Chapter 2

Oxidative Enzymes
GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Glucose-6-phosphate dehydrogenase (G-6-PDH) is a cytosolic enzyme of the pentose phosphate pathway that is responsible for the generation of NADPH, which is required in many detoxifying oxygen derived free radicals (Tian et al., 1998; Salvemini et al., 1999). G-6-PDH, the first and rate limiting enzyme of the pentose phosphate pathway, has long been regarded as important in the biosynthesis of sugar moiety of nucleic acids (Luzzatto and Metha, 1995) and determines the amount of NADPH by controlling the metabolism of glucose via pentose phosphate pathway. It has been traditionally thought that G-6-PDH was a typical "house keeping" enzyme that was regulated solely by the ratio of NADPH and NADP (Kletzien et al., 1994; Tian et al. 1999). The production of NADPH required for the regeneration of glutathione in the mitochondria is critical for scavenging the mitochondrial ROS through glutathione reductase and glutathione peroxidase systems (Jo et al., 2001).

G-6-PDH may have directly reduced the basis ROS formation and as a consequence, increased the cellular concentration of glutathione (Salvemini et al., 1999). G-6-PDH plays a critical role in cell growth by providing NADPH for redox regulation (Tian et al., 1998). A major role of NADPH in
erythrocytes is regeneration of reduced glutathione, which prevents the hemoglobin denaturation, preserves the integrity of red blood cell membrane sulphohydril groups and detoxify hydrogen peroxide and oxygen radicals in and on the red blood cells (Weksler, 1990).

Glucose-6-phosphate dehydrogenase enzyme is extramitochondrial in location and highly specific for NADP as an electron acceptor. G-6-PDH is known to occur in two distinct forms, one located in cytosol, which is specific for NADP (Meizer et al., 1977) and the other located in microsomes utilizes either NADP or NAD (Ashida et al., 1987). The overall equilibrium favors the formation of NADPH, which acts as electron donor in reductive biosynthesis.

G-6-PDH is an important enzyme in HMP shunt pathway, which occurs in the cytosol of the cell and is an alternate pathway of glucose oxidation. This pathway provides a major portion of the cell NADPH, which functions a biochemical reluctant. It is particularly important in liver, mammary glands which are active in the biosynthesis of fatty acids. The activity of G-6-PDH is an index for determining the efficiency of HMP shunt. G-6-PDH is inhibited by NADPH and the inhibition is relieved by NADP (Candy, 1980). Since, HMP shunt is an alternate pathway for the supply of energy and reduced co-enzymes (NADPH), which function in detoxification process, due to its importance an attempt has been made to examine the impact of G-6-PDH role in hepatic tissue with reference to ginger treatment, ethanol treatment, diabetic (STZ), ethanol treated diabetic and ethanol treated diabetic rats with ginger supplementation.

**Results and Discussion :**

G-6-PDH activity was assayed in the present study in all the experimental groups of control, ginger treated, ethanol treated, diabetic, ethanol treated diabetic and ethanol treated diabetic rats with ginger treatment. A significant decrease in specific activity of G-6-PDH was observed in
ethanol treated diabetic rats. Ginger treatment significantly elevated the specific activity of G-6-PDH. Where as, in ethanol treated, diabetic, and ethanol treated diabetic groups G-6-PDH activity was decreased. However, in the combination treatment i.e. ethanol treated diabetic rats with ginger supplementation G-6-PDH activity was elevated.

In the present study with ginger treatment G-6-PDH activity was increased, when compared to all the experimental groups. This could be due to elevation of mitochondrial enzymes by the compounds in ginger. Hence in ginger treated rats we observed elevated activity of G-6-PDH (Table: 10 and Figs: 19, 20).

G-6-PDH activity was decreased with ethanol treatment in the liver tissue of the present study. This suggests that decreased conversion of glucose-6-phosphate to 6-phosphogluconate leading to reduced formation of NADPH in HMP shunt. This cycle as an alternate source of energy impairment of glycolytic and Krebs cycle pathways. Inhibition of oxidoreductases in the mitochondria results in the decreased energy supply for normal metabolic functions. Inhibition of G-6-PDH by ethanol could also be attributed to the reduced availability of NADP. Markki et al., (1997) reported a decrease in G-6-PDH activity following the administration of ethanol to rats, the decrease in G-6-PDH activity by ethanol indicated reduced oxidation of glucose in HMP shunt. Similar decrease in G-6-PDH activity in rat during various induced toxic stress conditions was also reported (Cleary, 1991; Pugazhenthi et al., 1991). HMP shunt contributes the pentoses for the synthesis of ribonucleic acids. A decrease in G-6-PDH activity affects RNA synthesis and the nucleic acid metabolism, which is of vital importance.

