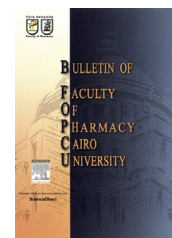HOSTED BY

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ORIGINAL ARTICLE

Alleviation of haloperidol induced oxidative stress in rats: Effects of sucrose vs grape seed extract

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Abstract Haloperidol (HP) is a classic antipsychotic drug known for its propensity to cause extrapyramidal side effects. HP is known to induce oxidative stress due to increased turnover of dopamine. The aim of the present study was to investigate the effect of sucrose (1 and 5 mg/kg; p.o.) and grape seed extract (GSE; 100, 200 and 400 mg/kg; p.o.) on the oxidative stress induced in rats by HP (1 mg/kg; p.o.) in the liver and the brain tissues. Oxidative stress was induced by injection of HP for 14 consecutive days which was concurrently administered with sucrose and GSE. Liver and brain levels of malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (nitrite) levels were determined in the brain and liver. Results of the present study revealed that HP-treated rats showed elevated levels of NO in the brain and MDA in the brain and liver. HP-treated rats showed also decreased levels of NO levels in the liver and GSH in the brain and liver. Treatment of HP-treated rats with GSE reversed all the oxidative stress markers in both the brain and liver due to its potent antioxidant property. On the other hand, sucrose attenuates the levels of NO in the brain and liver and the brain levels of MDA and GSH. It can be concluded that both GSE (a potent anti-oxidant) and sucrose (as a source of energy) have beneficial impacts on the brains of HP-treated rats. However, GSE is more potent in alleviating the oxidative stress associated with HP in the liver.

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1. Introduction

Haloperidol (HP), a butyrophenone, has been used as an antipsychotic drug in human. Unfortunately, the therapeutic effects of HP are accompanied by severe extrapyramidal side effects, resulting in movement disorders in patients. HP has been used clinically in the treatment of psychiatry, obstetrics, and anesthesiology, and its pharmacology has been widely reported.[1,2]

There has been increasing indication, implicating oxidative stress as a causative factor in neuropsychotic disorders including schizophrenia.[3,4] Free radicals have been implicated in the pathogenesis and clinical course of neuropsychiatric disorders such as schizophrenia and in the development of tardive dyskinesia. Increased superoxide dismutase (SOD) activity has been reported in the red blood cells of schizophrenic patients by some groups.[5–7] Abnormal activity of catalase (CAT) has also been reported.[8] Some studies have shown decreased CAT and increased SOD in schizophrenic patients.[6] Increased blood levels of malondialdehyde MDA have been

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found in schizophrenic patients relative to normal controls.[9] The brain is deficient in oxidative defense mechanisms and hence is at a greater risk of damage mediated by reactive oxygen species (ROS), resulting in molecular and cellular dysfunctions.[10] ROS can damage virtually any biological molecule in its vicinity, including DNA, essential proteins and membrane lipids.[11] There have been reports of membrane pathologies and alterations in membrane phospholipids, essential fatty acids and signal transduction,[12] which are believed to be ROS mediated.

Chronic treatment of HP is known to induce oxidative stress due to increased turnover of dopamine.[13] HP is cytotoxic to primary hippocampal neurons.[14] It was demonstrated that it causes necrotic death rather than apoptotic. Some studies reported the cytotoxic nature of HP but have not specified the type of cell death.[15] Others in their investigations, have demonstrated that amyloid beta resistant cells were opposed to HP toxicity, implying the role of free radicals in HP-induced cell death.[16] Typical neuroleptics such as HP and chlorpromazine are known to cause oxidative stress,[16] which is thought to be responsible for its extrapyramidal side effects.[16,17] It has also been shown that increasing doses of HP in rats[17] attenuate the extrapyramidal side effects caused by the same drug. Previous research has also shown that coadministration of haloperidol and antioxidants such as vitamin E resulted in a beneficial effect in patients with tardive dyskinesia.[18]

The aim of the present study was to investigate the effect of sucrose (as a source of energy) and grape seed extract (as a potent antioxidant) on the oxidative stress induced in rats by HP in both the liver and the brain. Oxidative stress was induced by injection of HP for 14 consecutive days which was concurrently administered with sucrose and GSE. Liver and brain levels of some relevant biomarkers for oxidative stress and nitric oxide (NO) were determined. Lipid peroxide levels (measured as malondialdehyde; MDA) were taken as *in vivo* reliable indices for the contribution of free radical generation in oxidative stress. Nitrate/nitrite levels were used as a convenient marker for NO formation.

