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Original Article

PROTEINACEOUS COMPOUNDS FROM FRAGARIA ANANASSA FRUIT ATTENUATES PARAQUAT INDUCED PARKINSON LIKE LOCOMOTOR AND MITOCHONDRIAL ALTERATIONS IN ZEBRAFISH

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ABSTRACT

Objectives: To assess the Parkinson like locomotor and mitochondrial alterations, associated with the exposure of paraquat (PQ), *in vivo* preventive effect of proteinaceous compounds extracted from *Fragaria ananassa* fruit (FA-D) against mitochondrial dysfunction induced by paraquat in zebra fish using brain mitochondria.

Methods: Parkinson like locomotor and mitochondrial alterations were resulted by intra peritoneal administration of 55 mM PQ alternatively for a period of 7 days. The water soluble proteinaceous compounds from *Fragaria ananassa* fruit were obtained by Ammonium sulphate fractionation. The molecular weight of FA-D fraction was determined by SDS-PAGE and we found three distinct bands at 20.0 kDa, 17.0 kDa and 14.4.0 kDa respectively. The *in vitro* antioxidant activity and the *in vivo* preventive effect of FA-D against PQ induced Parkinsonian symptoms were evaluated by different assay systems viz., *in vitro*: radical scavenging activity by DPPH reaction and *in vivo*: locomotion, dopamine levels, and complex-I activity, mitochondrial ROS levels, cytochrome c release and mitochondrial morphology.

Results: The results show that paraquat altered locomotor activity and increased dopamine levels. Mitochondria isolated from paraquat treated zebrafish showed a marked inhibition of complex-I activity, increase in mitochondrial reactive oxygen species (mt ROS) and cytochrome c release and disintegration of mitochondrial structure. Treatment of 0.25 mg/kg body weight of FA-D fraction once in alternative days, for 5 days subsequent to the administration of PQ alternatively for a period of 7days, substantially reduced mt ROS levels and markedly restored the complex-I activity, cytochrome c release and mitochondrial morphology.

Conclusion: The results strongly suggest that proteinaceous compounds from *Fragaria ananassa* fruit recuperate paraquat induced Parkinsonian like symptoms by protecting the mitochondria.

Keywords: Paraquat, Locomotion, Mitochondria, *Fragaria ananassa*, Proteinaceous compounds.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder has a prevalence of 1-2% in people over the age of 50 [1]. The disease is characterized predominantly by resting tremors, bradykinesia, muscular rigidity, and postural instability, along with several nonmotor symptoms resulted from loss of dopaminergic neurons [2]. Recent research has shown that oxidative stress and oxidative stress-targeted activation of mitochondrion-dependent programmed cell death pathways are the major contributors to the dopaminergic neuron degeneration in PD. Therefore, search for new and more effective antioxidant therapeutic approaches targeting mitochondrial dysfunction and oxidative stress can have a great promise so as to prevent the progression of the disease [3].

Recently, researchers indicated that neurotoxins such as rotenone, 6-hydroxydopamine (6-OHDA) and paraquat can induce Parkinson-like symptoms in zebrafish, and this may be a useful PD model because of the complete development of the zebrafish nervous system, low costs and low dosage. Experimental studies have demonstrated that chronic exposure of zebrafish to paraquat a toxic herbicide can result in Parkinsonian symptoms [4], including selective loss of dopaminergic neurons and locomotor deficits and that paraquat-based Parkinson's model can be used to screen a small volume of drugs or compounds for PD intervention.

New research discovered that foods that are antioxidant rich, like blueberries, strawberries and acai berries, may be beneficial in helping prevent Parkinson's disease. Strawberry and its anthocyanins has also been shown to reduce SH-SY-5Y neuroblastoma cell loss by decreasing the expression of pro-apoptotic genes bax, bad, gadd5 and fas-ligand [5].

Besides phenols, alkaloids and some plant secondary metabolites with known antioxidant properties and plant peptides could also be beneficial for this purpose [3, 4]. The findings of several studies have evidenced that protein/peptide from plant proteins can act as direct scavengers of diverse free radicals or behave as antioxidants in model systems [6-10]. In recent years, the antioxidant activities of proteins/peptides hydrolysates from plant-derived proteins, including *Sphenostylisstenocarpa* [8], hemp seed [6], phaseolin and bean [9], *Ziziphusjujuba* fruits and *Jatrophacurcas* [11], have been evaluated using several *in vitro* antioxidant evaluation systems.