Oh et al., (1997) reported a significant decrease in G-6-PDH activity in the ethanol treated rats. Recently Das and Vasudevan, (2005) in their dose dependent studies on ethanol toxicity reported depleted activities of G-6-PDH at a significant level in hepatic tissue of rats. Umadevi, (1992) also reported
decreased G-6-PDH activity levels in hepatic tissue of rats under acetaldehyde induced stress conditions.

The activity of G-6-PDH activity was found to be lowered in diabetic tissues (Saraswathi and Govindasawamy, 2002). Diabetes causes inhibition of glucose 6 phosphate dehydrogenase activity in liver. The decrease in the activity of this enzyme in diabetic condition may result in the diminished functioning of hexose monophosphate shunt and there by the production of reducing equivalents such as NADP and NADPH. The hyperglycemia results in decreased levels of citric acid cycle enzymes and pentose phosphate pathway enzymes as the phosphorylated glucose enters in to the other pathways like gluconeogenesis (Hue, 1987; Gomathy et al., 1990) and glycoprotein synthesis (Rathi et al., 1981; Camerini-Davalos et al., 1990). A similar decrease in the activities of citric acid cycle enzymes were also observed by Sener et al., (1990).

A decrease in G-6-PDH activity has been observed in diabetic rats by Ashok Kumar and Pari, (2005). The decreased activity of G-6-PDH affects the concentration NADPH and increases oxidative stress, leading to diabetic complications (Ugochukwu and Babady, 2003). Shibib et al., (1993) reported that the hyperglycemia decreases HMP shunt enzymes activity in diabetic animals, hence G-6-PDH activity was decreased.

Punitha et al., (2005) reported a decrease in G-6-PDH activity following the administration of ethanol to rats, the decrease in G-6-PDH activity by ethanol indicated reduced oxidation of glucose in HMP shunt. The decrease in the activity of this enzyme in diabetic condition may result in the diminished functioning of hexose monophosphate shunt and there by the production of reducing equivalents such as NADP and NADPH.

The major role of NADPH in liver is the regeneration of reduced GSH. Which preserves the integrity of liver tissue and detoxifies \( H_2O_2 \) and hydroxyl
radicals, oxygen radicals in the liver. G-6-PDH activity was increased in ethanol treated diabetic rats with ginger treatment. Where as, in ethanol treated diabetic rats due to diminished functioning of HMP cycle, G-6-PDH activity was decreased. With ginger treatment in ethanol treated diabetic rats the G-6-PDH activity was increased. The treatment of diabetic rats with curcumin drastically elevated G-6-PDH (Arun et al., 2002). Similar results were observed in *Momordica charantia* treatment, which is used for relief from diabetes (Leatherdale, 1981). Baizid et al., (1993) reported that the extracts of *Coccinia indica* stimulated the shunt enzyme G-6-PDH. This increase in G-6-PDH activity may be due to antioxidants like zingerone, gingerol, phytochemicals which are present in ginger, may elevate the G-6-PDH in ethanol treated diabetic rats

**ISOCITRATE DEHYDROGENASE (ICDH)**

(Isocitrate: NADP⁺ oxidoreductase: E.C:1.1.1.42)

