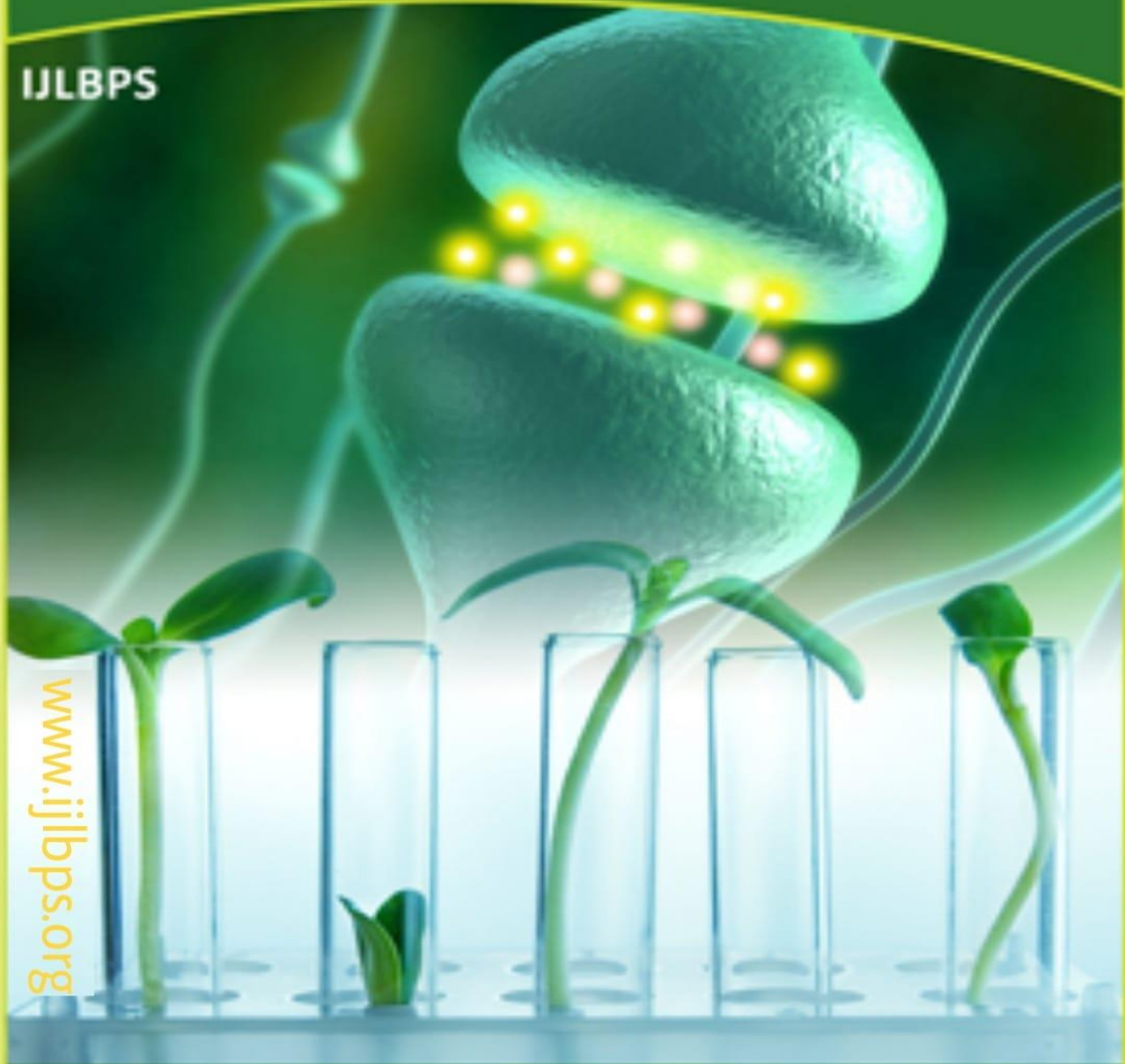




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# Ethanol with ginger: a preliminary screening for possible therapeutic effects