However, no study to our knowledge has investigated the characteristics of PD, such as mitochondrial dysfunction, in this model. Based on these observations, we first tested zebrafish with intraperitoneal paraquat injections and then reversed the locomotor disruptions and mitochondrial dysfunction by treatment with proteinaceous compounds from *Fragaria ananassa* fruit. The present study is an attempt to gain insight into the neuroprotective effect produced by proteinaceous compounds in *Fragaria ananassa* fruit against paraquat induced neuronal toxicity in zebrafish.

MATERIALS AND METHODS

Fruit material and Zebrafish

Fragaria annassa were procured from a local vegetable market for extract preparation. Zebra fishes (30 fishes, 1 month old) were housed in the Small Zebra fish Aquarium Facility of Sathyabama University. The zebra fishes were kept in aged oxygenated tap water in normal glass aquarium tanks under a 14:10 h light/dark cycle. Water temperature was kept at 26-29 °C and pH at 6.5-7.5. Fishes were fed twice a day with Artemia and once a day with dry food (Pet

Dry) during working days and once a day with Artemia on weekends.

Water soluble protein extraction from fruits (FA)

The extraction was performed essentially as per the protocol described in [12] with minor modifications. To obtain protein extract, frozen fruits were soaked in water, repeated until discoloration and then thoroughly homogenized with Remi motor homogenizer. The sample tube was kept in an ice bath while grinding. After grinding, samples were centrifuged at 10,000 × g for 10 min at 15 ˚C to pellet insoluble debris. The supernatant was treated with 1% insoluble polyvinylpyrrolidone (PVP) and stirred continuously for 4 h at 4 °C. PVP-bound phenolics were removed by centrifugation at 10,000 rpm and the supernatant after reduction its volume to about (1/3) by lyophilization, was kept for further investigation.

Ammonium Sulphate fractionation of the aqueous extract (FA-D)

The crude extract (FA) was next precipitated with 80% ammonium sulfate at room temprature [13]. Following centrifugation at 10,000 rpm, total proteinaceous compounds of *Fragaria ananassa* fruit were dissolved in water and dialyzed against distilled water. The protein was further precipitated using cold acetone method [14]. The protein content of FA and FA-D samples was quantified by Standard protocol and then lyophilized before storage at −20 °C.

SDS-PAGE analysis

FA-D was analyzed by SDS-PAGE according to the method of [15]. FA-D protein sample was mixed with sample buffer containing 2-Mercaptoethanol as reducing agent and heated for 5 min at 100 ºC and then loaded into the sample wells of 4% stacking gel. Electrophoresis was performed using 18% Tris-tricine gel along with standard marker protein and silver stained for visulaisation of protein bands.

***In vitro* antioxidant assay**

DPPH radical scavenging assay

Synthetic radical Diphenyl-1Picrylhydrazyl (DPPH) is widely used to assess radical scavenging activity of food extracts, due to having more stability than conventional natural radicals [16]**.** DPPH radical scavenging activity (RSA) was determined by using the method of [17]. The fractions were dissolved in distilled water at 1 mg/ml and 0.5 mg/ml. 200 µl of each sample was then mixed with 600 µl of methanol and 200 µl of DPPH (0.15 mM in methanol). The mixture was shaken vigorously for 2 min, kept for 30 min in the dark at room temperature. The absorbance of the mixture was measured at 517 nm using a UV–Vis spectrophotometer. A lower absorbance represents a higher DPPH scavenging activity. The control was conducted in the same manner, except that distilled water was used instead of the sample. The control contained 800 µl of methanol and 200 µl of DPPH (0.15 mM). Ascorbic acid was used as a standard. DPPH radical scavenging ability was calculated using the following equation:

$$\text{RSA (\%)} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100$$

Effect of proteinaceous compounds from *Fragaria ananassa* fruit paraquat induced zebrafish model of parkinson's disease

Intraperitoneal injection

The Zebrafish were grouped into experimental groups each containing minimum 15-20 in number. The dosage of FA-D was expressed in terms of their protein content. The experimental groups used for the tests comprise. A) Experimental Parkinsonism induced group (55 mM PQ was administered intraperitoneally (i. p.), alternatively for a period of 7days). B) FA-D control group (0.25 mg/kg body weight suspended in physiological saline (PS) was administered once in alternative days for 5 days). C) FA-D treated group ((FA-D+PQ) 0.25 mg/kg body weight of FA-D fraction was administered once in alternative days for 5 days subsequent to the administration of PQ alternatively for a period of 7days) D) Control group (PS-injected but otherwise identically treated fishes served as control group). The injections were conducted using syringes with a mean injection volume of 5 µl/g body weight.