ICDH is an important enzyme in Kreb’s cycle which catalyses the reversible oxidation of isocitrate to oxalosuccinate followed by decarboxylation leading to the formation of α-ketoglutarate. During oxidation-reduction it utilizes NAD/NADP to accept electrons. NAD-ICDH localized in the mitochondria is an allosteric enzyme which requires ADP, Mg²⁺ or Mn²⁺ for its activity. In the presence of the positive modulator (ADP) the monomeric form aggregates to form the dimer. Such ADP-induced aggregation is prevented by NADH, a negative modulator. NADP-ICDH catalyzes: 1) the formation of oxalosuccinate from isocitrate and concurrent reduction of NADP to NADPH and 2) the decarboxylation of oxalosuccinate to α-ketoglutarate. The first step requires NADP but not divalent metal ions. The second step requires divalent metal ions but not NADP. The rate of dehydrogenation observed in the absence of divalent ions is lower than the rate of the overall reaction. Approximately 75-90% of NADP-specific enzyme is located in non-particulate fraction of the cytoplasm and the remainder in the mitochondria (Plaut, 1963). The intra- and extra- mitochondrial enzymes
differ from each other in electrophoretic mobility and in immunological properties (Lowenstein, 1967). Owing to its differential distribution and important role played in mitochondrial and extramitochondrial pathways it was desired to study the specific activities of NADP-ICDH in ethanol, diabetic and ethanol treated diabetic rats under the influence of ginger treatment.

**Results and Discussion:**

From this study it was observed, a decrease in ICDH activity in the liver tissue in ethanol, diabetic and ethanol treated diabetic rats when compared to normal control. Ginger treatment significantly elevated the specific activity of ICDH in ethanol treated, diabetic, and ethanol treated diabetic groups in one month treatment period (Table.11; Fig: 21 to 22).

The liver NADP-ICDH was significantly decreased suggesting reduced mitochondrial oxidation of isocitrate in ethanol treated diabetic rat. This could be attributed to diminished supply of keto acids into citric acid cycle (Thalwar et al., 1989). From the data it was observed that, the ICDH enzyme activity was increased in the liver tissue of ethanol treated diabetic rats with ginger treatment. The possible reason for an increase in NADP-ICDH activity during ginger treatment is help to NADPH production needed for the detoxification of ammonia formed. NADP-ICDH activity increases during periods of increased energy demands and lowered ATP/ADP ratio (Stein et al., 1967). The increment in NADP-ICDH may restore the normal metabolic activity by the ginger treatment.

In the present study a significant elevation of ICDH activity was observed in the liver of ethanol treated diabetic rats with ginger treatment. The decrease in specific activity of NAD/NADP-ICDH as a consequence of induced acetaldehyde-treatment suggests reduced conversion of isocitrate to \(\alpha\)-ketoglutarate. Similar changes in ICDH activity was reported in different animals treated with various toxic compounds (Joseph and Rao, 1990; Reddy
Alcohol is known to affect the structural integrity of the mitochondria (Leo and Lieber, 1982). The changes in NADP-ICDH could be attributed to the mitochondrial damage caused by acetaldehyde treatment. Similar decrease in NADP-ICDH has been reported during induced ethanol treatment in dog (Pachinger et al., 1973) and rat (Chen and Plaut, 1963; Williamson et al., 1969). Interaction of enzyme with NADPH may result in an unfavorable conformation of the enzyme molecule (Plaut, 1963). It is possible that increased formation of NADPH as evidenced during acetaldehyde-treatment may be inhibiting NADP-ICDH activity. An increase in NADPH/NADP ratio has been reported in the animals treated with alcohol (Winston and Reitz, 1980; Crow et al., 1983). Inhibition of ICDH by acetaldehyde results in alteration of activities of TCA cycle enzymes (Umadevi, 1992). During acetaldehyde metabolism the oxygen consumption diminishes (Cederbaum et al., 1976) resulting in the decreased mitochondrial oxido-reductase activity.

Decreased NADP-ICDH activity as a consequence of acetaldehyde toxicity results in the reduced production of NADPH which plays a crucial role in the detoxification processes (Reed, 1986). Reports available in kidney reveals, an over utilization of cellular glucose occurs through elevated activities of glycolytic and NADP-linked lipogenic enzymes (Sochor et al., 1985; Raju et al., 2001).