2. Materials and methods

2.1. Animals

Rats with 120–150 g of body weight were used. Rats were obtained from animal house colony of the National Research Centre (Cairo, Egypt). Rats were housed under standardized conditions with free access to standard laboratory food and water. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Equal groups of 6 rats each were used in all experiments.

2.2. Drugs and chemicals

Grape seed extract (Mepaco, Egypt), haloperidol (ChemiPharm, Egypt), sucrose were used in the present investigation. They were freshly prepared in distilled water and given orally. Thiobarbituric acid (TBA) was purchased from Merck (Germany). All other chemicals were of the highest commercially available grade.

2.3. Experimental design

Rats were randomly allocated into seven groups (8–10 rats each) and the following groups were studied: Group I: containing rats receiving saline. Group II: containing rats receiving HP (1 mg/kg). Group III: containing rats receiving HP (1 mg/kg) and sucrose (1 mg/kg; p.o.). Group IV: containing rats receiving HP (1 mg/kg) and sucrose (5 mg/kg; p.o.). Group V: containing rats receiving HP (1 mg/kg) and GSE (100 mg/kg; p.o.). Group VI: containing rats receiving HP 1 mg/kg and GSE (200 mg/kg; p.o.). Group VII: containing rats receiving HP (1 mg/kg) and GSE (400 mg/kg; p.o.).

Twenty-four hours after the last dose, the rats were sacrificed by cervical dislocation, the liver and brain were isolated and homogenized in phosphate buffer.

2.3.1. Biochemical analysis

Rats were euthanized by decapitation under ether anesthesia, brains and livers were excised, washed with ice-cold saline solution (0.9% NaCl), weighed and stored at −80 °C for biochemical analyses. The liver was homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10% w/v for biochemical assays.

2.3.2. Determination of nitric oxide

Nitric oxide measured as nitrite was determined by using Griess reagent, according to the method of Moshage et al. (1995) where nitrite, stable end product of NO radical, is mostly used as an indicator for the production of NO.[19]

2.3.3. Determination of lipid peroxidation

Lipid peroxidation was assayed by measuring levels of malondialdehyde (MDA) in the brain and liver tissues. Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea et al. (1994), in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532 nm.[20]

2.3.4. Determination of reduced glutathione

Reduced glutathione (GSH) was determined by Ellman's method (1959). The procedure is based on the reduction of Ellman's reagent by –SH groups of GSH to form 2-nitro-s-mercaptobenzoic acid, the nitromercaptobenzoic acid anion has an in-tense yellow color which can be determined spectrophotometrically.[21]

2.4. Statistical analysis

Drug effects on HP-induced biochemical changes were expressed as the mean ± SEM. Data were analyzed with a one-way ANOVA followed by post hoc comparisons using Tukey's multiple comparisons test.

3. Results

3.1. Biochemical parameters measured in the brain

3.1.1. Effect of sucrose and GSE on nitric oxide

The oxidative stress induced in rats by HP is associated with an increase in the brain NO levels reaching about 138% of the normal value. Treatment of HP-treated rats with sucrose (1 and 5 mg/kg/day; p.o.) for 14 days showed a decrease in the elevated brain NO levels reaching nearly the normal value. Similar treatment of HP-treated rats with GSE (100, 200 and 400 mg/kg/day; p.o.) showed a decrease in the brain NO levels by about 17%, 25% and 36%, respectively (Fig. 1a).

3.1.2. Effect of sucrose and GSE on lipid peroxidation

The oxidative stress induced in rats by HP is associated with an increase in the brain lipid peroxidation levels (measured as MDA) reaching about 137% of the normal value. Treatment of HP-treated rats with sucrose (5 mg/kg/day; p.o.) for 14 days showed a decrease in elevated brain MDA levels by about 32%. Similar treatment of HP-treated rats with GSE (100, 200 and 400 mg/kg/day; p.o.) showed a decrease in brain MDA levels reaching nearly the normal value (Fig. 2a).

