EFFECT OF PANAX GINSENG ON THE ACTIVITY OF CHOLINESTERASE IN DIFFERENT TISSUES OF EXPERIMENTALLY-INDUCED DIABETES IN RATS

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ABSTRACT

In the present study cholinesterase (ChE) activity in seven brain regions (cerebral cortex, thalamus, hypothalamus, midbrain, cerebellum, pons and medulla oblongata), heart, liver and serum of adult male albino rats were determined following diabetes induction by a single subcutaneous injection of alloxan monohydrate (120 mg/kg body weight), oral administration of Panax ginseng extract (100 mg/kg body weight) for 12 consecutive days and the co-administration of both treatments. The enzyme activity was estimated after 2, 4, 8 and 12 days of alloxan and or Panax ginseng administration. Concomitant variation in blood glucose level and body weight of treated rats were also recorded.

The results showed that injection of alloxan provoked a highly significant rise in blood glucose level coupled with failure of the treated animals to gain weight. Moreover, diabetes induction resulted in a general increase in ChE activity in most of the brain regions studied. The increase was most prominent in the cerebral cortex while the cerebellum and pons were the less affected regions. This effect may be attributed to differences in the regulation of acetylcholine metabolism in these regions. In heart of diabetic rats, ChE activity exhibited a significant decrease after 12 days, however in the liver a significant increase was noticed after 4 days. Serum ChE was highly significantly elevated after 4 and 12 days.

Administration of ginseng alone or following alloxan injection was associated with an elevation in ChE activity in the cerebral cortex and thalamus. Meanwhile, a reduction in the enzyme activity appeared in the midbrain, cerebellum, and pons of ginseng treated rats and to a lesser extent in the cerebellum and pons of rats treated with both alloxan and ginseng.

In the heart tissue, administration of ginseng was characterized by an elevation in ChE activity, while combined treatment with alloxan and ginseng extract induced nonsignificant changes. Serum and liver ChE was markedly reduced in the ginseng treated rats and tended to be normalized in rats co-administered alloxan and ginseng thus showing an ameliorative effect of ginseng. It can be suggested that ginseng may possess a significant anti-hyperglycemic effect and may prove to be beneficial in improving the management of diabetes. Besides, it may have selective positive effect on the cholinergic system.

INTRODUCTION

Experimental diabetes induced in animals by alloxan or streptozotocin administration is a commonly used model system with several correlations with insulin dependent diabetes mellitus (Rerup, 1970 and Yamamoto et al., 1981). Oxygen free radicals are thought to be a major cause of β-cells dysfunction in animals with diabetes induced by alloxan or streptozotocin diabetic animals (Wilson et al., 1984 and Takasu et al., 1991).

Diabetes is associated with changes in the central nervous system (CNS) structure and function (Garris et al., 1984; DiGiulio et al., 1988; Rawland & Bellush, 1989 and Mooradian, 1997). Diabetes has also profound effects on brain chemistry, particularly on brain neurotransmitters and associated enzymes (Wahba & Soliman, 1988 and King & Rohrbach,
In addition, there are various reports of diabetic abnormalities in several neuropeptides, including neuropeptide Y, substance P, metenkephalin, somatostatin, and vasopressin (Abbracchio et al., 1989; Fernstrom et al., 1990 and Abe et al., 1991) as well as in the acetylcholine system (Khegai, 1990). In rats with malathion-induced hyperglycemia, an increase in the level of cortical and striatal acetylcholine (ACH) was recorded (Matin &Hussain, 1984). In addition, Khegai (1990) reported that the sensitivity to acetylcholine increased in alloxan-induced hypoinsulinemia. In the striatum and hippocampus of streptozotocin diabetic rats, the level of acetylcholine tended to increase by 7 weeks of diabetes induction but did not differ significantly from controls (Welsh & Wecker, 1991). Moreover, Szutowicz et al. (1994) found that acetylcholine synthesis in nerve terminals from diabetic rats was 50% higher than in healthy animals.

Acetylcholinesterase (AChE, E.C., 3.1.1.7) has been used as a marker of cholinergic activity. It plays an important physiological function by terminating the effects of acetylcholine at the cholinergic synapses (Atack et al., 1986). Wahba & Soliman (1988) found that AChE activity was increased in different brain regions of streptozotocin-induced diabetic rats. Moreover the results of Lakhman & Kaur (1994) revealed that in alloxan diabetic rats the activity of AChE was increased in the amygdale, thalamus, hippocampus, pons and medulla. However Dash et al., (1991) observed that hyperglycemia due to experimental diabetes induction in rats, resulted in a gradual decrease in ChE activity of the cerebral hemispheres, cerebellum and brain stem, a significant decrease in enzyme activity was also noticed in the heart tissue. On the other hand, Kutty (1994) noticed a significantly elevated ChE activity in liver and serum of diabetic mice, and genetically obese mice, which mimic non-insulin dependent diabetes mellitus in humans.