Increase in ICDH activity was observed in ethanol treated diabetic rats when they are treated with ginger. The restoration of ICDH activity reveals the normal operation of TCA cycle for high energy production to withstand the toxic conditions of ethanol in diabetic rats. This observation from this study elucidates ginger is beneficial to suppress hepatotoxicity due to ethanol in diabetic rats.
MALATE DEHYDROGENASE (MDH)

(L-Malate \textit{NAD}^+ oxidoreductase – EC: 1.1.1.37)

Malate dehydrogenase is an enzyme in the citric acid cycle that catalyzes the conversion of malate into oxaloacetate (using \textit{NAD}^+) and vice versa (this is a reversible reaction). Malate dehydrogenase is not to be confused with malic enzyme, which catalyzes the conversion of malate to pyruvate producing \textit{NADPH}. Malate dehydrogenase is also involved in gluconeogenesis, the synthesis of glucose from smaller molecules. Pyruvate in the mitochondria is acted upon by pyruvate carboxylase to form oxaloacetate, a citric acid cycle intermediate. In order to get the oxaloacetate out of the mitochondria, malate dehydrogenase reduces it to malate, and it then transverse the inner mitochondrial membrane. Once in the cytosol, the malate is oxidized back to oxaloacetate by cytosolic malate dehydrogenase. Finally, phosphoenol-pyruvate carboxy kinase (PEPCK) converts oxaloacetate to phosphoenol pyruvate.

It has been demonstrated that several mitochondrial soluble \textit{NAD}^+ dependent dehydrogenases such as MDH specifically associated with \textit{NADH} and Ubiquinone oxidoreductase. MDH catalyses the oxidation of L-malate to oxaloacetic acid using \textit{NAD} as cofactor and the reaction is reversible. Although the reaction is endergonic, it goes in the forward direction very rapidly in the cell, because of the rapid removal of reaction products, oxaloacetate and \textit{NADH} in subsequent steps. The MDH reaction is strictly stereo specific for the L-stereo isomer of malate. The cells of higher animals contain two forms of MDH; one is in the mitochondria and the other in the extra mitochondrial cytoplasm (Murray \textit{et al.}, 2007). The enzymes from these two sources differ in their electrophoretic mobility, molecular weight, amino acid composition and \textit{kinetic properties indicating}, a differential protein nature of the enzyme. As a member of the Kreb’s cycle, it participates in terminal oxidation and interlinks the electron transport system and oxidative phosphorylation system (Nelson and Cox, 2009). The activity of MDH depends on the rates of formation of oxaloacetate and phosphoenol pyruvate.
from malate. Any change in the mitochondrial structure inhibits the activity of MDH. The cytosolic and mitochondrial forms of malate dehydrogenase are key enzymes in the malate-aspartate shuttle (Chatterjea and Shinde, 1993). The activity levels of MDH indicate the status of prevailing oxidative metabolism.

Hence, an attempt has been made in the present study to evaluate the effect of *Gingiber officinale* ethanolic extract in correcting the ethanol treated diabetic rats associated alterations in mitochondrial MDH enzymes.

**Result and Discussion:**

The specific activity of Liver MDH was estimated in all the experimental groups of control, ginger treated, ethanol treated, diabetic, ethanol treated diabetic and ethanol treated diabetic rats with ginger treatment groups. The activity of MDH significantly elevated with ginger treatment. But a significant decrease in the MDH activity was observed in ethanol treated, diabetic and ethanol treated diabetic rats. Whereas in ethanol treated diabetic rats with ginger treatment we observed elevated levels of MDH (Table.12; Fig: 23 to 24).

In the diabetes mellitus, abnormalities of mitochondrial enzymes may impair the metabolism of glucose. As the rates of glucose oxidation normalize insulin secretion and subsequent release of β-cells, a defective insulin response to glucose stimulation may be due to respiratory chain deficiency in the pancreas of IDDM. This supposes that random partitioning of mitochondria during development might have resulted in the accumulation of mutated mitochondrial DNA-containing fragments in particular tissues including pancreas. The rearrangements produce potentially antigenic chimeric proteins. The reduction in the functioning of mitochondrial enzymes may lead to a defect in the mitochondrial energy production which would impair protein synthesis and energy production in β-cells.
The increased production of free radicals in mitochondrial cells in the tissue, also a decrease in oxygen consumption respiratory ratio were observed in mitochondria of diabetic rats (Puckett and Reddy, 1979). Lowering in the activity of MDH and increase in NAD$^+/NADH$ were reported by Obrosova et al., (1999). It has been suggested that the diabetogenicity of STZ is dependent on the inhibition of the activities of citric acid enzymes like MDH, $\alpha$-ketoglutarate dehydrogenase (Boquist et al., 1985). Diabetes decreased the expression of genes involved in carbohydrate and energy metabolism through effects on known pathways such as glycolysis, TCA cycle and oxidative phosphorylation. MDH activity was decreased in the tissues of diabetic animals in several studies (Ianuzzo and Aramstrong, 1976).