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## Abstract

The current investigation was designed to determine the methods by which ginger (*Zingiber officinale*) may prevent alcohol-induced heart damage in rats. Total carbs, pyruvate, total proteins, free amino acids, and lactate levels were analyzed in cardiac tissue to determine the metabolic profiles of carbohydrates. Rats that had consumed too much alcohol had significantly lower levels of free amino acids and lactate and significantly lower amounts of total carbs and pyruvate. While free amino acids and lactate levels decreased significantly in cardiac tissues, total carbs and proteins increased significantly after treatment with ginger (200 mg/kg body weight). Our findings suggest that ginger's abundance of bioactive chemicals may be responsible for its ability to shield rat cardiac tissue from the damaging effects of alcohol.

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**Keywords:** alcoholic beverages, ginger, cardiovascular system, rat carbohydrate metabolic profiles.

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## Introduction

recognized addictive substance (Guo, & Ren, 2010). Nevertheless, alcoholism is a fatal disease (American Psychiatric Association, 1994). Alcohol constitutes a substantial health and economic burden, costing an estimated \$184 billion in expenditures stemming from alcohol-related chronic diseases such as heart disease (George, & Figueredo, 2010), liver disease (Cederbaum, et al., 2009), cancer (Seitz, & Becker, 2007), diabetes (Baliunas, 2009). Multiple pathways exist in the digestive system for alcohol absorption (Holford, 1987). There is substantial evidence that drinking alcohol in moderation protects against vascular disease (Mukamal et al., 2005). Alcohol's ability to boost HDL cholesterol and reduce platelet aggregation likely accounts for these benefits. Alcohol, on the other hand, is well recognized as a hazardous substance when consumed in excess due to its metabolism by alcohol dehydrogenase, which results in the production of an abnormally high quantity of acetaldehyde via microsomal ethanol oxidation.

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(Ohkubo et al., 2009) system in the liver. Lieber (1995) reported that acetaldehyde; one of the products in alcohol metabolism alters the redox state in the cytosol, which in turn responsible for the abnormalities in antioxidant, lipid metabolism and carbohydrate metabolism. There is evidence that reactive oxygen species which are produced in alcohol toxicity (Robin, *et al.*, 2005) alters the mitochondrial structural, function in various organs including heart (Regan, 1990), liver (Klein, & Harnjanz, 1975).

Since ancient times, medicinal plants used to cure various ailments in humans (Butt *et al.*, 2009). A number of scientific investigations have led to the recognition of a safe status for such natural products. In Chinese, Ayurveda, Unani-Tibb like traditional medicines ginger have been used as medicinal plant (Rong *et al.*, 2009). Ginger possesses various pharmacological activities including hypoglycemia, anticancer, anticardiac antirenal and hepatoprotective and antioxidant (Nicoll and Henein, 2009). Ginger has many antioxidant compounds; these compounds may either mitigate or prevent generation of free radicals in toxic conditions. The active ingredients of ginger include gingerols, shagogals, phytochemicals and other compounds show antioxidant activity in various models (Dugasani *et al.*, 2010). The present study was designed to explore the cardiac protective property of ginger ethanolic extract against alcohol induced cardiac toxicity in the rats.

### Material and Methods

#### Ginger extracts preparation

The rhizomes of *Zingiber officinale* were purchased from local market in Tirupati with authenticated by botanist in the department of Botany, S.V. University, and Tirupati. The rhizomes were shade dried at room temperature and were crushed to powder. 120g of powder has taken and macerate in 1000 ml of 95% ethanol for 12 h at room temperature, then filtered and squeezed with muslin cloth to obtain ethanol extract juice. This process was repeated three times and finally collection of this juice were dried in rotary evaporator (Model: HS-2005V) from this we had get jelly and then this jelly was converted to powder in lyodel freezer. We has done dose dependent studies by using, 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg and 300 mg/kg. of this 200 mg/kg dose showed good antioxidant activity. So this study we selected dose of 200 mg/kg of ethanol extract of ginger.

