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Full Length Research Paper

Neuroprotective effect of Mulberry (*Morus nigra)* leaf extract on acrylamide – induced Zebrafish (*Danio rerio)*

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Based on previous studies, the possibility of harnessing the antioxidant potential of *Morus nigra* leaves to confer protection in acrylamide neurotoxicity was considered. This was studied in-vitro in acrylamide toxicity modelled Zebrafish (*Danio rerio*) by treatment with hydromethanolic extract of the leaf. This was supplemented by qualitative phytochemical analysis of the extract. The present study was designed to investigate the antioxidant activity of *Morus nigra* leaf extract using various assays. Out of these moderate increase in catalase activity, notable decrease in concentration of malondialdehyde (MDA) and a marginal increase in glutathione reductase activity was observed in zebrafish brain post treatment. This study highlights the potential of *Morus nigra* as a natural source of antioxidant or as an alternative drug source for the treatment of oxidative stress-related diseases such as Parkinson's, Alzheimer's, etc.

Keywords: *Morus nigra*, Zebrafish, Acrylamide, Oxidative stress, Antioxidants, Neurotoxicity

INTRODUCTION

At present there has been an unprecedented rise in the number of stress-related diseases that have been implicated in neurodegenerative disorders such as Alzheimer's, Parkinson's, etc. In the current scenario, lifestyle including dietary and environmental factors have hugely contributed to causing oxidative stress in the body cells which eventually lead to these diseased conditions. Promotion of a healthy lifestyle and proper nutrition are identified as factors to prevent the development of these diseases (Katarzyna et al., 2015). Incorporation of healthy functional foods containing bioactive components such as polyphenolic compounds, fibre, probiotics and prebiotics, active proteins and peptides, polyunsaturated fatty acids, minerals and vitamins can aid in the prevention and management of the aforesaid diseases (Katarzyna et al., 2015).

One of the known products containing bioactive ingredients is mulberry which is increasingly used for the production of functional foods (Katarzyna et al., 2015). *Morus nigra* is mainly cultivated for the purpose of sericulture. It belongs to the family *Moraceae* and is wildly cultivated in India, Southeast Asia, and China (Thanes et al., 2012). The presence of polyphenolic compounds like phenols, flavonoids, and anthocyanins confer the free-radical scavenging property to *M.nigra*. M.nigra leaves have been known to possess antibacterial. astringent, odontalgic, diphorectic, and ophthalmic properties.

Normal aging and other stresses cause decline in brain functions such as sensory and motor performance. This is due to accumulation of oxidative damage to lipids, proteins, nucleic acids and also make various neurotransmitters susceptible to oxidative stress. Among the many animal models used in the study of neurodegenerative disorders, *Danio rerio* is the vertebrate model increasingly used in human neuropharmacology and toxicology research. According to studies, 70 percent of protein-coding human genes are related to zebra fish genes, and 84 percent of the genes known to be associated with human disease have a counterpart in the zebra fish genome. These findings highlight the importance of the zebra fish model in human disease research (Jayanth et al., 2014). Acute exposure to acrylamide (ACR), a type-2 alkene, widely used in the paper and textile industries, cosmetics etc.,

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has been shown to cause oxidative stress in the human body. This toxicity can be effectively modelled in Danio rerio for the study of antioxidant properties of various bioactive substances.

The present study was designed to evaluate the neuroprotective role of *M.nigra* leaf extract against acrylamide toxicity in *Danio rerio*.

MATERIALS AND METHODS

Preparation of plant extract: 5% hydromethanolic extract of *M.nigra* leaves was prepared by homogenising with 1:1 water:methanol and incubated overnight in a shaking incubator. The filtrate was obtained and used for further analysis.

Phytochemical screening: Phytochemical examinations were carried out for the extract as per standard methods. The extract was screened for the presence of alkaloids, flavonoids, phenols, anthocyanin, terpenoids, tannins, phytosterols, carbohydrates, proteins and amino acids.

Experimental animal: The mutant variety of *Danio rerio* was obtained from Zooland Aquarium in Shivajinagar, Bengaluru. The fish were quarantined and stored in 5L aquarium and were fed with commercially available feed once every 24 hours.