In the current study MDH activity was decreased with ethanol treatment in the liver tissue. The decrease in specific activity of MDH in tissues of all groups of rats as a consequence of ethanol treatment suggests decreased utilization of malate. The reduced levels of TCA cycle intermediates may also be due to the decrease in MDH activity during ethanol treatment. Concisely the reduced MDH activity could be attributed to (1) Low availability of substrate (2) Lesser conversion of Succinate-Fumarate-Malate and (3). The changes in the structural integrity of mitochondria. A significant decrease in the specific activity of NADP-ICDH and as a consequence of ethanol-treatment observed in the present study indicates reduced formation of malate. The decrease in activity levels of dehydrogenases is in consistent with the decreased conformation (Cederbaum et al., 1976). An increase in proteolytic activity during ethanol intoxication may also be responsible for the decreased MDH activity (Klatskin, 1961).

In the combination treatment i.e. ethanol treatment in diabetic rats, we observed decreased levels of MDH activity. The decrease in specific activity of MDH in tissue of all groups of rats as a consequence of ethanol treatment suggests decreased utilization of malate. The reduced levels of TCA cycle intermediates may also be due to the decrease in MDH activity during ethanol treatment.
In the present investigation MDH activity was increased in ginger treated rats. This elevation may be due to ginger action on energy metabolism and also on free radicals. Hence in ginger treated groups MDH activity was increased.

In ethanol treated diabetic rats with ginger treatment MDH levels were elevated. This may be due to the decreased oxidative stress and increase in the activities of mitochondrial enzymes. Ginger has the capacity to increase the activities of mitochondrial enzymes, this may due to the compounds which are present in ginger has the capacity to decrease the oxidative stress and increase these mitochondrial enzymes activity. There are many reports on normalization of MDH activity with medicinal plants treatment in diabetic rats. The observed decrease in the activities of mitochondrial enzymes in liver and kidney of the diabetic rats were significantly enhanced upon molybdate therapy (Saraswathi and Govindaswamy, 2002). C-peptide rectified the mitochondrial defects and corrects many of the maladies associated with diabetes (Sima, 2003). Increase in succinate and malate dehydrogenase activities in ethanol treated diabetic rats with ginger treatment indicates better utilization of energy yielding intermediates by TCA cycle.

**GLUTAMATE DEHYDROGENASE (GDH – EC: 1.4.1.3):**

Glutamate dehydrogenase (GDH) is a homohexameric mitochondrial matrix enzyme that catalyses the reversible oxidative deamination of glutamate to \( \alpha \)-ketoglutarate plus free ammonia using either NAD or NADP as a co-factor. The enzyme is expressed at high levels in liver, brain, pancreas and kidney but low in muscle. Allosteric control of mammalian GDH activity by positive electrons (e.g. ADP and leucine) and negative electrons (e.g. GTP) has been studied extensively. The physiological significance of this regulation is highlighted by the recent identification of infants and children with an unusual hyperinsulinism / hyperammonaemia synodrome
(Stanley et al., 1998; Fang et al., 2002). In hepatocyte, glutamate is transported from the cytosol into mitochondria, where it undergoes oxidative deamination catalyzed by GDH (Nelson and Cox, 2001).

The enzyme that is linked to NAD is involved primarily in oxidation of glutamate, whereas the one linked to NADP is associated with biosynthetic process. This enzyme is also important because of its pivotal position in metabolism occupied by the glutamate and α-ketoglutarate and the ability of these compounds to enter into various metabolic pathways. Glutamate dehydrogenase (GDH) is a key pyridine nucleotide enzyme which is involved in the oxidative deamination of nitrogen into organic compounds and forms link between carbohydrate and amino acid metabolism. The changes in GDH activity indicate alterations in the production of ammonia and oxidative deamination of glutamate. Hence, its activity was assayed to assess the metabolic significance of this enzyme in ginger treated, ethanol treated, diabetic (STZ), ethanol treated diabetic and ethanol treated diabetic rats with ginger treatment groups.

