INTRODUCTION

Glioma is a highly invasive primary brain tumor that arises from glial cells. Malignant gliomas are invariably fatal and all efforts to develop new diagnostic and therapeutic modalities have failed to establish a curative regime. Gliomas almost spread locally along the neuronal fibers in the brain and rarely form metastatic lesions outside the central nervous system. Glioma patients are often characterized by broad depression of both humoral and cell-mediated immunities. Despite recent advances in diagnosis and treatment, the incidence and mortality from gliomas are increasing in many developing countries.

There is growing evidence that oxidative stress caused by free radical production is involved in brain injury. It has been suggested that free radicals are likely to be responsible for the neuronal changes mediating behavioural deficits in neurodegenerative disorders. Lipid peroxidation (LPO) is one of the major outcomes of free radical-mediated tissue injury and is an indicator of oxidative damage. Oxygen-derived free radicals produced by lipid peroxidation are believed to be implicated in cancer development. Antioxidants can act as reducing agents to prevent oxidative reactions by either scavenging reactive oxygen species (ROS) or inhibiting cellular signaling enzymes such as protein kinase C (PKC).

Sesamol (3,4-methylenedioxyphenol) is a major constituent of Sesamum indicum seeds, which makes the seed oil more resistant to oxidative deterioration than other vegetable oils. Sesamum indicum is a flowering plant of the genus Sesamum of the Pedaliaceae family. The precise natural origin of the species is unknown, although numerous wild relatives occur in Africa and a smaller number in India. It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds. Sesamol is a powerful antioxidant and inhibits UV- and Fe^{3+}/ascorbate-induced lipid peroxidation in rat brain. It has been shown to inhibit several steps in the generation of neoplasia and mutagenesis.
In this study we examined the anticancer activity of sesamol against gliomas induced in rats. Our results showed that sesamol effectively reduced the levels of free radicals with concomitant increases in the enzymatic and non-enzymatic antioxidants in the brain tissue of glioma-induced rats. It also restored the normal activities of lactate dehydrogenase (LDH) and creatine kinase (CK) in the brain tissue of these rats.

MATERIALS AND METHODS

Drugs and chemicals

Glutathione (oxidized and reduced), nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), chloro-2,4-dinitrobenzene (CDNB), 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sisco Research Laboratory, India. Sesamol was purchased from Sigma Aldrich. C6 cell line was purchased from NCCS, Pune, India. Other chemicals of analytical reagent grade were purchased from Qualigens, India.

Animals

Male Wistar rats weighing 250-300g were used for the study. Rats were obtained from the King Institute, Chennai, India. They were housed in polypropylene cages in an air conditioned room and allowed access to pellet diet and water ad libitum. Animals acclimatized under standard laboratory conditions for seven days were used for the experiments.

Experimental protocol

The animals were divided into four groups (n=6 per group):

Group I (control): Rats received only distilled water orally for 30 days.

Group II (glioma): Glioma was induced by injecting C6 cells and this group of animals received distilled water orally for 30 days.

Group III (glioma + sesamol): Glioma-induced rats were pretreated with sesamol (20 mg/kg body weight) dissolved in distilled water for 30 days.

Group IV (sesamol only): This group received sesamol (20 mg/kg body weight) dissolved in distilled water for 30 days.

Induction of glioma

The rats were anesthetized with ketamine (90 mg/kg body weight) and xylazine (10 mg/kg body weight). The animals were placed in a stereotactic frame, the scalp was opened and 4×10⁷ C6 cells mixed in 5 μl MEM were inoculated into the left striatum with a Hamilton syringe for 4 min (coordinates with respect to bregma: 1 mm anterior, 3 mm left lateral, 5 mm depth). Bone wax was used to seal the burr-hole and prevent spreading of neoplastic cells outside the needle track. The scalp incision was stitched with suture thread. Provo iodine and nephasulp were applied to the operated spot followed by an injection of gentamycin (5 mg/kg body weight, i.p.).

Tissue preparation

Animals were sacrificed by cervical dislocation and their brains were taken out quickly, washed with saline and weighed with an electronic balance. The brain was transferred to phosphate buffered saline (pH 7.4) and stored at -80 °C. Part of the brain was transferred to 4% formalin for 48 h.

About 500 mg of tissue was homogenized in 5 ml PBS (pH 7.4). The homogenate was centrifuged and the supernatant was used for biochemical measurements.