3.1.3. Effect of sucrose and GSE on reduced glutathione

The oxidative stress induced in rats by HP is associated with a decrease in brain GSH levels reaching about 57% of the normal value. Treatment of HP-treated rats with sucrose (1 and 5 mg/kg/day; p.o.) for 14 days showed an increase in reduced brain GSH levels by about 17% and 21%, respectively. Similar treatment of HP-treated rats with GSE (100, 200 and 400 mg/kg/day; p.o.) showed an increase in brain GSH levels by about 34%, 63% and 80%, respectively (Fig. 3a).
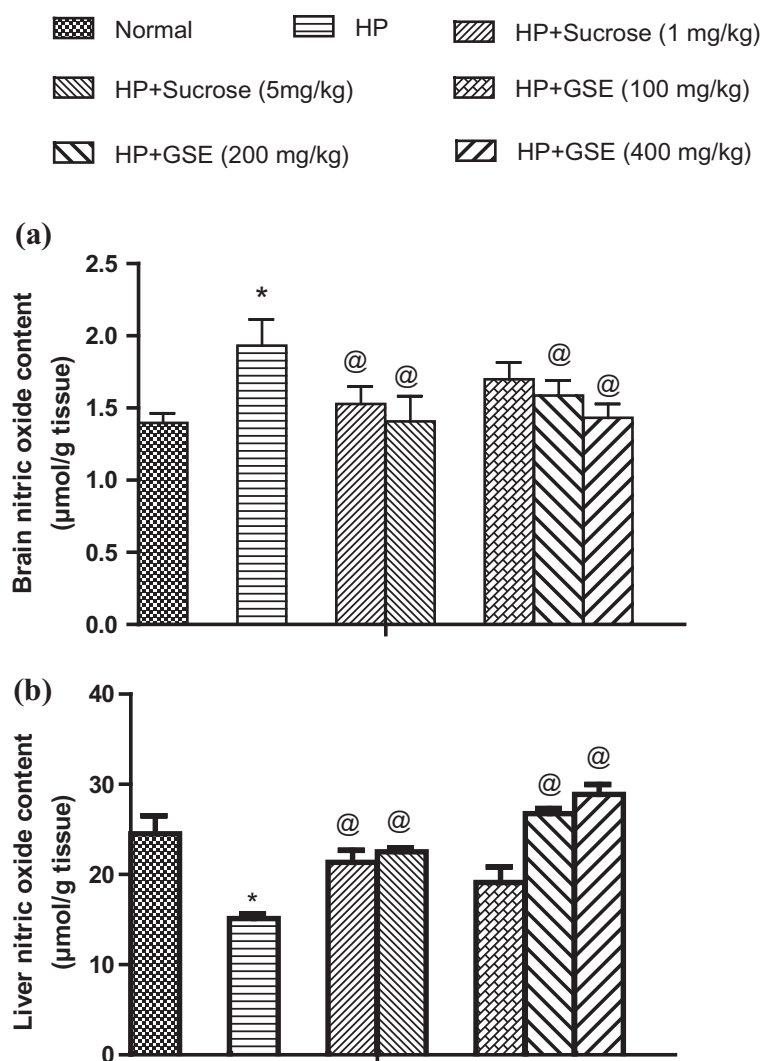