Results and Discussion:

The specific activity of glutamate dehydrogenase was assayed in the present study in all the experimental groups of control, ginger treated, ethanol treated, diabetic, ethanol treated diabetic and ethanol treated diabetic rats with ginger treatment groups. GDH activity was significantly elevated with one month period of ginger treatment. The GDH activity was decreased in ethanol, diabetic, ethanol treated diabetic rats. Whereas in ethanol treated diabetic rats with ginger treatment GDH levels were increased (Table: 13; Figs: 25 to 26).

The present examination reveals that the activity of GDH was significantly decreased in diabetic condition. The decrease in GDH activity is attributed to its inhibition by elevated ammonia levels (product-inhibitor),
which diminish the catalytic efficiency of the enzyme molecule (Dudley et al., 1983; Reddy and Rao, 1991), demonstrated that increase ammonia and lactate levels inhibit GDH activity. An increase in mitochondrial membrane permeability and consequent mitochondria swelling might be causing alteration in the activity of the mitochondrial enzyme GDH and GDH enzyme might be modifying the mitochondrial NAD reduction status during diabetes. The decrease in the activity of GDH suggests that regulation of ammonia toxicity in the kidney by the processes of deamination and amination is affected during the diabetic state. This was also reported in brain of diabetic rats by Telushkin et al., (2005) and Nayeemunisa et al., (2006). The decrease in activities of GDH in the brain of rats with enzyme dysfunction due to activation of lipidperoxidation (Telushkin and Nozdrachev, 1999) which attests serious disturbances in energy metabolism and contributes to the impairment of glutamate utilization in the brain and progression of glutamate induced toxicity. In diabetic rats GDH activity is decreased. This could be, due to diabetic condition ammonia metabolism is altered.

Dlugosz et al., (1991) correlate the changes in GDH activity induced by ethanol treatment to progressive alteration and degeneration of mitochondria. The decrease in GDH activity is attributed to its inhibition by elevated ammonia levels (product-inhibitor), which diminish the catalytic efficiency of the enzyme molecule (Dudley et al., 1983). Reddy and Rao, (1991) demonstrated that increase in ammonia and lactate levels inhibit GDH activity. The increased LDH also reported in the present study, consonance with that lactate inhibits the GDH activity. A high NADH/NAD was envisaged in the animals exposed to ethanol treatment, which might play a prominent role in the inactivation of GDH (Sugano et al., 1990). Dlugosz et al., (1991) reported that ethanol alter the mitochondrial enzymes activity. In ethanol treated diabetic rats due to oxidative stress and alteration in mitochondrial enzymes, GDH activity was decreased.
In ginger treated rats GDH activity was decreased. The elevated levels of GDH activity may be due to the antioxidants which are present in ginger are responsible for the increased activity of GDH. Ginger has the capacity to utilize energy yielding metabolites hence with ginger treatment, GDH activity was increased. In ethanol treated diabetic rats with ginger treatment the MDH activity was increased. The increased activity might be due to the decreased activities of oxidative stress by ginger and increase the mitochondrial enzymes. Ginger has the capacity to increase the activity of GDH in ethanol treated diabetic rats. Hence, normalizing of the activities of mitochondrial enzymes in ethanol treated diabetic rats with ginger treatment was observed. There are many reports on inhibition of GDH activity by medicinal plants in diabetic rats. Trigonella treatment for 21 days to diabetic rats, normalized mitochondrial enzymes in diabetic rats was observed by Thakaran et al., (2004).
Table – 10
Changes in Glucose-6-Phosphate Dehydrogenase (G-6-PDH) activity in the liver tissue of Normal Control (NC), Ginger treated (Gt) (200mg/kg body weight), Ethanol treated (Et) (2.0g/kg body weight), Diabetic (Di) (STZ = 50mg/kg body weight), Diabetic + Ethanol treated (Di + Et) and Diabetic + Ethanol + Ginger treated (Di + Et + Gt). The values are expressed in μ moles of formazan formed / mg protein / hour.