The use of herbal medicine as an unconventional health treatment is gaining considerable recognition and popularity worldwide (Goldbeck-Wood et al., 1996). One of the most widely used herbs is ginseng. The herb is obtained from the root of several species of the genus Panax of the family Araliaceae indigenous both to Asian and North America. Two of the most common types are the Asian ginseng, Panax ginseng C.A. Meyer and the American ginseng, Panax quinquefolius (Gillis,1997).

Panax ginseng has been noticed to affect the CNS having antistress, learning and memory increasing and antifatigue activities (Liu & Xiao, 1992 and Wang & Lee, 1998). Other pharmacological properties include neuroendocrine function (Gillis, 1997), immunostimulation (Kim et al., 1999), liver and cardiovascular system protective activities (Lin et al., 1995 and Gillis, 1997). The major active principles of Panax ginseng are saponins, which are called ginsenosides (Kim et al., 1998 and Attele et al., 1999). Minor components include amino acids, peptides and minerals (Tang & Eisenbrand, 1992).

The biochemical basis of the different influences exerted by Panax ginseng on the CNS may be related to its effects on the different neurotransmitters and related substances (Su et al., 2007). In vitro studies, Benishin et al. (1991) examined the effects of the ginsenoside Rb1 on central cholinergic metabolism and found that Rb1 has no effect on AChE activity but facilitates the release of acetylcholine from hippocampal slices. The authors added that the increase in acetylcholine release was associated with an increased uptake of choline into the nerve endings while calcium influx was unaltered. Moreover, the results of Su et al. (2007) suggest that Panax ginseng has the ability to increase the release of acetylcholine from nerve terminals in rats so as to stimulate muscarinic M3 receptors activity.

Ginseng saponins have also been reported to have protective effects on liver. Zain et al. (1987) noticed that the administration of ginseng extract clearly recovered liver damage induced by certain drugs including alcohol. Liver damage induced by carbon tetrachloride, thioacetamide, galactosamine and menadione were reported to be recovered by the ginseng extract and the ginsenoside Ro (Kim & Kim, 1996 and Jang et al., 1997).

Among the many different beneficial actions of ginseng is its effects on hyperglycemia (Vuksan et al., 2000). Wang et al., (1990) found that ginseng polypeptide isolated from the root of Panax ginseng decreased the level of blood sugar in rats after intravenous injection (50-200 mg/kg), in mice after subcutaneous injection (50 & 100 mg/kg for 7 days) and in various experimental hyperglycemia induced by injection of adrenaline, glucose and alloxan.

Gong et al. (1991) studied the hypoglycemic effect of the ginsenoside Rg1 in diabetic mice compared with that of insulin. Their results showed that Rg1 lowered the plasma glucose level. Furthermore, Joo et al. (1992) reported that treatment of streptozotocin-diabetic rats with ginseng saponins, which also had a hypoglycemic action, stimulated insulin biosynthesis by the liver. Lee et al. (2007) found that repeated injection of ginsenoside Rh2 (1 mg/kg, 3 times daily) into streptozotocin-diabetic rats for 10 days made an increase of the responses to exogenous insulin. They concluded that ginsenoside Rh2 has an ability to improve insulin sensitivity.

The objectives of the present work were to elucidate the effect of alloxan-induced, short-term diabetes on ChE activity in different tissues of adult male rats and to highlight the empirical use of Panax...
*Panax ginseng* extract in alleviating the ailments associated with *diabetes mellitus*, besides delineating its effects on the ChE activity.

**MATERIALS AND METHODS**

**Handling of Animals:**

The experimental animals used in this study were adult male albino rats (*Rattus norvegicus*) weighing 160-200 g. They were housed under normal environmental conditions of temperature and humidity with a lighting regime of 12 hours of light and 12 hours of darkness. Food and water were provided *ad libitum*.

Animals were assigned into 3 groups:

- **Group I:** animals receiving a single subcutaneous (s.c.) injection of alloxan (120 mg/kg body weight).
- **Group II:** animals receiving *Panax ginseng* extract (100 mg/kg body weight) daily for 12 consecutive days.
- **Group III:** animals receiving both alloxan (single dose) and *Panax ginseng* extract (daily for 12 consecutive days).

**Alloxan Administration:**

Animals were made diabetic by alloxan monohydrate. On the day of the experiment, overnight-fasted animals were injected subcutaneously with a single dose of alloxan monohydrate (120 mg/kg body weight) prepared freshly in 0.154 M sodium acetate buffer, pH 4.5. The corresponding control animals received an equivalent volume of buffer. Glucosuria was evidenced by "Gluke-Tur" test stripes (Boehringer Mannheim). Groups of diabetic and control animals were decapitated after 2, 4, 8 and 12 days of alloxan or acetate buffer injection.