Figure 1 Effects of sucrose and GSE on the brain (a) and liver (b) NO content in haloperidol-induced oxidative stress in rats. Oxidative stress was induced using haloperidol (1 mg/kg) then rats were given sucrose and GSE for 2 weeks. Twenty-four hours after the last doses of sucrose, rats were euthanized by decapitation and the brain and the liver were excised and homogenized. The homogenates were used for NO content determination. Statistical analysis was carried out by ANOVA followed by Tukey's multiple comparisons test. *Significant difference from the corresponding normal control group at $P < 0.05$. @Significant difference from the haloperidol-treated group at $P < 0.05$.
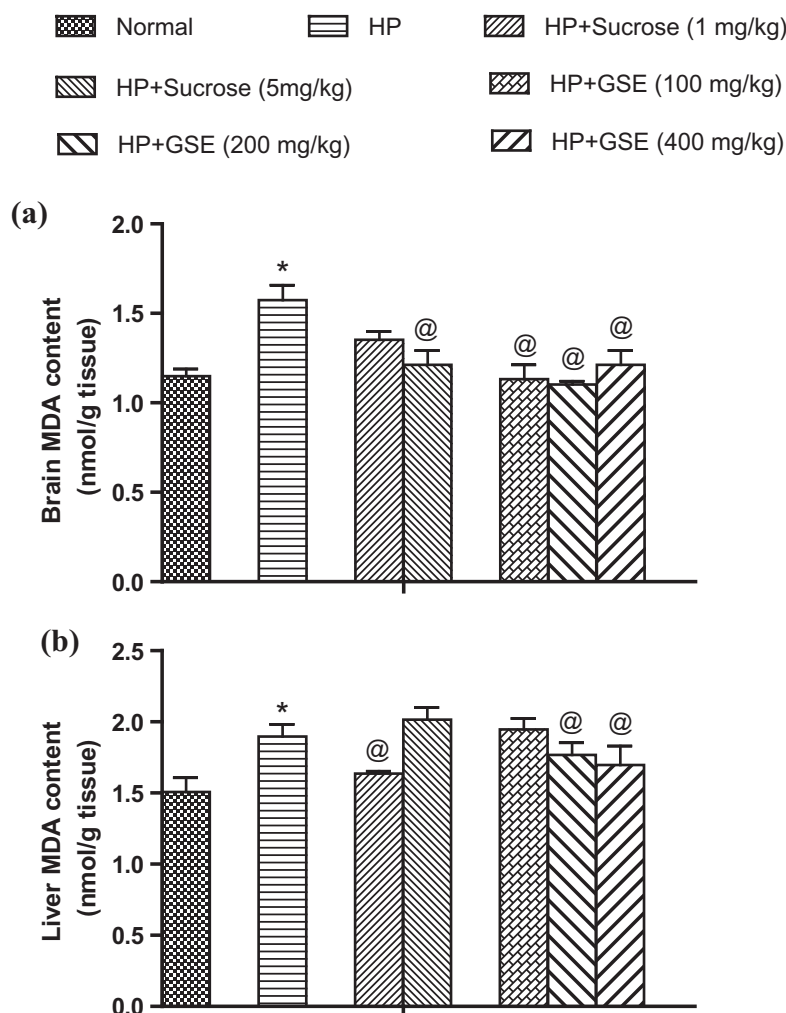
Figure 2 Effects of sucrose and GSE on the brain (a) and liver (b) NO content in haloperidol-induced oxidative stress in rats. Oxidative stress was induced using haloperidol (1 mg/kg) then rats were given sucrose and GSE for 2 weeks. Twenty-four hours after the last doses of sucrose, rats were euthanized by decapitation and the brain and the liver were excised and homogenized. The homogenates were used for NO content determination. Statistical analysis was carried out by ANOVA followed by Tukey's multiple comparisons test. [*]Significant difference from the corresponding normal control group at $P < 0.05$. [@]Significant difference from the haloperidol-treated group at $P < 0.05$.

3.2. Biochemical parameters measured in the liver

3.2.1. Effect of sucrose and GSE on nitric oxide

The oxidative stress induced in rats by HP is associated with a decrease in liver NO levels reaching about 61% of the normal value. Treatment of HP-treated rats with sucrose (1 and 5 mg/kg/day; p.o.) for 14 days showed an increase in the reduced liver NO levels by about 15% and 30%, respectively. Similar treatment with GSE (200 and 400 mg/kg/day; p.o.) showed an increase in liver NO levels by about 47% and 56%, respectively (Fig. 1b).

3.2.2. Effect of sucrose and GSE on lipid peroxidation

The oxidative stress induced in rats by HP is associated with an increase in liver lipid peroxidation levels (measured as MDA) reaching about 125% of the normal value. Treatment of HP-treated rats with sucrose (1 mg/kg/day; p.o.) for 14 days showed a decrease in elevated liver MDA levels reaching nearly the normal value. On the other hand, HP-treated rats with sucrose (5 mg/kg/day; p.o.) showed no change in liver MDA levels. Similar treatment of HP-treated rats with GSE (100 mg/kg/day; p.o.) showed no change in liver MDA levels, however treatment with GSE (200 and 400 mg/kg/day; p.o.) showed a decrease in liver elevated MDA levels by about 8% and 18%, respectively (Fig. 2b).

