

Quercetin reverses sodium arsenate induced oxidative stress, behavioural and histological alterations in brain of rat

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Abstract

Aim: Sodium arsenate is a neurotoxic agent present in the environment and human are exposed to it through contaminated drinking water, food, soil and air. Quercetin is potent antioxidant with possible neuroprotective properties. This study reports antioxidant and protective role of quercetin against neurotoxicity produced by sodium arsenate in brain of the rat.

Methods: Animals were divided into four groups: control, sodium arsenate (100ppm/kg BW), sodium arsenate (100ppm/kg BW)+Quercetin (20mg/kg BW) and Quercetin (20mg/kg BW). The treatments were delivered to rats for 15 days. After the treatment period, behavioural (Rotarod, Hotplate test) studies were conducted and then brains were collected used for oxidative stress markers and histopathological studies.

Results: The results showed that sodium arsenate significantly ($P<0.05$) increased prooxidant lipid peroxidation level, and decreased antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase activities. Further, a significantly ($P<0.05$) declined motor coordination (Rotarod test) and enhanced the paw withdrawal latency period (Hotplate test) were observed. Histological alterations like neuronal damage and neural cells with irregular shape in cerebral cortex region of the brain were observed in sodium arsenate treated group compared to the control group. Quercetin attenuated these effects by significantly ($P<0.05$) lowering lipid peroxidation level and increasing superoxide dismutase, catalase, glutathione peroxidase activities, motor coordination towards control and reversing the paw withdrawal latency period and histological alterations compared to sodium arsenate group.

Conclusion: These findings have demonstrated that quercetin can act as an antioxidant and neuroprotective agent against brain damage induced by sodium arsenate.

Keywords: Quercetin, Sodium arsenate, Oxidative stress, Behavioural alterations, Cerebral cortex.

INTRODUCTION

Flavonoids a group of polyphenolic compounds present in different parts of plants have multiple advantageous properties for health whereas most of them are attributed to their antioxidant properties. Recently, Daniela et al., (1), reported flavonoids can behave as antioxidants because of their ability to scavenge free radicals and chelate metal ions. This antioxidant activity of flavonoids associated with hepatoprotective, cardio protective, anti-inflammatory and anti-diabetic activities (2, 3). Polyphenolic compounds can protect the human body from free radicals and retard the progress of many chronic diseases as well as heavy metals induced oxidative damages (4, 5). Naturally occurring dietary compounds also proposed for development as effective neuroprotective agents because of their neuroprotective properties (6). Among flavonoids, quercetin is the most ample in human dietary sources. It is present in numerous vegetables and fruits, but the highest concentrations available in onion. Quercetin is a potent antioxidant and therefore one of the main interest in the compound has involved protection against heart, liver and kidney syndromes (7, 8). Previous studies reported quercetin directly quenches free radicals and indirectly enhances the generation of enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and non-enzymatic antioxidants like reduced glutathione (9, 3). Dietary administration of quercetin has been shown to inhibit the histological alterations in the liver of rats (10). Quercetin is the major bioflavonoid and easily traverse the blood brain-barrier and acts as promising agents for intervention in neurodegenerative illnesses such as

dementia, amyotrophic lateral sclerosis and Parkinson's diseases (11). Moreover, recent studies reported that quercetin abrogated the neurotoxicant induce neurotransmitters alterations in rat (12). Quercetin has been studied for its potential utilisation as a safe alternative to the antioxidant and anti-inflammatory drugs used for conditions like heart diseases. Swapnila et al., (13), reported quercetin is a more potent antioxidant than any other antioxidant nutrients, such as β -Carotene, vitamin E and vitamin C on a molar basis.

Sodium arsenate (Na_3AsO_4) toxicity is a worldwide health problem affecting many millions of people. Contamination of arsenate is with natural geological sources like contaminating drinking water, leaching into aquifers and industrial processes. In industry, sodium arsenate is used to manufacture pesticides, herbicides, fungicides, insecticides, wood preservatives, cotton desiccants and paints (14). Sodium arsenate is also exposed to human through drinking water, additive in food, industrial wastes and inhalation of sodium arsenate dust particles (15). Goudarzi et al., (16), reported common symptoms of acute sodium arsenate poisoning symptoms such as vomiting, nausea, anorexia, abdominal pain, muscle cramps, hepatotoxicity and cardiac abnormalities. Oxidative stress is one of the mechanisms of sodium arsenate prompted toxicity that suggest cellular injury mediated by free radicals involve in the pathological alterations (17). Previous histopathological studies have shown that sodium arsenate caused pathological alterations in kidney tissue and mitochondrial swelling (18). Though the effects connected with sodium arsenate exposure are various, of particular interest are those acting on the central nervous