**Panax ginseng Extract Administration:**

*Panax ginseng* extract is a yellowish brown powder (Muggenburg, France). It was dissolved in a physiological saline solution (0.9% NaCl). Animals received a daily oral dose of 100 mg /kg body weight/day for 12 consecutive days. Control animals received equivalent volumes of saline. Groups of treated and control animals were decapitated after 2, 4, 8 and 12 days of alloxan or acetate buffer injection.

**Determination of blood glucose:**

Glucose determination was carried out using kits supplied by Stambio Laboratory Inc. (San Antonio, Texas) according to the procedure described by Trinder (1969).

**Determination of ChE activity:**

The procedure used for the determination of ChE activity in the brain samples, heart, liver as well as serum was a modification of the method of Ellman *et al.* (1961) as described by Gorun *et al.* (1978).

**Data Presentation and Statistical Analysis:**

The blood glucose level, the body weight and the ChE activity in the different brain regions, heart, liver and serum are expressed as mean ± S.E.M. Comparisons between control and treated animals and the levels of significance were determined using Student's t-test (Hill, 1971).

Percentage difference representing the percent of variation with respect to the control or alloxan-treated animals was also calculated.

\[
\% \text{ Difference} = \left( \frac{\text{Treated value} - \text{Control value (or alloxan-treated values)}}{\text{Control value (or alloxan-treated values)}} \right) \times 100
\]

**RESULTS**

**Effect of Alloxan administration:**

The blood glucose level recorded for control animals was 119.00 mg/dl and increased markedly to reach its maximum (423.00 mg/dl) after 4 days of alloxan administration. Though a slight decline from this maximum appeared after 8 and 12 days, the level of blood glucose remained threefold (407.00 mg/dl and 402.00 mg/dl, respectively) above the control value (Table 1). Body weight was lost progressively throughout the study in alloxan-treated rats (Table 2).
As could be seen from Table (3) the induction of diabetes provoked a general increase in ChE activity of the different brain regions examined. The increase was highly significant after 4 days in the cerebral cortex (+13.20%) and hypothalamus (+28.4%) and after 12 days in cerebral cortex (+30.85%) and significant after 2 days in the cerebral cortex (+27.66%) and after 4 days in the thalamus (32.90%) and after 8 days in the cerebral cortex (+37.23%) and midbrain (+25.38%).

Heart ChE activity exhibited an increase after 2 days followed by a gradual decline which became significant (-47.91%) after 12 days. In liver, ChE activity increased nonsignificantly after 8 and 12 days. Moreover, a highly significant increase in serum ChE activity was recorded after 4 and 12 days (+59.18% and +53.06%, respectively).

**Effect of *Panax ginseng* extract:**

From Table (1) it can be noticed that daily oral administration of *Panax ginseng* extract (100 mg/kg body weight) for 12 days raised slightly the blood glucose level. The increase was highly significant after 4 days then tended to reach the control value after 12 days of treatment.

Administration of *Panax ginseng* extract caused a gradual sustained increase in the body weight of animals after 4, 8 and 12 days of administration (+2.42%, +6.10% and +9.25%, respectively) (Table 2).

It can be noticed from Table (4) that the changes in ChE activity in response to ginseng administration were not the same in the different tissues studied. In the cerebral cortex and thalamus a general increase in the enzyme activity was noticed. This increase was highly significant after 2 days in the cerebral cortex (+24.91%) and after 8 days in the thalamus (+37.38%). In the hypothalamus ChE activity increased significantly after 4 and 8 days (+16.95% and 31.80%, respectively). Midbrain ChE activity was significantly decreased after 2 days (-31.60%) and increased after 12 days (+19.37%). Cerebellar ChE exhibited a significant decrease after 2 days (-42.31%) and a highly significant decrease after 8 days (-55.38%). In pons, the enzyme activity was significantly reduced after 8 and 12 days (-20.11% and -20.24%, respectively). On the other hand, a nonsignificant increase was recorded for the enzyme activity in medulla at all the time intervals investigated.

Ginseng administration caused an increase in heart ChE activity, this increase was significant after 4 days (+46.67%). However liver ChE activity exhibited a highly significant decrease after 2, 4 and 8 days (-61.13%, -70.97% and -66.93%, respectively). In serum, a significant decrease after 8 days (-27.08%) and a significant increase after 12 days (+29.17%) were seen.

**Effect of Co-administration of alloxan and *Panax ginseng* extract:**

In comparison to control animals, administration of ginseng extract to alloxan-treated rats provoked a highly significant increase in the blood glucose level after 4 days. However, the continuous ginseng administration brought the blood glucose level back to the normal range (Table 1). Moreover, it is also clear that, if compared with alloxan-treated rats, co-administration of *panax ginseng* extract with alloxan had a hypoglycemic effect and greatly reduced the increase in blood glucose level caused by alloxan. The decrease was highly significant after 2, 8 and 12 days (-44.64%, -43.73% and -62.19%, respectively).