3.2.3. Effect of sucrose and GSE on reduced glutathione

The oxidative stress induced in rats by HP is associated with a decrease in liver GSH levels reaching about 70% of the normal value. Treatment of HP-treated rats with sucrose (1 and 5 mg/kg/day; p.o.) for 14 days showed no change in liver GSH levels. Similar treatment of HP-treated rats with GSE (100, 200 and 400 mg/kg/day; p.o.) showed an increase in liver GSH levels reaching about 92%, 111% and 124% of the normal value, respectively (Fig. 3b).
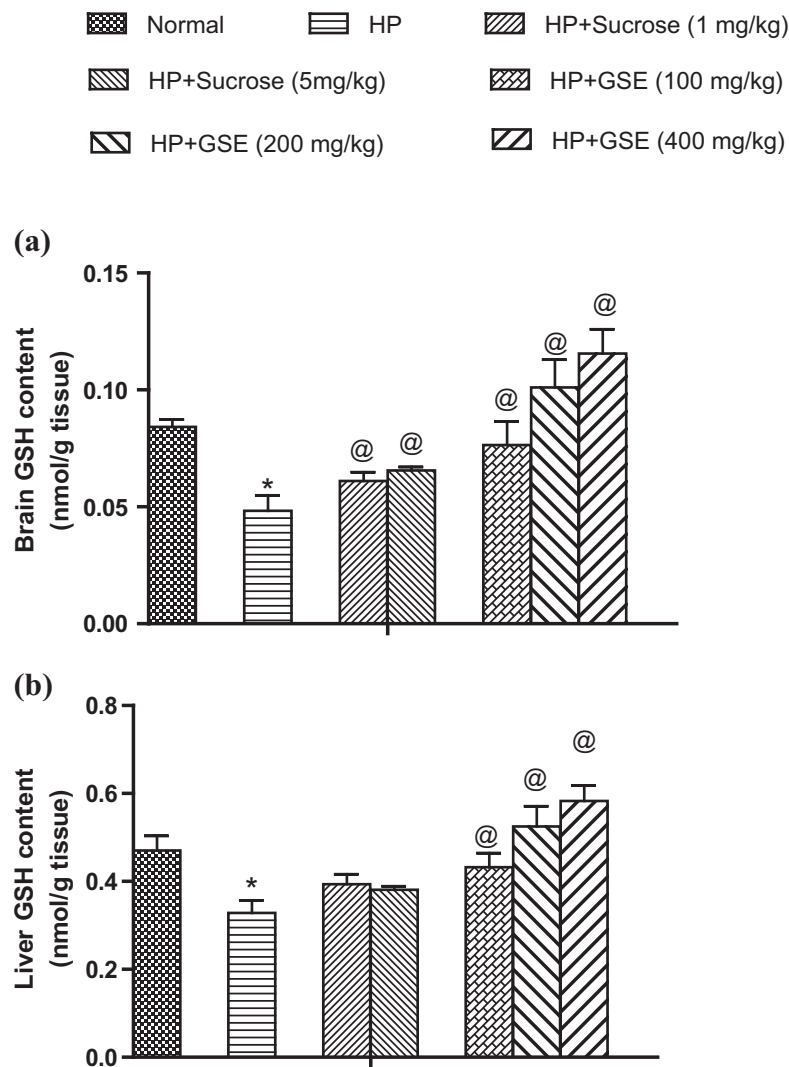
Figure 3 Effects of sucrose and GSE on the brain (a) and liver (b) GSH content in haloperidol-induced oxidative stress in rats. Oxidative stress was induced using haloperidol (1 mg/kg) then rats were given sucrose and GSE for 2 weeks. Twenty-four hours after the last doses of sucrose, rats were euthanized by decapitation and the brain and the liver were excised and homogenized. The homogenates were used for NO content determination. Statistical analysis was carried out by ANOVA followed by Tukey's multiple comparisons test. [*]Significant difference from the corresponding normal control group at $P < 0.05$. [@]Significant difference from the haloperidol-treated group at $P < 0.05$.