<table>
<thead>
<tr>
<th>Experimental Groups (Treatment)</th>
<th>NC</th>
<th>Gt</th>
<th>Et</th>
<th>Di</th>
<th>Di + Et</th>
<th>Di+Et+Gt</th>
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<tbody>
<tr>
<td>Mean</td>
<td>0.892</td>
<td>0.931</td>
<td>0.724**</td>
<td>0.614**</td>
<td>0.551**</td>
<td>0.825</td>
</tr>
<tr>
<td>SD (±0.0188)</td>
<td>(±0.0451)</td>
<td>(±0.0307)</td>
<td>(±0.0291)</td>
<td>(±0.0482)</td>
<td>(±0.0338)</td>
<td></td>
</tr>
<tr>
<td>Percent Change</td>
<td>(+4.353)</td>
<td>(-18.668)</td>
<td>(-31.123)</td>
<td>(-38.203)</td>
<td>(-7.491)</td>
<td></td>
</tr>
</tbody>
</table>

All the values are mean ± SD of six individual observations.
Values in parentheses denote per cent change over respective normal control.
Values are significant compared to normal control at (P<0.001) **

One way ANOVA

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Between Treatment</th>
<th>Within treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df (a)</td>
<td>Sum of squares (x)</td>
</tr>
<tr>
<td>Liver</td>
<td>5</td>
<td>0.702</td>
</tr>
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</table>
Table – 11

Changes in Isocitrate Dehydrogenase (ICDH) activity in the liver tissue of Normal Control (NC), Ginger treated (Gt) (200mg/kg body weight), Ethanol treated (Et) (2.0g/kg body weight), Diabetic (Di) (STZ – 50mg/kg body weight). Diabetic + Ethanol treated (Di + Et) and Diabetic + Ethanol + Ginger treated (Di + Et + Gt).

The values are expressed in μ moles of formazan formed / mg protein / hour.

<table>
<thead>
<tr>
<th>Experimental Groups (Treatment)</th>
<th>NC</th>
<th>Gt</th>
<th>Et</th>
<th>Di</th>
<th>Di + Et</th>
<th>Di+Et+Gt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.944</td>
<td>1.267**</td>
<td>0.812</td>
<td>0.707**</td>
<td>0.639**</td>
<td>0.917</td>
</tr>
<tr>
<td>SD</td>
<td>(±0.0455)</td>
<td>(±0.0741)</td>
<td>(±0.0382)</td>
<td>(±0.0097)</td>
<td>(±0.0664)</td>
<td>(±0.0165)</td>
</tr>
<tr>
<td>Percent Change</td>
<td>(+34.222)</td>
<td>(-14.0134)</td>
<td>(-25.15)</td>
<td>(-32.245)</td>
<td>(-2.842)</td>
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</tr>
</tbody>
</table>

All the values are mean ± SD of six individual observations.

Values in parentheses denote per cent change over respective normal control.

Values are significant compared to normal control at (P<0.001) **

One way ANOVA

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<thead>
<tr>
<th>Tissue</th>
<th>df (a)</th>
<th>Sum of squares (x)</th>
<th>Mean Squares</th>
<th>df (b)</th>
<th>Sum of squares (y)</th>
<th>Mean Squares</th>
<th>a+b</th>
<th>x+y</th>
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<tr>
<td>Liver</td>
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<td>1.488</td>
<td>0.298</td>
<td>30</td>
<td>0.069</td>
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<td>1.557</td>
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Table – 12

Changes in Malate Dehydrogenase (MDH) activity in the liver tissue of Normal Control (NC), Ginger treated (Gt) (200mg/kg body weight), Ethanol treated (Et) (2.0g/kg body weight), Diabetic (Di) (STZ-50mg/kg body weight), Diabetic + Ethanol treated (Di + Et) and Diabetic + Ethanol + Ginger treated (Di + Et + Gt).

The values are expressed in μ moles of formazan formed / mg protein / hour.

<table>
<thead>
<tr>
<th>Experimental Groups (Treatment)</th>
<th>NC</th>
<th>Gt</th>
<th>Et</th>
<th>Di</th>
<th>Di + Et</th>
<th>Di + Et + Gt</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
<td>0.674667</td>
<td>0.739</td>
<td>0.536**</td>
<td>0.511**</td>
<td>0.43**</td>
<td>0.614</td>
</tr>
<tr>
<td>SD</td>
<td>(±0.0093)</td>
<td>(±0.0293)</td>
<td>(±0.0351)</td>
<td>(±0.0218)</td>
<td>(±0.0481)</td>
<td>(±0.0216)</td>
</tr>
<tr>
<td>Percent Change</td>
<td>(+9.634)</td>
<td>(-20.578)</td>
<td>(-24.185)</td>
<td>(-36.265)</td>
<td>(-9.017)</td>
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</tbody>
</table>

All the values are mean ± SD of six individual observations.