From Table (2), rats treated with alloxan followed by ginseng administration showed a gradual decrease in their body weight at the beginning of the experiment. The decrease was maximum after 4 days (-7.06%), then increased to above the initial value after 12 days (+5.35%).

Table (5) show that the combined treatment with alloxan and *Panax ginseng* extract caused fluctuation in ChE activity in the different tissues examined. However, a significant or highly significant increase was observed after 2 days in the cerebral cortex (+32.22%) and thalamus (+45.93%), after 4 days in the thalamus (+65.15%) and liver (+89.26%) and after 8 days in the thalamus (+32.41%) and serum (+28.57%). On the other hand, a significant decrease in cerebellum after 12 days (-50.62%) were recorded.

**DISCUSSION**

In the present investigation, there is evidence that the alloxan-treated rats were fully diabetic. The results demonstrate a highly significant rise in blood glucose levels coupled with the failure of the experimental animals to gain weight and are thus strongly indicative of the successful induction of diabetes. The present results are consistent with those of many workers using alloxan or streptozotocin-treated rats as animal model for diabetes (Wahba & Soliman, 1988; Ucciali et al., 1993; Szutowicz et al., 1994; Khandkar et al., 1995; Ahren et al., 1996 and Bhardwaj & Kaur, 1999).

The diabetogenic agent alloxan induces damage to the insulin-producing β-cells due to its selective uptake in these cells (Malaise et al., 1982). The cause of the β-cells toxicity is thought to be due to inhibition of glucokinase activity (Lenzen & Panten, 1988) and/or to breaks of DNA strands with subsequent activation of the nuclear enzyme poly (ADP-ribose) synthetase, leading to depletion of NAD and cell death (Yamamoto et al., 1981).
Table (1): Effect of alloxan (120 mg/kg body weight, s.c.) and/or Panax ginseng extract (100 mg/kg body weight, for 12 consecutive days) on blood glucose level (mg/dl) of adult male albino rat after 2, 4, 8 and 12 days of treatment. The number of animals was seven in each experiment. Values given are Mean±S.E.M.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>2 Days</th>
<th>4 Days</th>
<th>8 Days</th>
<th>12 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/dl</td>
<td>% Difference</td>
<td>mg/dl</td>
<td>% Difference</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Alloxan</td>
<td>119±5.85</td>
<td>**229.40</td>
<td>423±48.8</td>
<td>**255.50</td>
<td>407±27.92</td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>120±5.56</td>
<td></td>
<td>**25.83</td>
<td>140±8.66</td>
<td>125±9.08</td>
</tr>
<tr>
<td>+ Alloxan + Panax ginseng</td>
<td>115±5.45</td>
<td># # **88.69 (#44.64)</td>
<td>**329±33.51</td>
<td>**186±10 (-22.22)</td>
<td>**229±47.4 (43.73)</td>
</tr>
</tbody>
</table>

*: Significant at p<0.05 versus control values. **: Highly Significant at p<0.01 versus control values. # #: Highly Significant at p<0.01 versus alloxan values. % Difference represents a comparison between control and treated values.

Table (2): Effect of alloxan (120 mg/kg body weight, s.c.) and/or Panax ginseng extract (100 mg/kg body weight, for 12 consecutive days) on initial and final body weights of adult male albino rats after 2, 4, 8 and 12 days of treatment. The number of animals was eight in each experiment. Values given are Mean±S.E.M.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial</th>
<th>Final</th>
<th>% Difference</th>
<th>Initial</th>
<th>Final</th>
<th>% Difference</th>
<th>Initial</th>
<th>Final</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alloxan</td>
<td>184±1.92</td>
<td>156±4.1</td>
<td>-7.89</td>
<td>167±2.24</td>
<td>154±4.05</td>
<td>-7.78</td>
<td>183±4.25</td>
<td>162±4.52</td>
<td>-10.00</td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>165±1.33</td>
<td>164±1.70</td>
<td>-0.60</td>
<td>165±2.22</td>
<td>169±0.35</td>
<td>+2.42</td>
<td>164±1.28</td>
<td>174±1.28</td>
<td>+6.10</td>
</tr>
<tr>
<td>Alloxan + Panax ginseng</td>
<td>181±3.74</td>
<td>169±8.10</td>
<td>-6.63</td>
<td>170±3.48</td>
<td>158±2.57</td>
<td>-7.05</td>
<td>179±4.27</td>
<td>168±8.21</td>
<td>-6.14</td>
</tr>
</tbody>
</table>

