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Possible Prophylactic Effect of Soy Isoflavones Genistein on Cigarette Smoke-Induced Neurotoxicity in Rats' Brains

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Abstract: Neurodegenerative diseases have long been linked to Cigarette Smoke (CS). The goal of this study was to examine the neurotoxicological effects of CS on rat brains as well as any potential ameliorative benefits of genistein as a neuroprotective agent. A total of 24 adult male Wistar rats were divided into 4 different groups. Group I served as control; group II was CS Exposed (Rats are exposed to side stream CS twice daily, the duration of each exposure was 3 h with an interval of 10 min between each cigarette, using 8 to 10 cigarettes per day); group III received: both CS Exposure and Gen (Genistein dissolved in cremophor EL orally, 10 mg/kg BW orally); Group IV received Gen (Genistein alone dissolved in cremophor EL orally, 10 mg/kg b.w./day). Rats treated with CS poisoning developed biochemical and histological abnormalities. The levels of enzymic and non-enzymic antioxidants as well as neuronal enzymes like acetylcholinesterase, creatine kinase, and Lactate Dehydrogenase within the brain and serum of treated rats were significantly improved by genistein, which also significantly reduced the elevation of lipid peroxidation and normalized the CS impact. Histology data further supported the findings that CS therapy resulted in neurodegenerative changes and that soy isoflavones genistein act as antioxidants and neuroprotective agents against CS toxicity. Therefore, Genistein should be a part of a healthy diet to prevent or reduce neurological diseases caused by long-term exposure to CS.

Keywords: Cigarette smoke, Neurotoxicity, Genistein, Prophylactic effect, Neurodegenerative disorders, Biochemical, Histology changes, Rats

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Introduction

In spite of initiatives to reduce its usage, cigarette smoke is the most often used licit substance globally. Cigarette smoking has a lot of seriously detrimental impacts on one's health, even though

the usage of tobacco has been seen to be increasing. (CDC, 2000). Despite the fact that there are 4000 distinct components of nicotine found in cigarettes, nicotine is the most common alkaloid in

tobacco (Djordjevic *et al.*, 1989, Benowitz 1996). While around 6 million individuals each year perish away from illnesses brought on by passive (second-hand) or active (cigarette) exposure to cigarette smoke (WHO, 2016). The CDC (2021a) estimates that smoking-related illnesses claim the lives of more than 7 million people worldwide and more than 16 million Americans each year (WHO, 2017). By 2030, it is predicted that if current worldwide smoking trends continue, more than 8 million people will per year pass away from diseases linked to tobacco smoke (CDC, 2021a). By the year 2025, 30% of men and 8% of women worldwide will smoke, according to a WHO global assessment of trends in tobacco use. It has been shown that long-term smoking contributes to BBB (Blood-brain barrier) failure by triggering inflammatory, oxidative, and immunological reactions that promote the development and spread of cerebrovascular and neurodegenerative diseases.

Due to their advantageous effects on brain function, isoflavones -- a subgroup of the polyphenolic chemical family known as flavonoids and found in large quantities in food sources-- have drawn a growing amount of interest (Lee *et al.*, 2005; Vauzour, 2012; Moosavi *et al.*, 2015). A number of the pathogenic processes connected to neurodegeneration have been demonstrated to be modulated by genistein (4',5,7-trihydroxy-isoflavone), one of the main isoflavones found in soy and soy products (Devi, 2017, Oliveira, 2016, Uddin *et al.*, 2019).

The high concentration of lipids in the brain makes it susceptible to oxidative radical damage while being exposed to CS. According to recent research, oxidative damage caused by free radicals in several organs may be linked to smoking-related illnesses. Of course, smoking Reactive Oxygen Species (ROS) are the main free radicals found in smoke, which is a complicated environment (Pryor, 1997). Numerous components in cells, such as membrane lipids, proteins, carbohydrates, and DNA, are known to suffer oxidative damage as a result of ROS

(Halliwell, 1987). The oxidation of protein thiols and lipid peroxidation (LPO) are both increased by smoking, and the levels of protein carbonyl (PCO) are also said to be altered (Frei *et al.*, 1991, Reznick *et al.*, 1992). Passive cigarette smoke has also been linked to several illnesses in previous research, according to many of them (Menziez *et al.*, 2006, Beadsmoore *et al.*, 2007, Metsios *et al.*, 2007). According to a recent assessment by the International Agency for Research on Cancer, involuntary smoking (passive smoking) increases the chance of developing lung cancer by 20% for women and 30% for men who have never smoked (IARC 2004). The objective of the current study was to determine the toxicological effects of CS on rat brains. To lessen or suppress the cytotoxic effects of CS on the neurological system, the study may also be expanded to examine the role of Genistein as a protective agent with antioxidant properties. We identified markers for oxidative stress and neuronal enzymes. The biochemical findings may be confirmed by looking at histopathological analyses of rat brain tissue.

Materials and Methods

Chemicals:

Genistein was bought from TCI, and Cremophor EL was bought from Sigma-Aldrich Co. (St. Louis, MO, USA). The only other substances and reagents that were employed in this study were of analytical grade. The cigarettes were purchased from a local store, Vaniyambadi. The same brand of locally available cigarette will be used throughout the experiment (Scissors Standard).

Animals:

Adult male albino Wistar rats weighing 200g-250 g were obtained from Saveetha Dental College, Chennai, India. Animals were separated and allowed to adapt for a week prior to experimentation. Water and feed were supplied. Six animals were housed per cage and maintained on a 12/12 h/day and night cycle in a temperature and humidity-controlled room. The rats were fed with a commercial pellet diet (Chennai, India). All animals were allowed free access to feed and

water *ad libitum* throughout the study. The Ministry of Social Justice and Empowerment of the Government of India, as well as the Institutional Animal Ethics Committee Guidelines, were followed for conducting the studies (Approval No. BRULAC/SDCH/SIMATIC/IAEC/02-2018/004).