system, as they can elicit an abundance of biochemical alterations and behavioural abnormalities (19). Sodium arsenate neurotoxicity is manifested as a peripheral neuropathy involving both motor and sensory nerves resulting in numbness and paraesthesia, diminished sensation of pain, heat, touch and cold and muscle faintness (20). Kassab et al., (21), reported neurotoxic action of sodium arsenate in rats. In view of this, the present study report the toxic effects of sodium arsenate on oxidative stress, behavioural and neuro histological alterations in brain of rats and protective role of quercetin supplementation against arsenate exposure.

MATERIALS AND METHODS

Chemicals

Quercetin and sodium arsenate were purchased from Sigma Aldrich Company. All other chemicals used in the investigation were of analytical grade.

Animals and treatment

Experimental protocols were conducted in accord with guidelines of the Committee for the purpose of control and supervision on experimental animals and were approved by the Animal Ethical Committee of the Department of Zoology, Osmania University (CPCSEA No: 383/01/a/CPCSE), Hyderabad, Telangana, India.

Twenty four Wistar rats with a mean body weight of 220 ± 20 g were used for the experiments. They were housed in an animal room with normal controlled temperature ($22 \pm 2^\circ\text{C}$) and a regular 12h light-dark cycle. Animals were divided into 4 groups of 6 animals each and these animals were maintained at standard laboratory conditions and given doses for 15 days as follows:

1. **Group I:** Control group-received normal water.
2. **Group II:** Sodium arsenate group-received sodium arsenate (100ppm/kg BW) through drinking water.
3. **Group III:** Sodium arsenate+Quercetin group-received drinking water with Sodium arsenate (100ppm/kg BW) and Quercetin (20mg/kg BW) fed orally with gavage.
4. **Group IV:** Quercetin group-received quercetin (20mg/kg BW) through oral gavage.

After the experiment period we conducted behavioural studies and the rats were sacrificed and brains were dissected out to perform biochemical and histopathological studies.

Biochemical determinations

Determination of Lipid peroxidation content

Lipid peroxidation (LPO), in homogenates of brain was assessed by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content, according to the method of Garcia et al., (22). Briefly, an aliquot of tissue homogenate was mixed with 1 ml of 5% trichloroacetic acid (TCA) and centrifuged at 4000g for 10 min. About 1 ml of thiobarbituric acid reagent (TBA) was added to 500 μl of supernatant and heated at 95°C for 15 min. The mixture was then cooled and was measured for absorbance at 533 nm. The results were expressed as nano mole MDA/gm weight of tissue.

Determination of superoxide dismutase activity

Superoxide dismutase (SOD), activity was assayed according to the method of Marklund and Marklund (23). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM ethylene-diaminetetraacetate (EDTA), 13 mM L-methionine, 2 μM riboflavin and 75 μM nitro blue tetrazolium. The developed blue colour in the reaction was measured spectrophotometrically at 420 nm. The enzyme activity was expressed as Units/mg protein.

Determination of catalase activity

Catalase (CAT), activity was assayed by the method of Aebi (24). Enzymatic reaction was started by adding an aliquot of 20 μl of the homogenised tissue and the substrate (H_2O_2) to a concentration of 0.5 mM in a medium containing 100 mM phosphate buffer, pH 7.4. The rate of H_2O_2 decomposition was followed by monitoring absorption at 240 nm. One unit of activity is equal to the moles of degraded/min/mg/ protein.

Determination of glutathione peroxidase activity

Glutathione peroxidase (GPx) activity was assessed by the method of Flohe and Gunzler (25). The supernatant was homogenised with the extraction buffer KNaHPO_4 (pH 7.8) and centrifuged during 10 min at 3000 rpm. To the enzymatic extract was added $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, and finally, GPx was estimated using 5',5'-dithiobis-2-nitrobenzoic acid (DTNB). Absorbance was recorded at 412 nm.