Values in parentheses denote per cent change over respective normal control.

Values are significant compared to normal control at (P<0.001)**

One way ANOVA

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Between Treatment</th>
<th>Within treatment</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df (a)</td>
<td>Sum of squares (x)</td>
<td>df (b)</td>
<td>Sum of squares (y)</td>
<td>Mean Squares (x)</td>
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</tr>
<tr>
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<td>0.388</td>
<td>30</td>
<td>0.027</td>
<td>0.001</td>
<td>35</td>
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</table>
Table – 13

Changes in Glutamate Dehydrogenase (GDH) activity in the liver tissue of Normal Control (NC), Ginger treated (Gt) (200mg/kg body weight), Ethanol treated (Et) (2.0g/kg body weight), Diabetic (Di) (STZ – 50mg/kg body weight), Diabetic + Ethanol treated (Di + Et) and Diabetic + Ethanol + Ginger treated (Di + Et + Gt).

The values are expressed in µ moles of formazan formed / mg protein / hour.

<table>
<thead>
<tr>
<th>Experimental Groups (Treatment)</th>
<th>NC</th>
<th>Gt</th>
<th>Et</th>
<th>Di</th>
<th>Di + Et</th>
<th>Di + Et + Gt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.529</td>
<td>0.502</td>
<td>0.459</td>
<td>0.385**</td>
<td>0.314**</td>
<td>0.510</td>
</tr>
<tr>
<td>SD (±0.0428)</td>
<td>(±0.0162)</td>
<td>(±0.0469)</td>
<td>(±0.0251)</td>
<td>(±0.0272)</td>
<td>(±0.0212)</td>
<td></td>
</tr>
<tr>
<td>Percent Change</td>
<td>(+13.724)</td>
<td>(-13.157)</td>
<td>(-27.258)</td>
<td>(-40.762)</td>
<td>(-3.6198)</td>
<td></td>
</tr>
</tbody>
</table>

All the values are mean ± SD of six individual observations.

Values in parentheses denote per cent change over respective normal control.

Values are significant compared to normal control at (P<0.001) **

One way ANOVA

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Between Treatment</th>
<th>Within treatment</th>
<th>a+b</th>
<th>x+y</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df (a)</td>
<td>Sum of squares (x)</td>
<td>Mean Squares</td>
<td>df (b)</td>
<td>Sum of squares (y)</td>
<td>Mean Squares</td>
</tr>
<tr>
<td>Liver</td>
<td>5</td>
<td>0.326</td>
<td>0.065</td>
<td>30</td>
<td>0.031</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Fig. 19: Effect of Gt, Et, Di (STZ), Di + Et and combination of Di + Et + Gt, on G-6-PDH activity in the liver tissue of male albino rats. The values marked with** ($P<0.001$) are of significant compared to normal control.

Fig. 20: Percent change in liver G-6-PDH activity in experimental rats over the control
Fig. 21: Effect of Gl, El, Di (STZ), Di + Et and combination of Di + Et + Gl, on ICDH activity in the liver tissue of male albino rats. The values marked with ** (P<0.001) are of significant compared to normal control.

Fig. 22: Percent change in liver ICDH activity in experimental rats over the control.
Fig. 23: Effect of Gt, Et, Di (STZ), Di + Et and combination of Di + Et + Gt, on MDH activity in the liver tissue of male albino rats. The values marked with ** (P<0.001) are of significant compared to normal control.

Fig. 24: Percent change in liver MDH activity in experimental rats over the control
Fig.25: Effect of Gt, Et, Di (STZ), Di + Et and combination of Di + Et + Gt, on GDH activity in the liver tissue of male albino rats. The values marked with** (P<0.001) are of significant compared to normal control.

Fig.26: Percent change in liver GDH activity in experimental rats over the control