*: Significant at p<0.05
**: Highly Significant at p<0.01
% Difference represents a comparison between initial and final body weight values.
Table (3): Effect of s.c. injection of alloxan (120 mg / kg body weight) on ChE activity (µM SH/min/g fresh tissue or ml serum) in different tissues of adult male albino rat after 2, 4, 8 and 12 days of injection. The number of animals was seven in each experiment. Values given are Mean±S.E.M.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>2 Days</th>
<th>4 Days</th>
<th>8 Days</th>
<th>12 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity</td>
<td>Activity</td>
<td>% Difference</td>
<td>Activity</td>
<td>% Difference</td>
</tr>
<tr>
<td>1- Cerebral cortex</td>
<td>8.46±0.60</td>
<td>10.8±0.56</td>
<td>+27.66</td>
<td>11.1±0.61</td>
<td>+31.20</td>
</tr>
<tr>
<td>2- Thalamus</td>
<td>6.23±0.55</td>
<td>7.43±0.28</td>
<td>+19.26</td>
<td>8.28±0.51</td>
<td>+32.90</td>
</tr>
<tr>
<td>3- Hypothalamus</td>
<td>7.64±0.44</td>
<td>8.73±0.37</td>
<td>+14.27</td>
<td>9.81±0.52</td>
<td>+28.40</td>
</tr>
<tr>
<td>4- Midbrain</td>
<td>9.73±0.67</td>
<td>9.26±0.62</td>
<td>-4.83</td>
<td>11.7±0.99</td>
<td>+20.25</td>
</tr>
<tr>
<td>5- Cerebellum</td>
<td>2.51±0.34</td>
<td>2.54±0.33</td>
<td>+1.19</td>
<td>2.77±0.56</td>
<td>+10.36</td>
</tr>
<tr>
<td>6- Pons</td>
<td>7.26±0.53</td>
<td>6.23±0.75</td>
<td>-14.19</td>
<td>7.31±0.69</td>
<td>+0.69</td>
</tr>
<tr>
<td>7- Medulla oblongata</td>
<td>4.94±0.41</td>
<td>5.78±0.44</td>
<td>+17.00</td>
<td>5.63±0.53</td>
<td>+13.97</td>
</tr>
<tr>
<td>8- Heart</td>
<td>2.15±0.36</td>
<td>2.72±0.21</td>
<td>+26.51</td>
<td>2.18±0.31</td>
<td>+1.39</td>
</tr>
<tr>
<td>9- Liver</td>
<td>1.15±0.17</td>
<td>1.34±0.12</td>
<td>+16.52</td>
<td>2.16±0.32</td>
<td>+87.83</td>
</tr>
<tr>
<td>10- Serum</td>
<td>0.49±0.04</td>
<td>0.47±0.03</td>
<td>-4.08</td>
<td>0.78±0.05</td>
<td>+59.18</td>
</tr>
</tbody>
</table>

*: Significant at p<0.05 versus control values
**: Highly Significant at p<0.01 versus control values
% Difference represents a comparison between control and treated values

Table (4): Effect of oral administration of Panax ginseng extract (100 mg / kg body weight) for 12 consecutive days on ChE activity (µM SH/min/g fresh tissue or ml serum) in different tissues of adult male albino rat after 2, 4, 8 and 12 days of administration. The number of animals was seven in each experiment. Values given are Mean±S.E.M.

<table>
<thead>
<tr>
<th>Tissue</th>
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<th>2 Days</th>
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<td>Activity</td>
<td>Activity</td>
<td>% Difference</td>
<td>Activity</td>
<td>% Difference</td>
</tr>
<tr>
<td>1- Cerebral cortex</td>
<td>8.55±0.54</td>
<td>10.68±0.4</td>
<td>+24.91</td>
<td>8.37±0.41</td>
<td>-2.10</td>
</tr>
<tr>
<td>2- Thalamus</td>
<td>6.34±0.48</td>
<td>7.5±0.65</td>
<td>+18.30</td>
<td>7.04±0.57</td>
<td>+10.41</td>
</tr>
<tr>
<td>3- Hypothalamus</td>
<td>7.73±0.40</td>
<td>6.16±0.10</td>
<td>-20.31</td>
<td>9.04±0.38</td>
<td>+16.95</td>
</tr>
<tr>
<td>4- Midbrain</td>
<td>9.81±0.64</td>
<td>6.71±0.81</td>
<td>-31.60</td>
<td>8.28±0.34</td>
<td>-15.60</td>
</tr>
<tr>
<td>5- Cerebellum</td>
<td>2.60±0.30</td>
<td>1.59±0.24</td>
<td>-42.31</td>
<td>1.80±0.42</td>
<td>-30.77</td>
</tr>
<tr>
<td>6- Pons</td>
<td>7.36±0.46</td>
<td>8.08±0.79</td>
<td>+9.78</td>
<td>6.42±0.46</td>
<td>-12.77</td>
</tr>
<tr>
<td>7- Medulla oblongata</td>
<td>5.03±0.37</td>
<td>5.21±0.77</td>
<td>+5.58</td>
<td>5.24±0.38</td>
<td>+4.17</td>
</tr>
<tr>
<td>8- Heart</td>
<td>2.25±0.31</td>
<td>2.40±0.16</td>
<td>+6.67</td>
<td>3.30±0.19</td>
<td>+46.67</td>
</tr>
<tr>
<td>9- Liver</td>
<td>1.24±0.13</td>
<td>0.42±0.07</td>
<td>-66.13</td>
<td>0.36±0.08</td>
<td>-70.97</td>
</tr>
<tr>
<td>10- Serum</td>
<td>0.48±0.04</td>
<td>0.41±0.04</td>
<td>-14.58</td>
<td>0.41±0.03</td>
<td>-14.58</td>
</tr>
</tbody>
</table>