Experimental design: The fishes were acclimatized for laboratory conditions and kept on normal diet for 1 week. Experimental fishes were divided into the following three groups of 10 fishes each:
Group I : Normal control group
Group II : Acrylamide control group. The fishes were exposed to 0.75mM acrylamide in 5L water for three consecutive days.
Group III : Acrylamide exposed fishes were given a dose of 1mL/L of hydromethanolic extract of *M.nigra* leaves daily for three consecutive days.

Biochemical studies: Five fishes were sacrificed from each group (I, II, III) after their respective periods of exposure. They were anaesthetized on ice and dissected. The brain was removed along with the skull case and homogenised using pestle and mortar with 5mL of 0.1M phosphate buffer each. The brain homogenate was centrifuged at 5000rpm for 15 minutes at 4℃ to remove cell debris. The supernatant was used for antioxidant assays of catalase, lipid peroxidation and glutathione reductase.

Assay for catalase: Catalase activity was measured by the method of Aebi (1974). The rate of decomposition of hydrogen peroxide was measured spectrophotometrically from changes in absorbance at 240 nm and catalase activity was expressed as rate constant (per second), (Figure 1).

Assay for Lipid peroxidation: The level of lipid peroxides was quantified by Thiobarbituric acid reaction method described by Ohkawa et al. (1979) by measuring the formation of thiobarbituric acid reactive substances (TBARS). The TBARS was extracted into 15:1 butanol:pyridine mixture and absorbance was measured in a UV-Visible spectrophotometer at 532 nm and expressed as nmoles of malondialdehyde (MDA) released(Figure 2) .

Assay for Glutathione Reductase: Glutathione reductase was assayed by the method of Stahl et al. (1963). The change in optical density of the reaction mixture, constituted by phosphate buffer, EDTA, GSSG, NADPH, and distilled water, after addition of brain homogenate was measured spectrophotometrically at 340nm for 2 minutes at 30 second intervals. The enzyme activity was expressed in micromoles per minute (Figure 3).

RESULTS

The phytochemical constituents screened are given in detail in Table 1. In general, an increase in oxidative stress was observed in the brain post-acrylamide exposure based on the antioxidant enzyme assays. A moderate decrease in catalase activity and a remarkable decrease in glutathione reductase activity was observed in Group II fishes in comparison with Group I. A highly significant increase in concentration of MDA was observed in group II.

The antioxidant properties of *M.nigra* leaves was ascertained by the following parameters:
Group III fishes showed moderate increase in catalase activity compared to Group II. A notable decrease in concentration of MDA and a marginal increase in glutathione reductase activity was observed in Group III fishes compared to Group II.

DISCUSSION

There is a requirement of advanced research about the mechanisms of acrylamide toxicity since humans are exposed to thermally processed foods such as chips, biscuits etc., which produce significant amounts of acrylamide (Tarake et al., 2002). Epidemiological studies of occupationally exposed human populations have identified neurotoxicity as an important outcome of acrylamide exposure, though carcinogenicity and reproductive toxicity have also been implicated. There

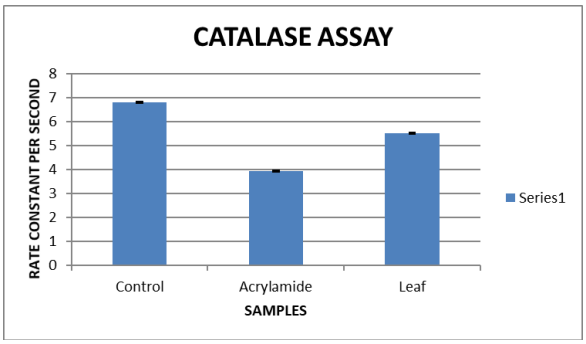

Figure 1. Catalase activity in control fish, acrylamide exposed fish and leaf extract treated fish.

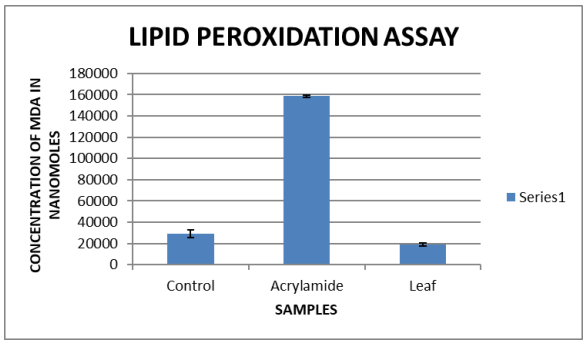

Figure 2. Malondialdehyde (MDA) concentration in control fish, acrylamide exposed fish and leaf extract treated fish.