4. Discussion

The present study provided further evidence that HP increases the oxidative stress in the brain tissue i.e. decreases the reduced GSH levels and increases the MDA levels in rat brain. HP is found to increase oxidative stress mediated neuronal damage in animals. This neuronal damage by pro-oxidant actions of HP has led to suggest that its side effects resulted from induced oxidative injury.[22]

Lipid peroxides, measured as MDA, which are expressed as thiobarbituric acid reactive substances reflect a condition of oxidative stress. MDA a marker of lipid peroxidation activity[23] was associated with a marked and significant decrease in the level of reduced glutathione, an important antioxidant defense mechanism. These results are in accordance with that reported by other authors.[24,25] Sagara had reported that HP increases brain oxidative stress due to increased turnover of dopamine.[24] The present study also shows that the administration of HP-induced a similar oxidative stress in the liver. Hepatic toxicity of the antipsychotic HP has been reported in cultured rat hepatocytes[26] and experimentally in Wistar rats.[27,28] Shivakumar et al. (1992) reported that HP blockade of dopamine receptor increases dopamine turnover resulting in excessive production of hydrogen peroxide and, thus, generates oxidative stress.[17]

Haloperidol-induced oxidative stress was associated with an increased generation of brain NO which is a free radical which can be deleterious to brain functions, where synthesis of inducible nitric oxide synthase contributes to the activation of apoptotic pathways in the brain. NO can react with many other free radical i.e. superoxide radical, generation peroxynitrite radical causing oxidative changes to numerous tissues.[29] On the other hand, HP-induced oxidative stress was associated with a decrease in the liver NO content.

Results of the current study revealed that treatment of HP-treated rats with GSE (100, 200 and 400 mg/kg/day; p.o.) reversed all the oxidative stress markers in both the brain and liver i.e. increased the reduced GSH and decreased the lipid peroxidation, measured as MDA. GSE is considered a very potent antioxidant and exhibits numerous interesting pharmacologic activities.[30] It has been suggested to be of use in treatment of several diseases.[31] GSE contains polyphenols including proanthocyanidins and procyanidins that showed antioxidant and free radical scavengers, being more effective than either ascorbic acid or vitamin E.[32] Their effects include the ability to increase intracellular vitamin C levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals. Several studies have indicated that extracts obtained from GSE inhibit enzyme systems that are responsible for the production of free radicals.[33,34]

Grape seed extract supplementation increased NO levels in the liver tissue. In accordance with other studies GSE scavenger NO[35] has a beneficial effect on the liver by protecting it against both tissue injury and the cytotoxic effects of invading microorganisms, parasites, and tumor cells. Thus, NO may prevent liver tissue damage caused by oxidative stress.[36] However, GSE (100, 200 and 400 mg/kg/day; p.o.) has a beneficial effect on the brain NO content.

On the other hand, treatment of HP-treated rats with sucrose attenuates the levels of NO in the brain and liver and the brain levels of MDA and GSH. However, low sucrose dose decreased elevated liver MDA levels reaching nearly the normal value. Many studies showed the anti-oxidative effects of sucrose and sucrosyl oligosaccharides.[37] The mechanism of OH· scavenging might be linked to the presence of stable oxidized sucrose free radical which are slower reacting radicals compared with OH· radicals and seem to undergo several possible reactions to form more stable non-radical compounds or, alternatively, to regain their reduced forms. A previous study reported that consumption of sucrose increased dopamine D_3 receptor mRNA levels and decreased dopamine D_2 receptor mRNA levels.[38] Sucrose fulfills various functional roles in plant metabolism. It might either directly detoxify reactive oxygen species or indirectly stimulate the classic anti-oxidative defense systems. On the other hand, treatment of HP-treated rats with sucrose (5 mg/kg/day; p.o.) showed no change in the liver contents of MDA and GSH. Spolarics and his co-worker proposed that sucrose-rich diet results in an increased hepatic sensitivity to oxidative stress.[39] It has also been suggested that sucrose-fed rats may predispose lipoproteins and subsequently oxidative stress.[40]

Finally, it can be concluded from all these findings that both GSE (a potent anti-oxidant) and sucrose (as a source of energy) have beneficial impacts on the brains of HP-treated rats. However, GSE is more potent in alleviating the oxidative stress associated with HP in the liver.

5. Conflict of interest

None.

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