The brain tissue fixed in 4% formalin was embedded in paraffin wax. Serial sections (4 μm thick) were obtained and mounted on glass slides. For morphometric analysis, hematoxylin–eosin (H&E) staining was performed according to the standard protocol.

Biochemical estimations

Activities of aspartate transaminase (AST) and alanine transaminase (ALT) were assayed using the method of Reitman and Frankel. The LDH activity was assayed by the method of King. Creatine kinase activity was assayed according to Okinaka et al. Tissue thiobarbituric acid reactive substances (TBARS) were estimated as described by Yagi. The tissue level of vitamin C was estimated by the method of Roe and Kuether. The vitamin E level was determined as described by Barker and Frank. The total reduced glutathione content of brain tissue homogenate was determined by the method of Moron et al. Superoxide dismutase activity was determined by the method of Marklund and Marklund, catalase by the method of Sinha, glutathione peroxidase by the method of Rotruck et al., and glutathione-S-transferase activity by the method of Habig and Jackoy. The activity of Na⁺-K⁺-ATPase in brain tissue was determined as inorganic phosphate (Pi) released from ATP, using the method of Svoboda and Mossiger.

STATISTICAL ANALYSIS

All statistical computations were performed using SPSS software. Values of experimental groups are
given as mean ± SD. One-way ANOVA with LSD post-test analysis was used to determine statistical significance, with P<0.05 as the criterion.

RESULTS

Histopathology

Figure 1a shows H&E-stained sections of the brain tissue of a control rat with normal architecture of the astrocytes. Microscopically, Fig. 1b shows a brain tissue section of a glioma-induced rat with the features of glioma: large polygonal cells of fibrillary background with round to oval acidophilic nuclei and partially scattered cytoplasm. The tumor was quite well differentiated, richly cellular, and showed typical nuclear changes with increased anaplasia. Figure 1c shows the brain tissue section of a sesamol-pretreated glioma-induced rat. There is a marked reduction in the number of neoplastic cells. Figure 1d shows a brain tissue section of a sesamol-only treated rat with normal morphology of glial cells.

AST, ALT, LDH and CK

Table 1 shows the activities of AST, ALT, LDH and CK in the brain tissues of control and experimental rats. Glioma-induced rats (Group II) showed significantly greater levels of all of these enzyme activities than control rats (Group I). Oral pretreatment with sesamol in glioma-induced rats (Group III) significantly decreased these activities in comparison with glioma-induced rats that had not received sesamol.

\[
\begin{array}{|c|c|c|c|}
\hline
\text{Groups} & \text{AST (IU/mg protein)} & \text{ALT (IU/mg protein)} & \text{LDH (U/mg protein)} & \text{CK (µg phosphorous liberated/mg protein)} \\
\hline
\text{I Control} & 4.53±0.13 & 1.76±0.18 & 5.74±0.20 & 6.51±0.09 \\
\text{II Glioma} & 6.56±0.27^a & 3.61±0.11^a & 8.52±0.52^a & 15.33±0.81^a \\
\text{III Glioma+sesamol} & 4.45±0.23^b & 2.36±0.18^b & 7.44±0.18^b & 9.52±0.09^b \\
\text{IV Sesamol only} & 4.40±0.17 & 1.62±0.13 & 5.70±0.19 & 6.55±0.26 \\
\hline
\end{array}
\]

Values are expressed as mean ± SD (n=6 in each group). Significance was determined as:

\(^a\)P < 0.001 – Group I Vs Group II

\(^b\)P < 0.001 – Group II Vs Group III
Table 2. Effect of sesamol on the levels of TBARS, Ascorbic acid, Tocopherol, and reduced glutathione in the brain tissue of normal and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (µmol MDA/g tissue)</th>
<th>Ascorbic acid (mg/g tissue)</th>
<th>Tocopherol (mg/g tissue)</th>
<th>GSH (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>2.81 ± 0.09</td>
<td>0.52±0.02</td>
<td>0.25±0.08</td>
<td>2.50±0.12</td>
</tr>
<tr>
<td>II Glioma</td>
<td>4.66±0.11</td>
<td>0.25±0.05</td>
<td>0.06±0.01</td>
<td>0.53±0.08</td>
</tr>
<tr>
<td>III Glioma+sesamol</td>
<td>3.52±0.21</td>
<td>0.45±0.01</td>
<td>0.15±0.02</td>
<td>1.61±0.27</td>
</tr>
<tr>
<td>IV Sesamol only</td>
<td>2.65±0.18</td>
<td>0.55±0.02</td>
<td>0.26±0.01</td>
<td>2.61±0.26</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n=6 in each group). Significance was determined as:
*P < 0.001 – Group I Vs Group II
*P < 0.001 – Group II Vs Group III
*P < 0.003 - Group II Vs Group III.