Behavioural tests

Rotarod test:

The rotarod test was performed according to the method Hutter et al., (26). The rotarod test is widely used to measure the fore and hind limb coordination, motor skills, and continues to be a primary method for the study of motor learning. The time of the instrument (DolphinTM instruments) adjusted to 0 s and the rotational speed to 20 RPM before the experimentation. The time was noted, and the results were analysed. The results were expressed as time in seconds.

Hot plate test:

The hot plate test was performed according to the method Gunn et al., (27). Rat's response latency to either a hind-paw lick or a jump on the hot plate (Analgesiometer - Eddy's Hot Plat) was recorded. In the absence of a response, the animals were removed from the hot plate at 60 seconds (cut off time) and a 60 seconds latency was assigned as the passive response. The results were expressed as time in seconds.

Histopathology:

The Golgicox stock solution-fixed brain tissues were sliced at 4-10 μm thickness, mounted on silanized slides, and subjected to golgicox staining according to the histopathological methods (28). Histopathological changes were observed using a light microscope (Magnification 40X).

Statistical Analysis:

The results are expressed as the mean \pm standard error of the mean (SEM). Comparison of means were conducted using one way analysis of variance followed by least

significant difference post hoc test to compare means between the different groups. Differences were considered as significant ($P < 0.05$). Statistical analyses were performed using SPSS version 20 software.

RESULTS

Effect of Quercetin on sodium arsenate induced oxidative stress in brain

The effects of sodium arsenate exposure on brain lipid peroxidation, superoxide dismutase, catalase and

glutathione peroxidase the protective efficacy of Quercetin in these rats are shown respectively in **Figure-1, 2, 3 & 4**. MDA level significantly ($P < 0.05$) increased, while superoxide dismutase, catalase and glutathione peroxidase activities significantly declined in sodium arsenate exposed rats. Oral administration of Quercetin significantly ($P < 0.005$) reversed elevated level of MDA and decreased SOD, CAT and GPx activities in sodium arsenate pre-exposed rats.

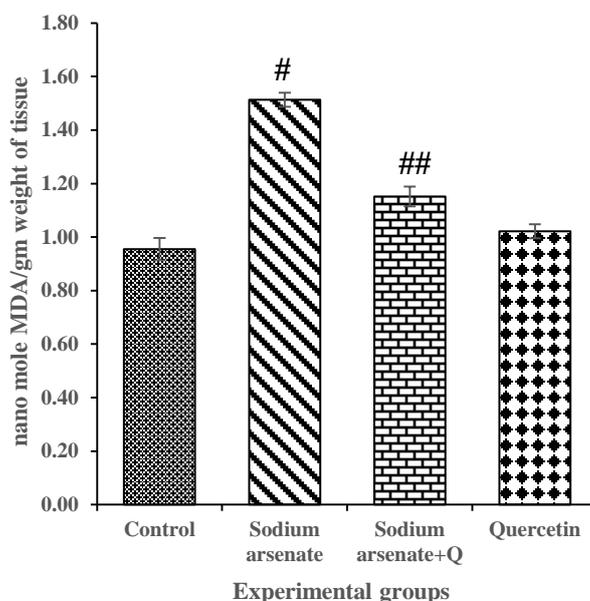


Figure-1: Effect of quercetin treatment on lipid peroxidation content in rats subjected to sodium arsenate treatment for 15 days. [#] $P < 0.05$ as compared to control group and ^{##} $P < 0.05$ as compared to sodium arsenate treated group. Data expressed as the mean \pm S.E.M (n=6) and results expressed in nano mole MDA/gm weight of tissue.