#### Animals and treatments

The study was approved by Institutional Animal Ethics Committee (IAEC) [Reg No:

10/i/a/CPCSEA/IAEC/SVU/KSR-GVS/dt 15/11/2011].

Twenty four Wistar male albino rats weighting  $200 \pm 25$  g were used. They obtained from Indian Institute of Science, Bangalore. Animals were kept in laboratory at constant room temperature ( $26 \pm 2^{\circ}\text{C}$ ) at least one week before and throughout the experimental period. Commercial pellet diet and water were provided *ad libitum*. After acclimatization 24 rats were divided into four groups of 6 animals each and treated as follows:

**Group I: Normal control (NC):** rats received 2 % of Tween- 80 in normal saline. **Group II: Ginger treatment (Gt) :** rats received ethanol extract of ginger (200 mg/kg body wt) orally for 30 days.

**Group III: Alcohol treatment (At) :** rats received alcohol at dose of 2g/kg for 30 days. **Group IV:**

**Ginger+Alcohol treatment (At+Gt) :** rats received ginger for 30 days followed by alcohol (2g/kg) for 30 days. At the end of 30 days treatment period, the rats were sacrificed by cervical dislocation and heart tissues were isolated, washed with ice cold saline, immersed in liquid nitrogen and stored in deep freezer at  $-80^{\circ}\text{C}$  for further biochemical analysis. The carbohydrate metabolic profiles such as total proteins, total carbohydrates, total free amino acids, pyruvate and lactate levels were estimated in cardiac tissue by the methods of Lowry *et al.*, 1951, Carroll *et al.*, 1956, Moore and Stein, 1954, Friedmann and Hangen, 1942 and Barker and Summerson (1941) as modified by Huckabee 1961 respectively.

#### Chemicals

In the present study all chemicals used were of Analar Grade (AR) and purchased from the following scientific companies: Sigma (St. Louis, MO, USA), Fischer (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India).

#### Statistical analysis

The data has been analyzed by using SPSS (Version 16.0; SPSS Inc., Chicago, IL, USA) and M.S. Office, Excel Software for the significance of the main effects (factors), and treatments along with their interactions. The data has been compared using one way ANOVA with Dunnett's multiple comparison test and differences were considered significant at  $p < 0.05$ .

#### Results and Discussion

Ginger phenol compounds like gingerols, shagogals Possesses antioxidant properties due to

their free radical scavenging activities. (Stadtman, 2004; Siddaraju & Dharmesh, 2007; Shanmugam *et al.*, 2010). Ginger has been used to cure diarrhea, dysentery, fever, cough, ulcers boils and wounds (Young, HV *et al.*, 2005). Ajith *et al.*, (2007) reported that ginger ameliorates cisplatin-induced nephrotoxicity. A combination of ginger and garlic has been reported to produce hypoglycemic and hypolipidemic effects. In another study ginger has protected the tissue from oxidative stress.

The data obtained from our study demonstrate that total proteins (Figure D) were significantly decreased in the alcohol treated rats than normal control group; meanwhile total proteins were increased in the ginger treated group compared to ethanol group. Proteins are most important to maintain the structure of the body. Feinman, 1998 & Lieber, 2003 reported that in alcohol subjects, protein levels are decreased. Chronic exposure of rats to a diet containing ethanol inhibits the protein synthesis as a result of declined messenger RNA translation consecutively 25% loss in cardiac protein per heart (Vary *et al.*, 2001). The major ethanol metabolite acetaldehyde may contribute to cardiac dysfunction, hypertrophy and heart failure by either its direct toxicity or promoting elevated levels of catecholamines and reactive oxygen species (ROS) (Zhang *et al.*, 2010). Although, alcohol metabolite acetaldehyde has cytotoxic effect within the cells or tissues due to reacting with nucleic acids, proteins, peptides, amino acids, lipids and carbohydrates (Bartsch and Nair, 2000). Other early reports also implicated enhanced lipid peroxidation or generation of ROS could result in reduced tissue protein synthesis (Matias *et al.*, 1999). On the other hand total protein levels are increased in ginger treated and ginger treatment in alcoholic rats. Many reports confirmed that ginger's antioxidant activity. Siddaraju and Dharmesh (2007) reported that ginger-free phenolic and ginger hydrolysed phenolic fractions exhibited free radical scavenging activity. Thus ginger prevented the oxidative modification of proteins by its antioxidant property.