Table 3. Effect of sesamol on the activities of SOD, catalase, GPX, GST and Na⁺-K⁺ ATPase in the brain tissues of normal and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/ mg protein)</th>
<th>Catalase (µmol H₂O₂/min/mg protein)</th>
<th>GPX (µmol glutathione oxidized/mg protein)</th>
<th>GST (µmol CDNB/mg protein)</th>
<th>Na⁺-K⁺ ATPase (µg phosphorous liberated/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>7.61 ± 0.17</td>
<td>5.74 ± 0.28</td>
<td>4.41 ± 0.07</td>
<td>43.03 ± 0.48</td>
<td>4.52 ± 0.17</td>
</tr>
<tr>
<td>II Glioma</td>
<td>5.38 ± 0.25</td>
<td>3.60 ± 0.24</td>
<td>1.76 ± 0.07</td>
<td>47.04 ± 1.40</td>
<td>2.46 ± 0.19</td>
</tr>
<tr>
<td>III Glioma+sesamol</td>
<td>6.64 ± 0.16</td>
<td>4.52 ± 0.19</td>
<td>3.46 ± 0.18</td>
<td>45.03 ± 0.65</td>
<td>3.55 ± 0.24</td>
</tr>
<tr>
<td>IV Sesamol only</td>
<td>7.57 ± 0.21</td>
<td>5.51 ± 0.24</td>
<td>4.51 ± 0.06</td>
<td>43.21 ± 0.71</td>
<td>4.47 ± 0.24</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n=6 in each group). Significance was determined as:
*P < 0.001 – Group I Vs Group II
*P < 0.001 – Group II Vs Group III
*P < 0.003 - Group II Vs Group III.

The sesamol-only group (Group IV) showed no significant differences from the control rats (Group I) in the activities of these enzymes.

**TBARS, ascorbic acid, tocopherol and GSH**

Table 2 shows the levels of TBARS, ascorbic acid, tocopherol and reduced glutathione (GSH) in the brain tissues of normal and experimental rats. The TBARS levels were significantly higher and the levels of ascorbic acid, tocopherol and GSH were significantly lower in glioma-induced rats (Group II) than control rats (Group I). Oral treatment with sesamol (Group III) significantly decreased the TBARS levels when compared with glioma-induced rats without sesamol (Group II). Sesamol pretreatment also caused a significant restoration in ascorbic acid, tocopherol and GSH levels in the glioma + sesamol group (Group III) compared to Group II. Sesamol treatment alone (Group IV) did not significantly alter the status of TBARS, ascorbic acid, tocopherol and GSH found in control rats (Group I).

**Antioxidant enzymes and Na⁺-K⁺ ATPase**

The activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), GST and Na⁺-K⁺ ATPase in the brain tissues of normal and experimental rats are given in Table 3. The activities of SOD, catalase, GPX and Na⁺-K⁺ ATPase were significantly lower and the GST activity significantly higher in the glioma group (Group II) than in controls (Group I). Sesamol pretreatment (Group III) significantly restored the activities of antioxidant enzymes and Na⁺-K⁺ ATPase compared with Group II. There were no significant differences in these activities between the sesamol-only group (Group IV) and controls (Group I).

**DISCUSSION**

One of the universally accepted etiologies of brain tumors is imbalance between factors promoting free radical formation and the maintenance of neuronal integrity through endogenous defense mechanisms. In the present study, the major cellular defenses (both enzymatic and non-enzymatic) were markedly different in the glioma-induced rats and the control group because of the increased formation of ROS and decreased levels of radical scavengers. However, this imbalance was prevented by sesamol supplementation. A substantial elevation in LPO level along with the depletion of various protective antioxidant enzyme activities in glioma-induced rats was reported by Umadevi-Subramanian et al. However, pretreatment with sesamol resulted in decreased LPO levels and up-regulation of the depleted antioxidant enzymes in comparison with the glioma group, suggesting decreased formation of ROS.