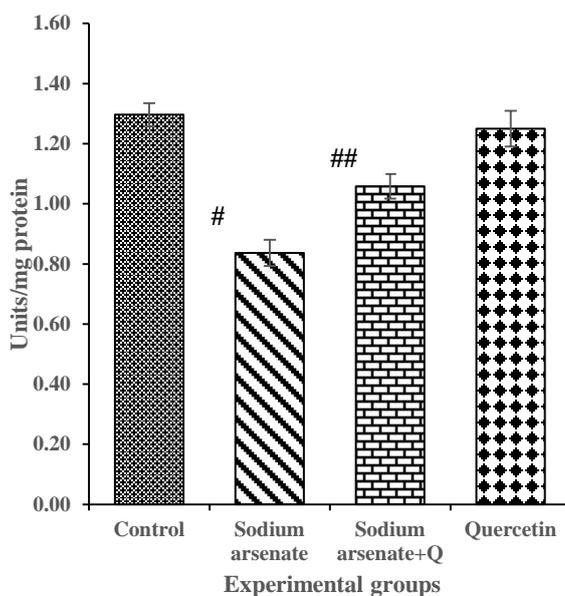


Figure-2: Effect of quercetin treatment on superoxide dismutase activity in rats subjected to sodium arsenate treatment for 15 days. [#] $P < 0.05$ as compared to control group and ^{##} $P < 0.05$ as compared to arsenate treated group. Data expressed as the mean \pm S.E.M (n=6) and results expressed in Units/mg protein.

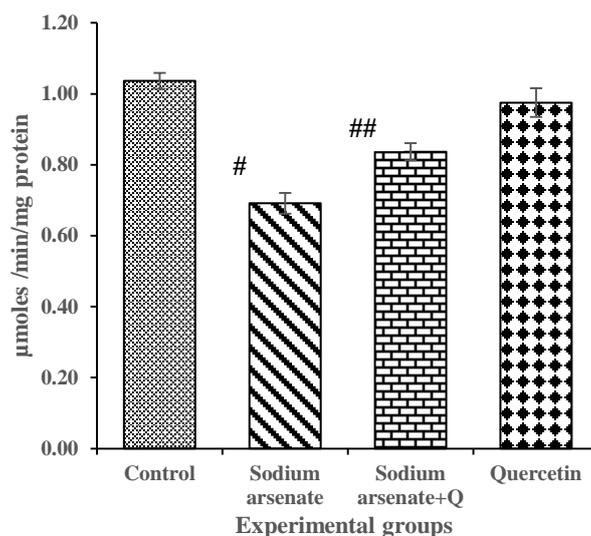


Figure-3: Effect of quercetin treatment on Catalase activity in rats subjected to sodium arsenate treatment for 15 days. [#]P<0.05 as compared to control group and ^{##}P<0.05 as compared to sodium arsenate treated group. Data expressed as the mean±S.E.M (n=6) and results expressed in µmoles /min/mg protein.

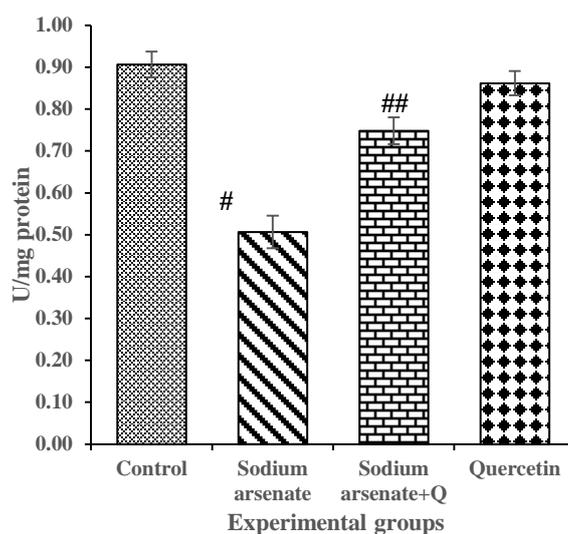


Figure-4: Effect of quercetin treatment on glutathione peroxidase activity in rats subjected to sodium arsenate treatment for 15 days. [#]P<0.05 as compared to control group and ^{##}P<0.05 as compared to sodium arsenate treated group. Data expressed as the mean±S.E.M (n=6) and results expressed in U/mg protein.

Effect of Quercetin on sodium arsenate induced behavioural alteration

Figures 5 & 6 shows effect of Quercetin on sodium arsenate induced behavioural alteration in rats. The motor coordination (Rotarod test) significantly decreased, while paw withdrawal in hot plate test latency period significantly (P<0.05) increased in sodium arsenate intoxicated rats compared to normal rats, and sodium arsenate+quercetin and quercetin treated rats showed significantly (P<0.05) improved the motor coordination and paw withdrawal latency period compared to sodium arsenate exposed rats.