Experimental Design:

The toxicity of CS was evaluated by a literature study and Rats were divided into four groups, each consisting of six animals.

Group I - Control rats (Rats received no treatment and served as a control group)

Group II - Rats induced with Cigarette smoke (Rats are exposed to sidestream cigarettes (CS) twice daily for a total of 3 h, with a 10-min gap between each cigarette (equivalent to 8–10 cigarettes per day) for 12 weeks as described by Gokulakrishnan *et al.* (2013)

Group III- Drug-alone rats (Genistein alone dissolved in Cremophor EL orally (10 mg/kg b.w. /day) (Maryam Bagheri *et al.*, 2010)

Group IV- Rats exposed to CS and simultaneously administered Genistein dissolved in Cremophor EL orally for 12 weeks (10 mg/kg b.w. /day).

When the experiment was complete, the animals were killed by cervical decapitation after a 24 h fast. The blood sample was collected from the jugular vein for serum isolation. For biochemical examination, the blood samples were centrifuged at 5000 rpm for 20 min at 4°C, the serum was separated for biochemical investigation using Biosystem BTS 350 Biochemistry analyzer. The brain tissue from the experimental rats was carefully removed and rinsed with ice-cold phosphate buffer 1 M (pH 7.4). At 4°C, the homogenate was centrifuged for 20 min at 3000 rpm. For biochemical examination, the supernatant was aliquoted and kept at -20°C. Parts of the brains had been preserved in a 10% neutral buffered formalin solution for histopathological study.

Biochemical investigations in the brain tissues:

Superoxide dismutase SOD (Misra and Fridovich, 1972), catalase CAT (Takahara *et al.*, 1960), glutathione peroxidase GPx (Rotruck *et al.*, 1973), glutathione reductase GR (Staal *et al.*, 1969), and non-enzymatic antioxidants reduced glutathione GSH (Ellman, 1959), vitamin C (Omaye *et al.*, 1979), and vitamin E (Desai, 1984) were estimated using the serum. The brain tissue homogenate was analysed for lactate dehydrogenase (LDH) using the method of King (1965), and creatine kinase by using the method of Hall and Deluca (1976). Malondialdehyde (MDA) was measured to estimate the amount of lipid peroxidation using the method described by Slater (1984). The method of Ellman *et al.* (1961) was used to measure the activity of AChE in the homogenate.

Histopathological studies:

To assess the histomorphological alterations in several experimental groups, brain tissues were inspected. Brain tissue samples were obtained and preserved in 10% formaldehyde for 24 h. Hematoxylin and eosin were used to stain some sample sections, and these sections were then viewed using a high-power light microscope.

Statistical analysis:

Standard Error Mean values were used to represent the results of various biochemical investigations (S.E.M). A one-way analysis of variance was used in the statistical analysis of the comparisons between the parameters of the experimental group and the control group (ANOVA). When the p-value was $P < 0.05$, differences were deemed to be meaningful. Statistical analysis was carried out using the Statistical Package for the Social Sciences Program, and all graphs were obtained using Microsoft Excel (SPSS, version 25.0).

Results

Effect of Genistein on lipid peroxidation:

Malondialdehyde (MDA), a byproduct of lipid peroxidation, was analysed in the homogenate of the brains of control and experimental rats to

LIPID PEROXIDATION

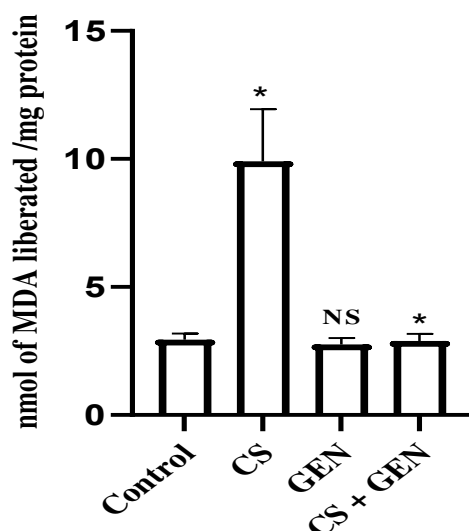


Fig. 1: Number of lipid peroxides in the brain tissues of rats in the control and experimental groups. Each value represents mean $\pm$ SD (n=6). These statistically significant differences are contrasted: CS-induced vs. control; CS exposed vs. CS exposed + Genistein-treated; *denotes $P < 0.05$, NS denotes non-significant.

Table 1: Effect of CS and Genistein on the activities of SOD, CAT, GPx, GST, and GR in the serum of control and experimental rats

Enzymic Antioxidant (Serum)				
	Control	CS	GEN	CS + GEN
SOD	4.82 $\pm$ 0.42	2.53 $\pm$ 0.53*	5.08 $\pm$ 0.37 ^{NS}	4.03 $\pm$ 0.41*
CAT	86.19 $\pm$ 6.81	57.68 $\pm$ 11.76*	89.74 $\pm$ 6.53 ^{NS}	72.36 $\pm$ 10.17*
GPx	6.81 $\pm$ 0.68	3.99 $\pm$ 0.89*	6.35 $\pm$ 0.56 ^{NS}	5.63 $\pm$ 0.58*
GR	2.01 $\pm$ 0.15	0.72 $\pm$ 0.17*	2.30 $\pm$ 0.15 ^{NS}	1.51 $\pm$ 0.18*
GST	13.11 $\pm$ 1.13	7.41 $\pm$ 1.50*	13.42 $\pm$ 0.93 ^{NS}	9.98 $\pm$ 1.39*