*: Significant at p<0.05 versus control values
**: Highly Significant at p<0.01 versus control values
% Difference represents a comparison between control and treated values
It is clear from the present investigation that induction of diabetes by alloxan injection resulted in a general increase of ChE activity in most of the brain regions examined. This increase was most prominent in the cerebral cortex, while the cerebellum andpons were the less affected regions. The results of Wahba & Soliman (1988) showed a significant increase of AChE activity in the cerebellum, bulbus olfactorius and medulla oblongata of streptozotocin diabetic rats. They reported that the increase in enzyme activity may reflect an increased synthesis of the enzyme, possibly due to changes in retrograde axonal transport, which normally delivers information to the nerve body concerning the state of the axon and its terminals under diabetic conditions (Niakan et al., 1986).

However, Dash et al. (1991) noticed that hyperglycemia was associated with an initial decrease in ChE activity followed by its recovery with the duration of diabetes in the cerebral hemispheres, cerebellum and brainstem of rats. They assumed that this could be partially due to the decreased glucose oxidation caused by hyperglycemia (Ruderman et al., 1974). Lakhman & Kaur (1994) found a marked and significant increase in AChE activity from discrete areas of the rat brain during the early development of diabetes. They attributed this increase to increased substrate level (i.e. of acetylcholine) during acute hyperglycemia. Meanwhile, Welsh & Wecker (1991) did not find any significant change in the enzyme activity of the rat striatum after 7 weeks of hyperglycemia.

In heart of diabetic rats, the present results showed that ChE activity was increased after 2 days of alloxan administration then decreased gradually to a significant value after 12 days. Dash et al. (1991) noticed decreased ChE activity in the heart of diabetic rats. This decrease was also gradual and reached its maximum on day 14 of diabetes. Earlier reports (Neubaur & Christensen, 1976 and Akiyama et al., 1989) confirmed significantly increased ACh concentration in the heart of diabetic rats. It was proposed that the alteration in the concentration of the cholinergic neurotransmitter is perhaps the mechanism by which the organism copes with the crisis of hyperglycemia (Dash et al., 1991).

The results of Vadlamudi & McNeill (1983) showed that during the initial phase of diabetes, there were no major alterations in the autonomic regulation of the heart. Meanwhile, it was found that metabolic abnormalities of cardiac parasympathetic nerves in acutely streptozotocin-induced diabetic rats include down-regulation of cholinergic receptors (Carrier & Aronstam, 1987) and an increase in the synthesis and decrease in metabolism of ACh (Ganguly et al., 1987) with no change in the rate and