Effect of Quercetin on sodium arsenate induced histopathological changes

Microphotographs of neurons in rat brain are shown in Figure 7. The neural cells with irregular shape and cyton, axon and dendrite were degenerated in sodium arsenate exposed rat brain. The sodium arsenate+quercetin, quercetin alone administrated groups were noticed with neurons arranged closely, axon, cyton and dendrite was clear in the cerebral cortex region of brain as compared to sodium arsenate intoxicated group.

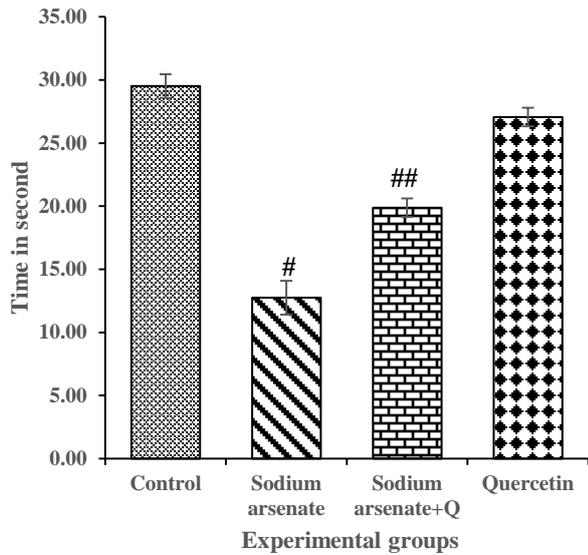


Figure-5: Effect of quercetin treatment on motor coordination (Rotarod test) in rats subjected to sodium arsenate for 15 days. #P<0.05 as compared to Control group and ##P<0.05 as compared to sodium arsenate treated group. Data expressed as the mean±S.E.M (n=6) and results shown in time in seconds.

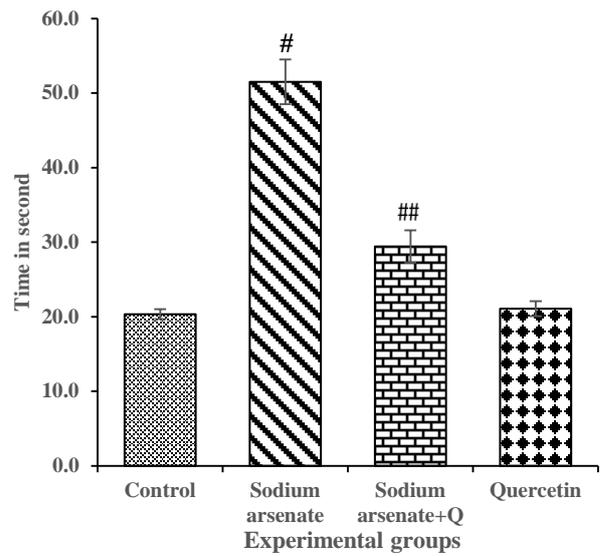


Figure-6: Effect of quercetin treatment on latency period (Hotplate test) in rats subjected to sodium arsenate for 15 days. #P<0.05 as compared to Control group and ##P<0.05 as compared to sodium arsenate treated group. Data expressed as the mean±S.E.M (n=6) and results shown in time in seconds.