Units: SOD - 50% inhibition of epinephrine auto-oxidation/min/mg protein; CAT - mole H₂O₂ consumed/min/mg protein; GPx - g of GSH consumed/min/mg protein; GST - moles of 1-chloro-2, 4-dinitrobenzene formed/min/mg protein; and GR - g GSSH utilized. Each value represents mean $\pm$ SD (n=6). The following comparisons are made between statistically significant variations: CS exposed vs. control; CS exposed + Genistein treatment vs. CS-exposed; *indicates $P < 0.05$ and NS indicate non-significant.

assess the potential effects of oxidative stress. Comparing the rats who smoked cigarettes with the control rats, there was a substantial rise in the MDA levels (Fig.1). Genistein treatment for CS rats reduced the mean MDA concentration to values that were almost identical to those observed in control rats, most likely through reducing lipid

peroxidation in the brain. When compared to control rats, Genistein-only treated rats did not exhibit any appreciable changes in MDA levels (Fig. 1).

Effect of Genistein on enzymic antioxidant:

Table 1 demonstrates the antioxidant enzyme

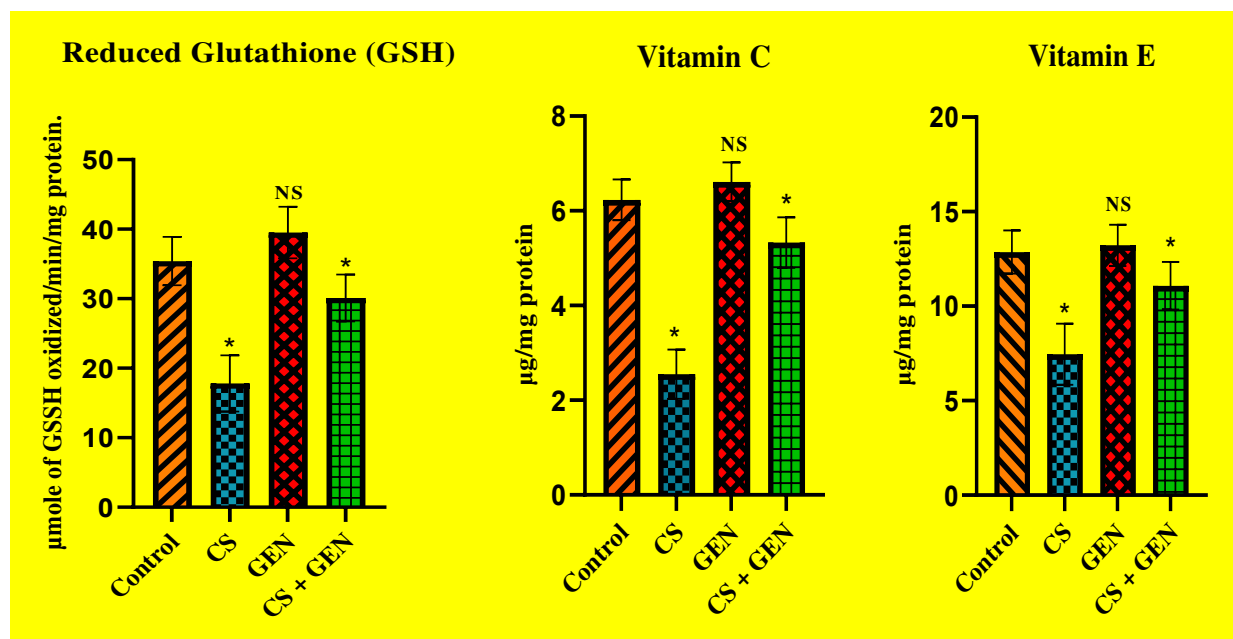


Fig. 2: Levels of GSH, Vitamin C, and Vitamin E in the serum of experimental and control rats. Each value represents mean $\pm$ SD (n=6). These statistically significant differences are contrasted: CS-induced vs. control; CS exposed vs. CS exposed + Genistein-treated; *denotes P<0.05, NS denotes non-significant.