Transaminases are markers employed in the diagnosis of cancer. They are important enzymes in the brain and are implicated in the maintenance of amino acid homeostasis, and they might be indicators of mitochondrial injury. The AST and ALT activities were higher in the brain tissue of glioma-induced rats than in controls. We previously reported elevated activities of serum transaminases in cervical cancer patients. Treatment with sesamol significantly decreased the activities of AST and ALT, suggesting that sesamol offers protection against glioma.
The marker enzymes are major indicators of tissue injury. Elevated enzyme release from damaged tissues has become a definitive diagnostic and prognostic criterion for various diseases and disorders. LDH is a fairly sensitive marker for solid neoplasms and it is elevated in sera from cancer patients. In the present study, the glioma-induced rats showed higher tissue LDH and CK activities than the control group. LDH might be elevated because of the higher rate of glycolysis, which is the major energy-producing pathway in malignant cells proliferating without control. Our results are consistent with the increased activities of LDH and CK in the brain tissue of glioma-induced rats reported by Umadevi Subramanian et al. These activities were significantly decreased in sesamol-pretreated rats.

ROS generated in tissues is efficiently scavenged by enzymatic and non-enzymatic antioxidants. The decrease in antioxidant enzyme activities is closely related to the induction of lipid peroxidation. Antioxidants play a major role in protecting biological systems from ROS-derived species and reflect the antioxidant capacity of the system. In this study, brain tissue levels of ascorbic acid and tocopherol were significantly decreased in glioma-induced rats. Ascorbic acid has been shown to scavenge superoxide, hydrogen peroxide, hypochloride, hydroxyl radicals and peroxyl radicals efficiently and to restore the antioxidant properties of fat-soluble α-tocopherol; therefore it interrupts the radical chain reaction of lipid peroxidation. The reduction in tissue ascorbic acid and tocopherol concentrations might be due to enhanced lipid peroxidation. Previous reports have demonstrated significantly reduced serum tocopherol levels in brain tumor patients. Reduced levels of ascorbic acid and tocopherol have been reported in cervical cancer patients. Sesamol pretreatment effectively restored the tissue levels of ascorbic acid and tocopherol compared with the glioma-induced rats.

GSH is a major endogenous antioxidant that counters free radical-mediated injury. We observed decreased GSH and GPX levels in the brain tissue of glioma-induced rats. GPX is a major component in the disposal of peroxides. The decrease in GSH levels might be due to increased utilization in protecting SH-containing proteins from lipid peroxides. Decreased SOD and GPX activities were reported by Aggarwal et al. Our findings are in agreement with the results of other authors. In our study, pretreatment with sesamol significantly increased the tissue levels of GSH and GPX.

SOD is important in protecting the cells from oxidative damage by converting superoxide radicals into hydrogen peroxide, which is further metabolized by catalase to molecular oxygen and water. In the present study, SOD and catalase activities were lower in the brain tissue of glioma-induced rats than controls, and this decrease was antagonized by sesamol treatment. SOD is the first enzyme of the scavenger enzyme series that prevents injury to cells by free radicals, while catalase is one of the several cellular antioxidant enzymes that provide a defense system for scavenging ROS.

Glutathione-S-transferase (GST) is important for detoxifying exogenous and endogenous substances and protecting cells from the toxic effects of ROS. In our study, GST activity was significantly greater in glioma-induced rats than control rats. A number of polymorphic GST enzymes are expressed in the human brain, including GSTP1 and GSTM3. GSTP1 protein is the most highly expressed GST enzyme in the brain, and it is over-expressed in several precancerous and malignant tumors, including malignant glioma. Over-expression of GST has been reported in malignant astrocytoma cell lines. Sesamol pretreatment significantly restored GST activity in the brain tissue of glioma-induced rats.

CONCLUSION

This study shows that sesamol administration to control rats had no effect on the activities of endogenous antioxidant enzymes or oxidative stress markers in brain tissue. However, sesamol pretreatment restored the levels of antioxidants and free radicals in the brain tissue of glioma-induced rats. From the above findings, it is evident that sesamol exhibits anticancer potential and neuroprotection in glioma-induced rats.

REFERENCES