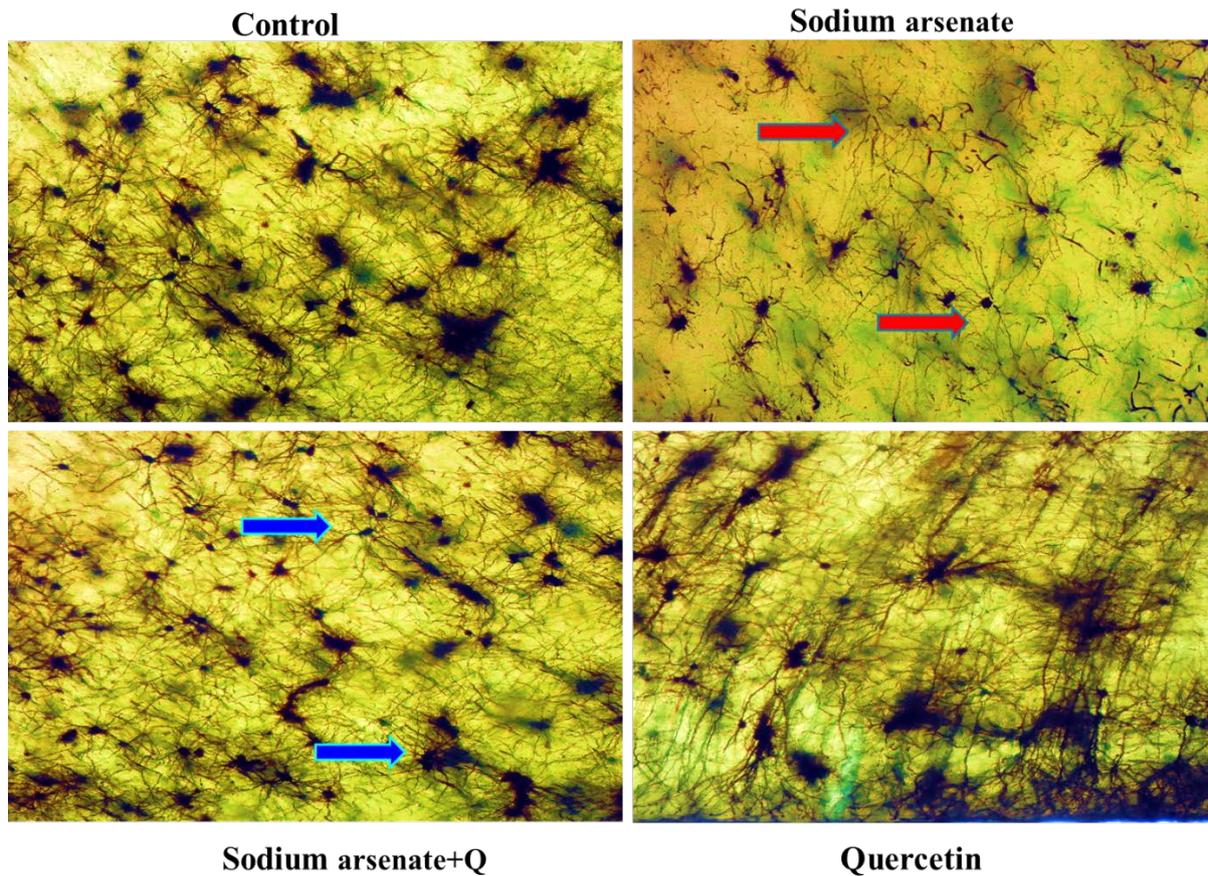


Figure-7: Brain Histopathological studies in cerebral cortex by golgicox stain in control, sodium arsenate, sodium arsenate + quercetin, quercetin alone treated groups. (Magnification 40x). blue colored arrow mark showing the neurons arranged closely, axon, cyton and dendrite was clear in sodium arsenate+quercetin group compared to sodium arsenate group and red colored arrow mark showing the neural cells with irregular shape and axon, cyton, and dendrite was degenerated in the sodium arsenate group compared to sodium arsenate+quercetin group.

DISCUSSION

The purpose of this study was to evaluate the efficacy of quercetin on oxidative stress, behavioural and histopathological alterations induced by sodium arsenate in brain of rat. The results suggest generation of reactive oxygen species, lipid peroxidation, behavioural and histological changes might contribute to sodium arsenate induced toxic effects in the experimental rats. Treatment with quercetin provided significant protection to the sodium arsenate induced oxidative stress, behavioural impairment besides histological alteration in brain of rat.

Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the body's natural antioxidant defense systems causing damage to deoxyribonucleic acid, lipids and proteins. Earlier report suggest the lipid peroxidation, in sodium arsenate exposure (29). Because brain is rich in polyunsaturated lipids, and is dependent on aerobic metabolism, it is highly vulnerable to oxidative damage. Earlier studies revealing increased lipid peroxidation content in the hepatic tissue of sodium arsenate intoxicated rats (30). Sodium arsenate induced free radical toxicity has been reported recently in the soft tissues of animals (18). Antioxidant defense systems activate in our body to scavenge ROS for the protection from body against oxidative stress. The catalase, superoxide dismutase, GSH and Glutathione peroxidase are considered as key antioxidant components playing vital role in oxidative stress (31). Accumulation of sodium arsenate in brain can disrupt the synthesis and release of certain oxidative stress markers and histological alteration such as irregular shape of neurons and neural damage (21). Acute administration of sodium arsenate altered antioxidant enzymes system (32). Previous study of Wafa et al., (17), suggest that antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase activities altered in sodium arsenate exposed rats. Flavonoids, including quercetin, comprise the most common group of polyphenolic compounds in the human diet. Quercetin has strong antioxidant properties and may be beneficial to endogenous antioxidative enzyme position. Nabavi et al., (2), reported quercetin beneficial effects on liver damage by enhancing antioxidant enzyme activity and decreasing pro-oxidant effect.