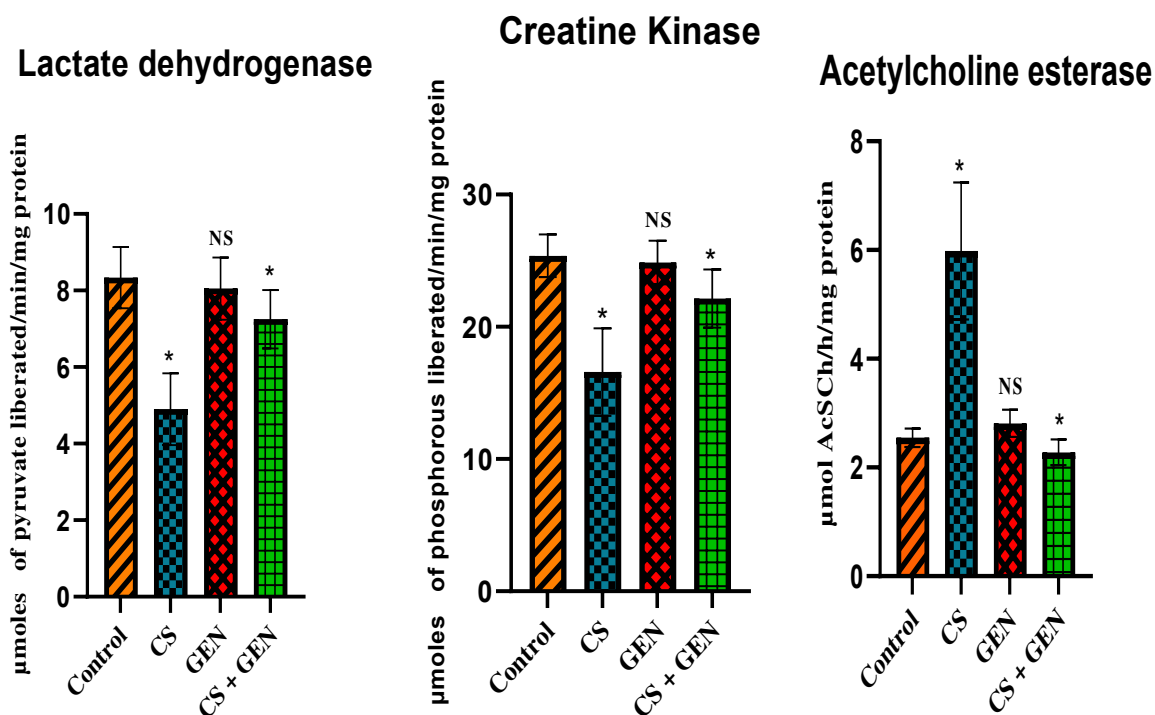


Fig. 3: Influence of CS and Genistein on the activities of LDH, CK, and AChE in the brain tissue homogenates of experimental and control rats. Each value represents mean $\pm$ SD (n=6). These statistically significant differences are contrasted: CS-induced vs. control; CS exposed vs. CS exposed + Genistein-treated; *denotes P<0.05, NS denotes non-significant.

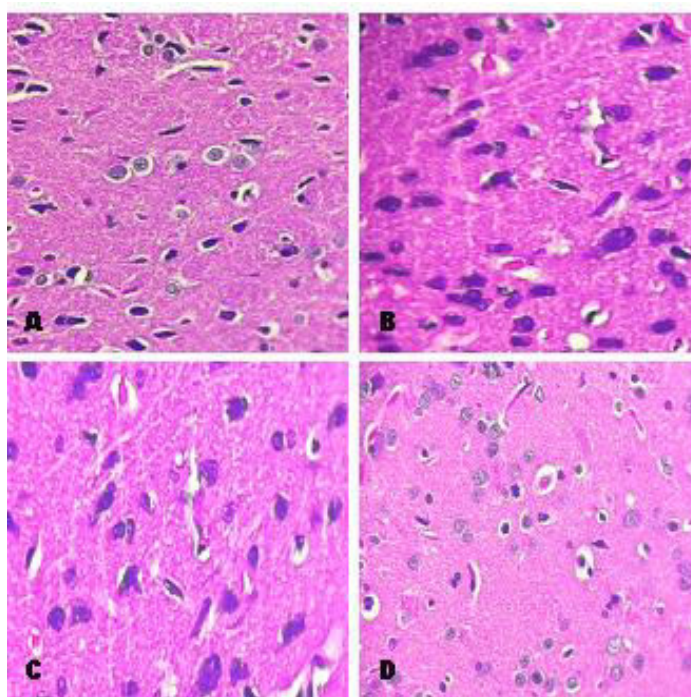


Fig. 4: Impact of CS and Genistein on histological structure of rat brain HE x 100. The cortex of CS exposed neurotoxicity had atypical cellular morphology and hydropic degenerative modifications; the architecture of the cortex in the CS rat brain (cortex) after treatment with genistein was near to normal, and hydropic degenerative alterations were diminished (C). No abnormal alterations to the cortex were seen in normal rats given genistein treatment.

activity of the control and experimental rat groups (SOD, CAT, GPx, and GR). In the CS exposed group, a substantial decline in antioxidant status was seen. These modifications show how the antioxidant level of the CS exposed group is declining. Genistein treatment caused antioxidant levels to significantly increase toward the control range, demonstrating that it has a protective impact against oxidative stress caused by smoking.

Effect of Genistein on non-enzymic antioxidant:

Measurements of non-enzymatic antioxidant systems have also been used to determine the protective mechanism against CS-related free radical-induced harmful oxidative damage to cells. When compared to the levels in control rats, the serum of CS exposed rats had considerably lower amounts of GSH, vitamin C, and vitamin E (Fig. 2). The levels of GSH, vitamin C, and vitamin E significantly increased after Genistein administration to CS exposed rats and returned

close to the levels of control rats. Comparing treated rats with Genistein alone to control rats, these levels were not noticeably different. (Fig. 2).

Effect of Genistein on LDH, CK and AChE:

In contrast to the control group, LDH and CK activity was considerably ($P < 0.05$) higher in rat brains treated with CS, indicating that treatment with Genistein corrected the aberrations of the LDH and CK enzyme. CS and Genistein have various impacts on tissue AChE activity, as depicted in Figure 3. In brain tissue homogenate, it was noticed that AChE activity varied considerably between the experimental groups. AChE inhibition activity was highest in the CS exposed rat (group II) when compared to control (group I), although Genistein administration's neuroprotective effects caused recovery that was near to control values.

Effect of Genistein on histopathology of the brain:

Figure 4 shows histopathological observations of

the control and experimental rats' brains. The control rat's brain exhibited no pathological alterations, but the CS exposed rat's brain displayed hydropic degenerative alterations (Fig. 4A, B). The architecture of the CS exposed rat brain after Genistein treatment was close to normal, and hydropic degenerative alterations were reduced (Fig. 4D). Normal rats treated with Genistein did not exhibit any pathological changes in the brain (Fig. 4C).