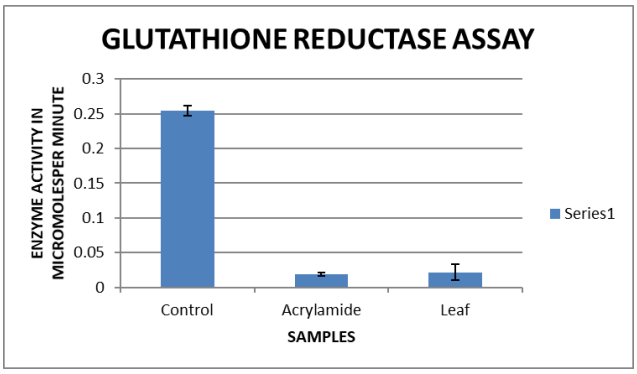

Figure 3. Glutathione reductase activity in control fish, acrylamide exposed fish and leaf extract treated fish

Table 1. Qualitative phytochemical screening of *M.nigra* leaf extract

Phytochemical tests	Leaf
Alkaloids	+
Phenols	+
Flavonoids	+
Terpenoids	+
Tannins	+
Phytosterols	+
Carbohydrates	+
Proteins	+
Amino acids	+
Saponins	+

is a pertinent need for approaches to evolve novel strategies to protect against ACR-mediated neurotoxicity since chronic exposure to ACR is capable of inducing neuropathic changes in humans (Lopachin, 2005).

The present study investigates the use of Danio rerio as an in-vivo model to ascertain if acrylamide toxicity involves oxidative damage and whether natural products such as M.nigra leaf extract could play a role in the amelioration of the acrylamide toxicity mediated via oxidative damage. Danio rerio serves as an excellent model to study neurotoxicity and neuroprotection since it provides a quick and cost-effective approach for large scale and high-throughput screening of natural products against various toxins that have direct pathological implications in humans (Allan et al., 2014).

Acrylamide toxicity might occur by mechanisms such as progressive decrease in protein content (Yousef et al., 2006). Oxidative stress is an important mechanism underlying acrylamide neurotoxicity (George et al., 2013). Melissa et al. (2018) has suggested that acrylamide neurotoxicity may occur via formation of adduct or modification of nucleophilic sulfhydryl groups on proteins which might affect the synaptic activity and also alter the function of antioxidant enzymes .This sort of oxidative damage has been implicated in the findings of our present study by the decrease in the levels of catalase activity and glutathione reductase activity, and increase in lipid peroxidation in the zebrafish brain.

The treatment of the acrylamide-exposed group of fishes with M.nigra leaf extract provided antioxidant enzyme activity values comparable to those of the control group of fishes with a significant increase in catalase activity, marginal increase in glutathione reductase activity, and a remarkable decrease in the level of lipid peroxidation as compared to the acrylamide control group. The propensity of M.nigra leaf extract to mitigate acrylamide induced oxidative stress is consistent with the data obtained from the preliminary qualitative phytochemical analysis of the extract, which reveals the presence of several bioactive compounds viz. terpenoids, phenols, alkaloids, flavonoids, tannins etc, which have been seen to possess free radical

scavenging property. Katsube et al. (2006) and Kim et al. (1999) isolated quercetin 3-(6-malonylglucoside) and rutin which are chief flavonol glycosides in mulberry leaves shown to possess free radical scavenging potential and confirmed to be antioxidative.

In summary, this investigation tested the neuroprotective propensity of M*orus nigra* leaf extract in *Danio rerio* with implications for neuropathy in humans. This extract significantly mitigates acrylamide-induced neurotoxic effects via elevated antioxidant function suggesting that it can be exploited for therapeutic application against neuropathy.

CONCLUSION

It can be concluded that as *M.nigra* leaves contain many bioactive compunds which possibly quenched the reactive oxygen species (ROS) produced by acrylamide hence showing protection against acrylamide neurotoxicity. Thus, the neuroprotective effect of compounds present in *M.nigra* leaves might be attributed to the possible augmentation of the acrylamide target in the antioxidant enzymes and thereby restoring normal function.

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