The variations in carbohydrates and pyruvate levels in cardiac tissue are shown in figure B and C. There was a significant decrease ( $P < 0.05$ ) in the carbohydrate and pyruvate levels in ethanol treated rats when compared to normal control rats. Alcohol metabolites acetaldehyde, acetate and their end product acetyl CoA levels were increased. NADH/NAD<sup>+</sup> ratio, which reflects to deplete the glycogen levels due to a blockage of gluconeogenesis in the hepatic tissue (Lieber, 1991). Randle *et al.*, (1988) has confirmed that

acetyl CoA inhibits the pyruvate dehydrogenase in alcoholic subjects, so in our study pyruvate levels also decreased in ethanol subjects. Alcohol metabolism increases the NADH levels resulting to induce the lactate dehydrogenase activity (Phyper & Tom Pierce 2006). On the other hand alcohol metabolite acetaldehyde induced the insulin resistance to the cardiac tissue which reflects on various metabolic abnormalities such as hyperlactacidemia, inhibits the lipolysis in adipocytes (Caballena, 2003) which results in decreased levels of the pyruvate in alcohol subjects. However with ginger treatment in ethanolic rats, carbohydrates and pyruvate levels are increased. Shanmugam *et al.*, (2009) have established that ginger modulates the pyruvate and carbohydrate levels in diabetic rats. Our results were confined to above reports, due to the bioactive compounds in ginger, these compounds may increase the levels of carbohydrates and pyruvate in ethanol treated rats.

There was a significant increase ( $P < 0.05$ ) in the lactate and free amino acids levels in the ethanol treated rats. Conversely, the previous reports have been demonstrated that alcohol upshot on NADH/NAD<sup>+</sup> ratio which falls out cellular redox state in turn increases lactate/ pyruvate ratio results in hyperlactacidemia (Greenway & Lutt 1990). Oxygen radicals and other ROS alter the proteins (Grune, 1997). These oxidative modifications may lead to changes in protein function, chemical fragmentation, or increased susceptibility to proteolytic attack (Freeman, 1982). The previous reports also demonstrated that chronic alcohol consumption can increase amino acid levels in alcoholic patients (Bleich *et al.*, 2004). Ginger intentionally condenses the proteolytic process and increases the gluconeogenesis (Chakraborty *et al.*,

According to a study conducted in 2012, ginger therapy reduced levels of free amino acids in ethanol-treated rats.

The levels of lactate and free amino acids are reduced by ginger therapy, in contrast to the ethanol treatment group. Ginger, however, has restored the cellular redox state by reducing the NADH/NAD<sup>+</sup> ratio and, as a result, lowering free radicals via its antioxidant capacity (Shanmugam *et al.*, 2010). Bioactive ginger molecules, such as gingerol, have been shown to exhibit antioxidant action in a number of experimental models, as described by Dugasani *et al.*, (2010).

According to a 2011 study (Morakinyo *et al.*) it's possible that the same thing happened in our

research, which is why we found that the ginger-treated group had lower levels of lactate and free amino acids than the ethanol-treated group.

#### Conclusion

In the current investigation, the influence of ginger on carbohydrate metabolic profiles was determined with reference to alcohol toxicity. To investigate its potential function in alcohol-induced myocardial infarction, researchers can isolate bioactive molecules.

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