Discussion

Smoking cigarettes is linked to a rise in the amount of lipid peroxidation in the brain (Luchese *et al.*, 2009). Lipid peroxidation, which manifests in ischemia injuries, neurotrauma, and neurological diseases, is a substantial contributor to neuronal damage (Coyle and Puttfarcken, 1993). MDA is one of the primary degrading byproducts of lipid peroxidation and is utilized as a measure of oxidative tissue damage. Smoking cigarettes is thought to accelerate the breakdown of membrane lipids, which then results in the production of peroxide radicals (Pryor and Stone, 1993; Anbarasi *et al.*, 2005). According to the current study, this breakdown of membrane lipids may have contributed to the observed rise in MDA levels in the brains of CS exposed rats as compared to control rats. The elevated levels of MDA in the CS exposed rats may potentially be related to insufficient antioxidant levels that were needed to scavenge peroxy radicals produced by smoking cigarettes. This study derives support from earlier reports which also observed similar findings (Anbarasi *et al.*, 2005; Ozkol *et al.*, 2012). Free radicals can damage lipids, therefore antioxidants are crucial for preventing their generation as well as for containing some of the harmful effects of ROS. MDA concentrations in the brain of CS exposed rats treated with genistein significantly dropped. These findings imply that genistein can lower the free radicals caused by smoking while also supporting normal cellular activity. Previous studies (Maulik *et al.*, 2012, Zhang *et al.*, 2013) have demonstrated the positive impact of genistein on antioxidant enzymes in a

neurotoxic environment.

In response to oxidative stress, antioxidant enzyme levels are raised to deal with the massive increase in ROS generation (Gutteridge and Halliwell, 1994). Due to an adaptive response, acute cigarette smoke exposure increases the synthesis of these antioxidant enzymes, which helps to reduce the harm caused by cigarette smoke (Hilbert and Mohsenin, 1996). Following a 12-week exposure to cigarette smoke, an increase in the activity of antioxidant enzymes has been seen (Baskaran *et al.*, 1999). Although a decrease in the quantities of these enzymes suggest that chronic exposure to cigarette smoke appears to overwhelm the human tissues' adaptive response (Hulea *et al.*, 1995). Our findings support the findings mentioned above, demonstrating lower superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) activity in smoke-exposed rat serum.

The first enzyme in antioxidant defense, SOD scavenges superoxide radicals to produce H_2O_2 and so lessens the radical's damaging effects. It is possible for the quinone-semiquinone radicals in tar-phase cigarette smoke to convert molecule oxygen into superoxide radicals, whose excessive production renders this enzyme inactive (Duthie and Arthur, 1994). Therefore, tar phase oxidants' deactivation of SOD could have caused a decrease in SOD activity after smoke exposure.

CAT is involved in the detoxification of high concentrations of H_2O_2 , whereas GPx is sensitive to lower concentrations. The serum contains fewer CAT levels and hence GPx has a major role in quenching H_2O_2 and other peroxides which otherwise will lead to the production of hydroxyl and peroxy radicals in the presence of iron (Bast and Barr, 1997; Gutteridge and Halliwell, 1994). Inhibition of CAT activity in rat brains and liver by cigarette smoke has been reported (Mendez-Alvarez *et al.*, 1998). The presence and production of free radicals from smoke lower this enzyme, leading to the accumulation of H_2O_2 and lipid hydroperoxides further worsening the damage

(Pryor, 1997). During acute smoke exposure, an increase in CAT activity was observed by Baskaran *et al.* (1999).

In contrast to GPx, which is sensitive to lower quantities of H₂O₂, CAT is engaged in the detoxification of high concentrations of H₂O₂. Since CAT levels in the brain are lower than in other tissues, GPx plays a critical function in scavenging H₂O₂ and other peroxides that, absent GPx, would otherwise produce hydroxyl and peroxy radicals when the iron is present (Gutteridge and Halliwell, 1994; Bast and Barr, 1997). There is evidence that cigarette smoke reduces CAT activity in rat brains and livers (Mendez-Alvarez *et al.*, 1998). Smoke's free radicals inhibit this enzyme and produce more of them, which worsens the damage by causing H₂O₂ and lipid hydroperoxides to build up (Pryor, 1997). A rise in CAT activity was noticed by Baskaran *et al.* (1999), during acute smoke exposure. The present study's finding that CAT activity has decreased shows that the host's antioxidant defenses are unable to adequately counteract the oxidative damage caused by repeated exposure to cigarette smoke.

When CAT activity is insufficient to handle oxidative stress, an increase in GPx activity is anticipated. The lack of an increase in GPx activity following smoke exposure in our study suggests that this is due to a drop in GSH levels, which are necessary for the conjugation of lipid peroxides. According to Gutteridge and Halliwell (1994), GR is a crucial enzyme for maintaining the intracellular content of reduced glutathione, hence smoke exposure may have decreased GR activity since there was less GSH available.

The current investigation demonstrated that genistein administration significantly increased antioxidant status while maintaining low oxidant levels, providing neuroprotective effects against CS exposed damage. Sonee *et al.* (2004) first reported that genistein can prevent oxidative damage by acting as an antioxidant. As a result of genistein's free radical scavenging and antioxidant processes, these levels were dramatically lowered in rats given the supplement (Wei *et al.*, 1993;

Arora *et al.*, 2000;). Its capacity to traverse the blood-brain barrier, the presence of processes similar to those found in estrogen, and a longer half-life inside the human system are all factors contributing to this protective mechanism (Chang *et al.*, 2000).