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**Table (5):** Effect of co-administration of alloxan (120 mg/kg body weight, s.c.) and *Panax ginseng* extract (100 mg/kg body weight, for 12 consecutive days) on ChE activity (µM SH/min/g fresh tissue or ml serum) in different tissues of adult male albino rat after 2, 4, 8 and 12 days of treatment. The number of animals was seven in each experiment. Values given are Mean±S.E.M.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control Activity</th>
<th>2 Days Activity</th>
<th>% Difference</th>
<th>4 Days Activity</th>
<th>% Difference</th>
<th>8 Days Activity</th>
<th>% Difference</th>
<th>12 Days Activity</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Cerebral cortex</td>
<td>8.3±0.57</td>
<td>7.08±0.8</td>
<td>-13.24</td>
<td>9.91±0.84</td>
<td>+18.26</td>
<td>10.16±0.98</td>
<td>+21.24</td>
<td>0.78±0.63</td>
<td>+16.71</td>
</tr>
<tr>
<td>2- Thalamus</td>
<td>6.1±0.52</td>
<td>5.96±0.52</td>
<td>+5.93</td>
<td>10.14±0.7</td>
<td>+65.15</td>
<td>8.13±0.45</td>
<td>+32.41</td>
<td>5.58±0.61</td>
<td>-9.12</td>
</tr>
<tr>
<td>3- Hypothalamus</td>
<td>7.56±0.40</td>
<td>7.10±0.40</td>
<td>-6.08</td>
<td>8.16±0.89</td>
<td>+7.94</td>
<td>7.94±0.82</td>
<td>-6.88</td>
<td>8.23±0.59</td>
<td>+8.86</td>
</tr>
<tr>
<td>4- Midbrain</td>
<td>9.6±0.62</td>
<td>11.08±0.6</td>
<td>+14.94</td>
<td>9.66±0.56</td>
<td>+0.21</td>
<td>10.36±0.56</td>
<td>+7.47</td>
<td>8.03±0.83</td>
<td>-16.70</td>
</tr>
<tr>
<td>5- Cerebellum</td>
<td>2.4±0.28</td>
<td>2.80±0.42</td>
<td>+15.23</td>
<td>2.23±0.25</td>
<td>-8.23</td>
<td>2.24±0.33</td>
<td>-7.82</td>
<td>1.29±0.10</td>
<td>-50.62</td>
</tr>
<tr>
<td>6- Pons</td>
<td>7.20±0.59</td>
<td>6.41±0.52</td>
<td>-10.97</td>
<td>5.41±0.57</td>
<td>-24.86</td>
<td>7.13±0.47</td>
<td>-9.07</td>
<td>5.46±0.72</td>
<td>-24.17</td>
</tr>
<tr>
<td>7- Medulla oblongata</td>
<td>4.88±0.37</td>
<td>5.44±0.51</td>
<td>+11.47</td>
<td>4.81±0.83</td>
<td>-1.43</td>
<td>5.76±0.36</td>
<td>+18.03</td>
<td>4.74±0.64</td>
<td>-2.87</td>
</tr>
<tr>
<td>8- Heart</td>
<td>2.09±0.33</td>
<td>1.95±0.35</td>
<td>-6.70</td>
<td>2.26±0.20</td>
<td>+8.13</td>
<td>2.18±0.24</td>
<td>+4.31</td>
<td>1.63±0.23</td>
<td>-22.01</td>
</tr>
<tr>
<td>9- Liver</td>
<td>1.21±0.11</td>
<td>1.05±0.30</td>
<td>-13.22</td>
<td>2.29±0.27</td>
<td>+89.26</td>
<td>1.93±0.22</td>
<td>-14.88</td>
<td>1.08±0.08</td>
<td>-70.74</td>
</tr>
<tr>
<td>10- Serum</td>
<td>0.49±0.04</td>
<td>0.47±0.05</td>
<td>-4.08</td>
<td>0.53±0.04</td>
<td>+8.16</td>
<td>0.63±0.04</td>
<td>+28.57</td>
<td>0.47±0.04</td>
<td>-4.08</td>
</tr>
</tbody>
</table>

*: Significant at p<0.05 versus control values
**: Highly Significant at p<0.01 versus control values
% Difference represents a comparison between control and treated values
extent of the neuronal choline uptake (Carrier & Aronstam, 1987).

In light of the present data, liver ChE activity exhibited a significant increase after 4 days then decreased nonsignificantly whereas serum ChE was highly significantly elevated after 4 and 12 days of diabetes induction. It has been reported that ChE activity in the plasma of alloxan-diabetic rats increased significantly with a concomitant increase in the activity in liver (Oreskovic & Kunec-Vajic, 1992). Elevated serum ChE activity was also recorded in diabetic patients (Ragoo-Birsingh et al., 1992) and in streptozotocin-induced diabetic rats (Kutty, 1994). Increased serum ChE activity may be attributed to increased synthesis by the liver and/or increased secretion into the blood.

Another aspect of the present study was to examine the effects of oral administration of crude Panax ginseng extract and its co-administration with alloxan on the blood glucose level, body weight as well as the activity of the ChE in the selected tissues of rats.

Ginsenosides, the bioactive ingredients of the ginseng root, have been found to exert many beneficial effects (Salim et al., 1997). Still an intriguing property of ginseng is its hypoglycemic effect. According to the present data continuous daily oral administration of Panax ginseng extract to normal rats provoked a slight rise in blood glucose level though keeping it within the normal range. However, following co-administration of alloxan and Panax ginseng extract blood glucose levels were significantly elevated in comparison to control levels but exhibited a gradual decrease towards the control value if compared with diabetic levels. The ginseng hypoglycemic effect is supported by several studies in humans and animals (Oshima et al., 1987; Wang et al., 1990; Gong et al., 1991; Joo et al., 1992; Liu & Xiao, 1992; Sotaniemi et al., 1995; Ohnishi et al., 1996 and Vuksan et al., 2000 & 2001). Xie et al. (2004) suggested that ginseng extract possesses a significant anti-hyperglycemic activity and may prove to be beneficial in improving the management of diabetes.