The fishes were sacrificed after 24 hours, following PQ injections, to observe the effects of FA-D. The dopamine levels, morphology of mitochondria by TEM, complex-I activity, Cytochrome C release and mitochondrial ROS levels were analyzed.

Locomotor activity assessment

The locomotor activity of zebra fish was measured as per the protocol followed by [17] with slight modifications. Small experimental tank (30 cm X 10 cm X 15 cm) containing 3 l waters was used to assess the locomotor activity of zebrafish. A transparent plastic film was placed in front of the tank indorder to divide the tank into four segments. Fish were placed individually in the tank and their behavior was video recorded for 5 min after a 10 min habituation period. Spontaneous swimming activity was measured by recording the distance.

Dopamine measurement

The content of dopamine was determined according to the method of [19]. Briefly, whole brain tissue was homogenized in 0.5 ml of cold perchloric acid (0.4 M). Subsequently, the sample was centrifuged at 20,000×g for 10 min at 4 ◦C, and the supernatant was transferred to a clean tube and measured for volume. One-half volume of a solution containing 0.02 M potassium citrate, 0.3 M potassium dihydrogen phosphate, and 0.002 M Na_2EDTA was added to the supernatant and mixed thoroughly to deposit perchloric acid. After incubation in an ice bath for 60 min, the mix was centrifuged at 15,000 g, for 20 min at 4 ◦C. Supernatants were analyzed for dopamine by HPLC (125 mm × 3 mm I.D. column, packed with Nucleosil 100 C 18; 3 5m particle size) and electrochemical detection (INTRO, ANTEC Leyden, The Netherlands; cell potential= 800mV). The mobile phase consisted of 5% acetonitrile, 10g/lcitricacid,4g/lKH2PO4, 0.1g/lEDTA and 0.175g/l octane sulfonicacid; pH=3.0.

Isolation of mitochondria from zebrafish

50 Zebrafish (*Danio rerio)* were sacrificed and the brain was dissected. The brain mitochondria were isolated as per the procedure given by [20] with slight modifications. Brain tissues were homogenized, in 10 ml of ice-cold grinding buffer. The homogenate was enriched by a three-step centrifugation: two centrifugations at 3,000 rpm for 5 min (organelles in supernatant) and one centrifugation at 10,000 rpm for 10 min (organelles in pellet). The pellet was re suspended in washing buffer and layered on top of three-step sucrose gradients (gradients of 30 ml containing 10 mL of 18% [w/v], 10 mL of 23% [w/v], and 10 mL of 40% [w/v] sucrose in 10 mM KOH, pH 7.2). After centrifugation for 45 min at 10,000 *rpm*, the mitochondria was isolated from the 23%/40% interphase. To remove the sucrose the purified mitochondria were centrifuged twice in "resuspension buffer" for 10 min at 5,000 *rpm*. Thus the mitochondria were isolated. The protein content was quantified by Standard protocol.

Analysis of mitochondrial morphology by transmission electron microscopy

10 µl brain mitochondria suspension was taken and rinsed in 0.1 M phosphate buffer (pH 7.2). The mitochondria pieces were trimmed and immediately fixed into 3% ice-cold glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and kept at 4 °C for 12 h. Then, tissue processing for TEM study was carried out. The grids containing sections were stained with 2% uranyl acetate and 0.2% lead acetate., These sections were examined under a TEM (×20,000). Samples were visualized using JEOL 1230 transmission electron microscopy. All microscopy measurements were performed using the TEM Facility available at Centralized Instrumentation Laboratory (CIL) In Tamil Nadu Veterinary College, Vepery, Chennai.

Measurement of complex I activity

Complex I activity was determined by monitoring the decrease in absorbance at 340 nm due to the oxidation of NADH [21]. The reaction mixture contained 250 mM sucrose, 1 mM EDTA, 50 mM Tris–HCl, pH 7.4, 2 µg/ml Antimycin A, 2 mM KCN, 0.15 mM

coenzyme Q1, and 20–40 ug mitochondrial homogenate. The total assay volume was 1 mL and the reagents were pre-warmed for 2 min at 30 °C. The reaction was initiated by addition of 0.1 mM NADH and the rate of decrease in absorbance was monitored spectrophotometrically at 340 nm for 3 min.

Measurement of cytochrome C (Cyt C)

CytC content in the mitochondrial suspension was determined using spectrophotometer essentially according to the protocol described in [22]. Both reference and sample cuvette contained the specimen, 5 mM NADH and 1 µM antimycin A, whereas the sample cuvette also contained 3 mM ascorbate, 100 µM N,N,N′,N′-tetramethyl-p-phenylenediamine, and 2 mM KCN.