Rats exposed to cigarette smoke had lower GST activity in their serum, it was discovered. The findings of earlier studies are consistent with the results of the present study (Ozkan *et al.*, 2007). Lower GSH concentrations and higher levels of lipid peroxidation in CS exposed rats may be the cause of the GST's reduced action. Following genistein administration to CS exposed rats, serum GST activity was found to be enhanced. In CS exposed rats, brain GSH levels may have been higher, which may account for the enhanced GST activity. Additionally, the enhanced activity of GST may hasten the interactions of the ROS and aldehydes with GSH to produce fewer harmful conjugates that enter the serum. Rats treated with CS had lower serum G6PDH activity. The lungs, heart, liver, kidney, and other organs of CS rats showed a similar outcome (Ramesh *et al.*, 2007; Ramesh *et al.*, 2008; Ramesh *et al.*, 2010). GSH regeneration was hindered because G6PDH activity was reduced. By generating more reducing equivalents and converting more oxidized glutathione into reduced glutathione, genistein administration in CS exposed rats showed enhanced G6PDH activity.

In the present study, the CS exposed group had decreased levels of the non-enzymic antioxidants (GSH), Vitamin C, and Vitamin E (Rani and Panneerselvam, 2001). By acting as non-enzymic antioxidants through direct interaction of their sulfhydryl groups, GSH, Vitamin C, and Vitamin E form the first line of defense against oxidative damage, which inactivates oxidative stress (Ramesh *et al.*, 2014). By preventing lipid peroxidation and interacting with lipid peroxy radicals to create a non-reactive tocopherol radical, vitamin E protects cell membranes. Vitamin E is renewed by vitamin C (Kallner *et al.*, 1981). In this study, genistein supplementation to

CS exposed rats preserves non-enzymic antioxidants, reduces oxidative stress, maintains non-enzymic antioxidant levels in CS-treated rats, and reduces their harmful accumulation in tissue.

In this study, we found that smoking-exposed rats had significantly higher LDH and CK activity. A greater degree of permeability and injury to neural tissue is suggested by the elevated enzyme levels. LDH and CK activity levels were, however, markedly decreased after treatment with genistein. This shows that Genistein, a dietary supplement, prevents the enzymatic changes caused by cigarette smoke in neural tissue. The anti-lipoperoxidation activity of genistein has also been shown to reduce the enzymes during morphine-induced kidney damage (Jalili *et al.*, 2020).

We examined the activity of AChE to determine the impact of genistein on the oxidative stress caused by cigarette smoking in rats and to determine whether it is connected to the alternations in cholinergic enzymes in the brain. The brain's AChE activity was higher in the cigarette smoke-exposed mice. Smoking causes a drop in the level of acetylcholine (ACh), which may contribute to concentration and memory problems and other cognitive problems smokers experience (Jacobsen *et al.*, 2005). By tonally firing action potentials at a rate of roughly 5 Hz, cholinergic interneurons maintain a continuing ACh signal (Zhou *et al.*, 2002). Primarily across the brain, cholinergic neurons create extensive projections. Information flow from the prefrontal cortex to the nucleus accumbens is gated by excitatory hippocampus afferents (Goto and O'Donnell, 2001; Grace, 2000).

The current investigation proved that therapy with Genistein reduced the neurotoxicity that exposure to cigarette smoke had caused in the rats' brains. The brain's AChE activity was reduced by Genistein to a normal level. Additionally, it should be taken into account that Genistein may have neuroprotective effects on these cholinergic system structures by reducing AChE activity in animal smokers. The results of the study, which

are consistent with those of Eidi *et al.* (2006), have led to the suggestion that Genistein may prevent the reduction of ACh levels in the analyzed brain areas.

Neuronal architecture in the brains of the control and Genistein-treated rats was normal. In the cortex of the brains of CS rats, hydropic degenerative alterations were found. These alterations could be the result of enhanced lipid peroxidation caused by free radicals and heavy metals produced in cigarette smoke. The hydropic degenerative alterations were lessened and nearly resembled those of the normal architecture in the CS exposed rats after treatment with genistein. Genistein's neuroprotective effects may have been enhanced by the synergistic interactions between all of its constituent parts.

Conclusion

The biochemical and histological findings of this investigation demonstrated the substantial risk of neurotoxicity caused by cigarette smoke. Genistein should be explored on alternative heavy metals and ecologically hazardous chemicals since it functions as an antioxidant compound with neuroprotective and therapeutic effects vs CS toxicity. The histologic findings supported the biochemical analysis. It is highly recommended to incorporate Genistein in your diet to prevent or reduce the occurrence of neurodegenerative illnesses brought on by long-term exposure to cigarette smoke.

References

- Anbarasi K, Vani G, Balakrishna K and Devi CS. (2005) Effect of bacoside A on membrane-bound ATPases in the brain of rats exposed to cigarette smoke. *J Biochem Mol Toxicol*. 19: 59-65.
- Arora A, Valcic S, Cornejo S, Nair MG, Timmermann BN and Liebler DC. (2000) Reactions of Genistein with alkylperoxyl radicals. *Chem Res Toxicol*. 13(7): 638-645.
- Bagheri M, Rezakhani A, Nyström S, Turkina MV, Roghani M, Hammarström P and Mohseni S. (2013) Amyloid beta(1-40)-induced astrogliosis and the effect of genistein treatment in rat: a three-dimensional confocal morphometric and proteomic study. *PLoS One* 8(10): e76526.