Vuksan et al. (2000) reported that the mechanism by which ginseng lowers the blood glucose concentration may be due to: 1- Slow digestion of food and a decreasing rate of carbohydrate absorption into portal hepatic circulation following treatment with ginseng. 2- An effect on glucose transport. This effect may be mediated by nitric oxide. Enhanced nitric oxide synthesis by ginseng in endothelium of lung, heart and kidney has been noticed (Gillis, 1997). 3- Modulation of insulin secretion, some ginseng fractions have been noticed to increase the blood insulin level and glucose-stimulated insulin secretion in alloxan diabetic mice (Kimura et al., 1981). This effect may also be mediated by nitric oxide. It was shown that nitric oxide stimulates glucose-dependent secretion of insulin in rat islet cells (Spinias et al., 1998). However, Tchilian et al. (1991) studied the effect of ginsenoside Rg1 on insulin binding in liver and brain membranes of mice. They recorded significantly increased insulin binding in both tissues and concluded that this increase can be ascribed to an increase in the total number of insulin binding sites rather than to a change in the receptor affinity.

Moreover, Broadhurst et al. (2000) reported that, because the major biologically active phytochemicals in Panax ginseng are the steroidal saponins ginsenosides, which are known to be mild adrenal cortex stimulants (Tang & Eisenbrand, 1992 and Huang, 1993), this may be the primary action of ginseng with respect to decreasing plasma glucose, rather than a direct stimulation of cellular glucose metabolism. It has also been suggested that ginseng may increase adrenal steroidogenesis via the pituitary gland (Ng et al., 1987).

The present results revealed also that co-administration of alloxan and Panax ginseng extract ameliorated the decrease in body weight caused by diabetes and on day 12 of treatment an increase was recorded. Yokozawa et al. (1987) noticed that the ginsenoside Rb1 isolated from the roots of Panax ginseng increased the body weight of diabetic rats and markedly improved symptoms such as polyuria and over-eating.

Data from the present study concerning the effect of ginseng on ChE activity showed regional variations. The enzyme activity was mainly elevated in the cerebral cortex and thalamus following administration of ginseng or the combined treatment with alloxan and ginseng and in the hypothalamus of ginseng-treated rats. Meanwhile, a reduction in ChE activity appeared in midbrain, cerebellum and pons of ginseng-treated rats and to a lesser extent in the cerebellum and pons of rats treated with both alloxan and ginseng. In comparison with alloxan-treated rats, co-administration of alloxan and ginseng induced variable mostly nonsignificant changes in the ChE activity. Ginsenosides (like all classes of steroids) may exert their effects at the membrane level. They may modify membrane protein structure by changing membrane dynamics and modulating activity of ion channels, membrane-bound receptors and enzymes (Attele et al., 1999).

Several authors found that ginsenosides affect the parameters of the cholinergic system. Benishin et al. (1991) showed that in vitro studies the ginsenoside Rb1 has no effect on AChE activity but facilitates the release of acetylcholine from hippocampal slices. The increase in acetylcholine release is associated with an increased uptake of choline into nerve endings. The results of Su et al. (2007) suggest that Panax ginseng
has the ability to increase the release of ACh from nerve terminals in rat so as to stimulate muscarinic M(3) receptors activity.

The present data also showed that, in the heart, administration of Panax ginseng extract to intact rats was characterized by an elevation in ChE activity. Meanwhile, combined treatment with alloxan and Panax ginseng extract induced slight nonsignificant changes in the heart ChE activity if compared either to control or diabetic rats. These slight changes may confirm the organ-protective actions of ginseng. Several studies have suggested that antioxidant and organ-protective actions of ginseng are linked to enhanced nitric oxide synthesis in endothelium of heart (Kim et al., 1992; Zhan et al., 1994 and Maffei-Facino et al., 1996).

The results of the present study indicated that, in liver and serum, following oral administration of ginseng extract or the co-administration of alloxan and ginseng the ChE activity was markedly reduced in the ginseng-treated rats and tended to be normalized in rats co-administered alloxan and Panax ginseng extract thus showing an ameliorative effect on liver damage in rats which have been treated with carbon tetrachloride for 8 weeks.

Jeong et al. (1997) showed that oral administration of red ginseng saponins for 7 consecutive days partially recovered the hepatotoxicity induced by carbon tetrachloride in male rats. They suggested that one possible mechanism of the hepatoprotective effects of ginseng saponins against carbon tetrachloride-induced hepatotoxicity would be an anti-oxidative property of certain ginsenosides in the saponins.

It can be concluded that ginseng may possess a significant anti-hyperglycemic effect and may prove to be beneficial in improving the management of diabetes. Besides, it may have selective positive effect on the cholinergic system.

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