the JRF during her study period. She expressed special thanks to Mr. Mahadeva Nayak, Sami Labs Limited, Bengaluru, for providing me Ginger Soft SCF Extract 35% as free gratis.

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