- Beadsmoore C, Cheow HK, Szczepura K, Ruparelia P and Peters AM. (2007) Healthy passive cigarette smokers have increased pulmonary alveolar permeability. *Nucl Med Commun.* 28: 75-77.
- Benowitz NL. (1996) Pharmacology of nicotine: addiction and therapeutics. *Annual Rev Pharmacol Toxicol.* 280: 1173-1181.
- Cataldo JK, Prochaska JJ and Glantz SA. (2010) Cigarette smoking is a risk factor for Alzheimer's disease: an analysis controlling for tobacco industry affiliation. *J Alzheimer's Disease* 19(2): 465-480.
- Center for disease control (2000) State-specific prevalence of current cigarette smoking among adults and their proportion of adults who work in a smoke-free environment, USA. <https://www.cdc.gov/mmwr/preview/mmwrhtml/mm4943a2.htm>
- Chang HC, Churchwell MI, Delclos KB, Newbold RR and Doerge DR. (2000) Mass spectrometric determination of Genistein tissue distribution in diet-exposed Sprague-Dawley rats. *J Nutr.* 130(8): 1963-1370.
- Cojocaru IM, Cojocaru M, Sapira V and Ionescu A. (2013) Evaluation of oxidative stress in patients with acute ischemic stroke. *Rom J Inter Med.* 51(2): 97-106.
- Coyle JT and Puttfarcken P. (1993) Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 262: 689-695.
- Desai I. (1984) Vitamin E analysis methods for animal tissues. *Meth Enzymol.* 105: 138-147.
- Devi KP, Shanmuganathan B, Manayi A, Nabavi SF and Nabavi SM. (2017) Molecular and therapeutic Targets of Genistein in Alzheimer's disease. *Mol Neurobiol.* 54: 7028-7041.
- Djordjevic MV, Brenneman KD and Hoffman D. (1989) Identification and analysis of nicotine derived N-nitrosaminoacid and other nitrosamine acids in tobacco. *Carcinogenesis.* 10: 1725-1731.
- Eidi A, Eidi M, Mahmoodi G and Oryan S. (2006) Effect of vitamin E on memory retention in rats: Possible involvement of cholinergic system. *European Neuropsychopharmacol.* 16: 101-106.
- Ellman GL, Courtney KD, Andres JV and Featherstone RM. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol.* 7: 88-95.
- Ellman GL. (1959) Tissue sulfhydryl groups. *Arch Biochem Biophys.* 82: 70-77.
- Farzane S, Faleh A, Ali S and Luca C. (2020) The cerebrovascular and neurological impact of chronic smoking on posttraumatic brain injury outcome and recovery: an in vivo study. *J Neuroinflammation* 17: 133.
- Frei B, Forte TM, Ames BN and Cross CE. (1991) Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma: protective effects of ascorbic acid. *Biochem J.* 277: 133-138.
- Gokulakrishnan A, Magendira MV, Liyakath AA, Thirunavukkarasu C. (2013) Epigallocatechin-gallate (EGCG) stabilizes the mitochondrial enzymes and inhibits the apoptosis in cigarette smoke-induced myocardial dysfunction in rats. *Mol Biol Rep.* 40: 6533-6545.
- Goto Y and O'Donnell P. (2001) Synchronous activity in the hippocampus and nucleus accumbens in vivo. *J Neurosci.* 21: 1-5.
- Grace AA. (2000) Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Res Rev.* 31: 330-341.
- Hall N and Deluca M. (1976) Electrophoretic separation and quantitation of creatine kinase isozymes. *Anal Biochem.* 76: 561-567.
- Halliwell B. (1987) Oxidants and human disease: some new concepts. *FASEB J.* 1: 358-364.
- International Agency for Research on Cancer: IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 83: Tobacco Smoke and Involuntary Smoking. International Agency for Research on Cancer, WHO, Lyon, France, 2004.
- Jacobsen LK, Krystal JH, Mencl WE, Westerveld M, Frost SJ and Pugh KR. (2005) Effects of smoking and smoking abstinence on cognition in adolescent tobacco smokers. *Soc Biol Psychiatry* 57: 56-66.
- Jalili C, Rashidi I, Roshankhah S, Jalili F and Salahshoor MR. (2020) Protective effect of Genistein on the morphine-induced kidney disorders in male mice. *Electron J Gen Med.* 17(3): em213.
- Kaisar MA, Sivandzade F, Bhalerao A and Cucullo L. (2018) Conventional and electronic cigarettes dysregulate the expression of iron transporters and detoxifying enzymes at the brain vascular endothelium: In vivo evidence of a gender-specific cellular response to chronic cigarette smoke exposure. *Neurosci Letts.* 682: 1-9.
- King J. (1965) The dehydrogenases or oxidoreductases—lactate dehydrogenase. In: *Practical clinical enzymology*, (ed.) Van D., London: Nostrand, pp. 83-93.
- Lee YB, Lee HJ and Sohn HS. (2005) Soy isoflavones and cognitive function. *J Nutr Biochem.* 16: 641-649.