Quercetin is known to attenuate oxidative stress in tissues of rats (33, 34). Federico et al., (35), demonstrated that the free radical scavenger activity of quercetin depends on the arrangement of functional groups on its core structure. Quercetin supplementation reversed the antioxidant enzymes activities altered by fluoride (36). Hence, quercetin also mitigated the rise in protein carbonyls, a general marker of oxidative damage to amino acids in proteins. In the current study, superoxide dismutase, catalase and glutathione peroxidase activities were depleted in sodium arsenate treatment, accompanying the over generation of lipid peroxidation in brain, which were in agreement with other studies (37). Sodium arsenate along with quercetin exposed rats showed significantly improved activities of lipid peroxidation, superoxide dismutase, catalase and GP_x enzymes suggesting its antioxidant action.

The hippocampus, cerebral cortex and cerebellum parts of the brain and involved in tasks such as motor coordination, learning and memory. Previous research has reported that sodium arsenate exposure may lead to neurodegenerative disease, including neurobehavioral alteration, behavioural abnormality and learning impairment caused by brain damage (38). Hence, the present results demonstrated sodium arsenate induced brain damage via behavioural tests (Rotarod and hotplate tests) of rat, and the protective effect of quercetin was confirmed. According to earlier studies, quercetin showed an ameliorating effect on cognitive function against heavy metals-induced behavioural alterations in animal models (12). In our results, the sodium arsenate exposed group showed declined motor coordination ability and enhanced latency period compared with the control group, and this cognitive alterations affected by sodium arsenate was consistent with the findings of a previous study (39). The sodium arsenate+Quercetin, Quercetin treated groups showed improved motor coordination ability and decreased latency period against sodium arsenate induced behavioural deficit, and these useful effects may be considered as affected by quercetin. Moreover, brain injuries are associated to behavioural impairment. According to Mehdi et al., (20), sodium arsenate exposed rats showed decreased behavioural activities that is due to altered spatial memory and locomotor activities. In addition, quercetin treatment showed enhanced motor coordination and memory in animals exposed to neurotoxicant (40). These results suggest that quercetin may have a significant effect on improving behavioural activities.

In the present study light microscopic examination of brain from sodium arsenate treated rats showed histopathological alterations. These finding are similar to sodium arsenate induced histological alterations in kidney of rat (18). Also Singh et al., (41), found histopathological changes that arsenic caused a significant injury to the kidney resulting in marked tubular damage, tubular dilatations, loss of brush border and tubular necrosis, nephritis along with mitochondrial swelling and acute renal failure. Quercetin, a polyphenolic flavonoid had recently drawn attention due to their antioxidant properties. Earlier studies reported quercetin has ameliorative role against toxicant generated histological alterations (42, 43). According to Chan-Min et al., (44), quercetin mitigated the liver histological changes, including leukocyte infiltration and extensive hepatocellular necrosis. This study reports sodium arsenate induced altered histopathological changes such as neurons, axon structure, density and morphology as well as neuron degeneration in sodium arsenate intoxicated rats. Whereas quercetin treatment abrogated the histopathological alterations induced by sodium arsenate. Thus, quercetin improves antioxidant status, behavioural function and reverses histological alterations generated by sodium arsenate, exhibiting quercetin neuroprotective effect on sodium arsenate induced toxicity.

CONCLUSION

In conclusion, this study demonstrates that quercetin has potent protective effects against sodium arsenate generated oxidative stress along with behavioural and histopathological alterations in the brain of rat. Quercetin attenuated sodium arsenate induced oxidative stress markers alterations such as lipid peroxidation, superoxide dismutase, catalase and glutathione peroxidase in the brain of rat. Quercetin treatment successfully reversed sodium arsenate induced declined motor coordination and increased paw withdrawal latency period in rat. Thus, quercetin is more potent neuroprotectant against sodium arsenate induced neurotoxicity.

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