- Lucchese C, Pinton S and Nogueira CW. (2009) Brain and lungs of rats are differently affected by cigarette smoke exposure: antioxidant effect of an organoselenium compound. *Pharmacol Res.* 59: 194-201
- Maulik SK, Prabhakar P, Dinda AK and Seth S. 2012 Genistein prevents isoproterenol-induced cardiac hypertrophy in rats, *Can J Physiol Pharmacol.* 90 1117-1125.
- Mendes AA, Souza L F, Walz R and Dafre AL. (2014) Perspectives on molecular biomarkers of oxidative stress and antioxidant strategies in traumatic brain injury. *BioMed Res Int.* 2014: 723060.
- Menzies D, Nair A, Williamson PA, Schembri S, Al-Khairalla MZ, Barnes M, Fardon TC, McFarlane L, Magee GJ and Lipworth BJ. (2006) Respiratory symptoms, pulmonary function, and markers of inflammation among bar workers before and after a legislative ban on smoking in public places. *JAMA* 296: 1742-1748.
- Metsios GS, Flouris AD, Jamurtas AZ, Carrillo AE, Kouretas D, Germenis AE, Gourgoulisanis K, Kiropoulos T, Tzatzarakis MN, Tsatsakis AM and Koutedakis Y. (2007) A brief exposure to moderate passive smoke increases metabolism and thyroid hormone secretion. *J Clin Endocrinol Metab.* 92: 208-211.
- Misra HP and Fridovich I. (1972) The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.* 247: 3170-3175.
- Moosavi F, Hosseini R, Saso L and Firuzi O. (2015) Modulation of neurotrophic signaling pathways by polyphenols. *Drug Des Devel Ther.* 10: 23-42.
- Mutinati M, Pantaleo M, Roncetti M, Piccinno M, Rizzo A and Sciorsci R. (2014) Oxidative stress in neonatology. A review. *Reproduction Domestic Animals* 49(1): 7-16.
- Oliveira MR. (2016) Evidence for genistein as a mitochondriotropic molecule. *Mitochondrion* 29: 35-44.
- Omaye ST, Tumball JD and Sauberlich HE. (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. *Method Enzymol.* 2: 1-11.
- Ozkol H, Tuluze Y and Koyuncu I (2012) Subacute effect of cigarette smoke exposure in rats: protection by pot marigold (*Calendula officinalis* L.) extract. *Toxicol Ind Health.* 28: 3-9.
- Paulson JR, Yang T, Selvaraj PK, Mdzinarishvili A, Van der Schyf CJ, Klein J, Bickel U and Abbruscato TJ. (2010) Nicotine exacerbates brain edema during in vitro and in vivo focal ischemic conditions. *J Pharmacol Exp Ther.* 332(2): 371-379.
- Pryor WA and Stone K (1993) Oxidants in cigarette smoke: radicals, hydrogen peroxide, peroxyhydrate, and peroxyhydrate. *Ann New York Acad Sci.* 686: 12-27
- Pryor WA. (1997) Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. *Environ Health Perspect.* 105(Suppl 4): 875-882.
- Reznick AZ, Cross CE, Hu ML, Suzuki YJ, Khwaja S, Safadi A, Motchnick PA, Packer L and Halliwell B. (1992) Modification of plasma proteins by cigarette smoke as measured by protein carbonyl formation. *Biochem J.* 286: 607-611.
- Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra WG. (1973) Selenium: biochemical role as a component of glutathione peroxidase. *Science* 79: 588-590.
- Shagirtha K, Muthumani M and Prabu SM. (2011) Melatonin abrogates cadmium-induced oxidative stress-related neurotoxicity in rats. *Eur Rev Med Pharmacol Sci.* 15: 1039-1050.
- Shahsavari F, Ghanadi K, Jafarzadeh M, Nasiri B and Forutani SH. (2013) Opium addiction decreases the T3 uptake amount. *Life Sci J.* 10(5s): 608-610.
- Sivandzade F and Cucullo L. (2019) Anti-diabetic countermeasures against tobacco smoke-dependent cerebrovascular toxicity: use and effect of rosiglitazone. *Int J Molec Sci.* 20(17): 4225.
- Slater TI. (1984) Overview of methods used for detecting lipid peroxidation. *Method Enzymol* 105: 283-293.
- Sonee M, Sum T, Wang C and Mukherjee SK. (2004) The soy isoflavone, genistein, protects human cortical neuronal cells from oxidative stress. *Neurotoxicol.* 25(5): 885-891.
- Staal GE, Visser J and Veeger C. (1969) Purification and properties of glutathione reductase of human erythrocytes. *Biochim Biophys Acta* 185 :39-48.
- Takahara S, Hamilton BH, Neell JV, Kobra TY, Ogura Y and Nishimura ET. (1960) Hypocata-lasemia: a new genetic carrier state. *J Clin Invest.* 29: 610-619.
- Uddin MS and Kabir MT. (2019) Emerging signal regulating potential of genistein against Alzheimer's disease: A promising molecule of interest. *Front Cell Dev Biol* 7: 197.
- Vauzour D. (2012) Dietary Polyphenols as modulators of brain functions: biological actions and molecular mechanisms underpinning their beneficial effects. *Oxid Med Cell Longev.* 2012: 914273.
- Wang JW, Wang HD, Cong ZX, Zhou XM, Xu JG, Jia Y and

- Ding Y. (2014) Puerarin ameliorates oxidative stress in a rodent model of traumatic brain injury. *J Surg Res.* 186(1): 328-337.
- Wei H, Wei L, Frenkel K, Bowen R and Barnes S. (1993) Inhibition of tumor promoter-induced hydrogen peroxide formation in vitro and in vivo by genistein. *Nutr Cancer* 20(1): 1-12.
- WHO (2016). Tobacco. <https://www.who.int/news-room/fact-sheets/detail/tobacco>.
- World Health Organization. (2019) WHO Global Report on Trends in Prevalence of Tobacco Use 2000–2025. 3rd ed. World Health Organization; Geneva, Switzerland, 2019.
- Zhang T, Wang F, Xu HX, Yi L, Qin Y, Chang H, Mi MT and Zhang QY. (2013) Activation of nuclear factor erythroid 2-related factor 2 and PPAR γ plays a role in the genistein-mediated attenuation of oxidative stress-induced endothelial cell injury. *Br J Nutr.* 109(2): 223-235.
- Zhou F, Wilson CJ and Dani JA. (2002) Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J Neurobiol.* 53: 590-605.