Assay for mitochondrial reactive oxygen species

5 µM Dihydrodichlorofluorescindiacetate (DCFDA) in 1.0 mL assay containing 50 µg of proteins from mitochondria was used to assay the levels of ROS in mitochondria as described before [23].

RESULTS

Extraction of proteinaceous compounds from fruits of *Fragaria ananassa* (FA-D)

Our initial effort was to extract proteinaceous compounds from *Fragaria ananassa* fruits. The extraction was performed using 200 g of fruit weight. 500 mg precipitate was obtained from fraction after 80% ammonium sulphate precipitation. The protein concentration of strawberry fruit extracts after ammonium sulphate precipitation was 12.06 mg with 50% DPPH radical scavenging activity using 0.45 mg/ml protein. The ammonium sulfate precipitation (FA-D) was submitted to SDS-PAGE analysis to resolve its molecular weight. Based on silver staining, we observed three bands ~ 20.0 kDa, ~ 17.0 kDa and ~ 14.4.0 kDa respectively depicted by (fig. 1). The FA-D precipitate was dialyzed and lyophilized for subsequent analysis.

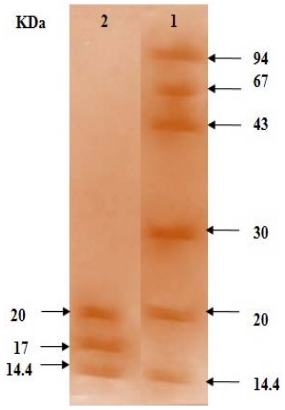


Fig. 1: **Silver Stained electrophoretogram of Ammonium Sulphate fractionation of the aqueous extract of *Fragaria ananassa* fruit (FA-D). Lane 1: Silver stained electrophoretogram (on 10% SDS-PAGE) of FA-D fraction showing different protein bands. Lane 2: shows standard protein markers (94KDa-10KDa)**

Intraperitoneal administration of paraquat (PQ) Caused PD-Like alterations in zebrafish

We generated a paraquat-based animal model using wild type zebrafish. Paraquat was administered intraperitoneally (i. p), at various concentrations for a period of 7 days. There was a progressive dose dependent decline in locomotor activity in zebrafish with paraquat exposure. Paraquat at 55 mM caused significant locomotor impairment and early mortality when compared to the untreated group (fig. 2 and inset). Thus, we used this concentration to conduct further studies.

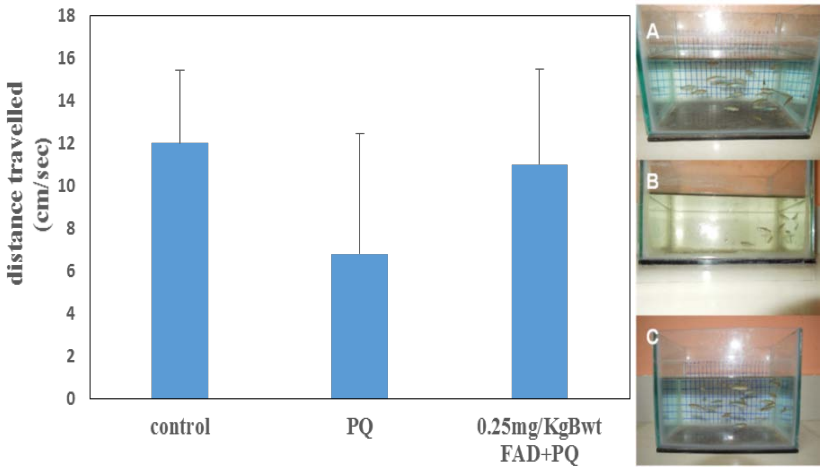


Fig. 2: **Effects of Ammonium Sulphate fractionation of the aqueous extract of *Fragaria ananassa* fruit (FA-D) on zebrafish locomotor activity. The inset shows three fish tanks with (A) Control group (B) Experimental Parkinsonism induced group (55 mM PQ) (C) FA-D treated group (FA-D+PQ)**

We also analyzed PQ zebrafish for possible alterations in the brain content of dopamine as behavioral paradigms sensitive to dysfunction of the dopaminergic pathway. Our results showed that the dopamine contents in PQ zebrafish brain were markedly higher than those of control fish (fig. 3).

Mitochondrial dysfunction has long been implicated in the pathogenesis of PD. Hence, the alterations of mitochondrial biology such as complex-I activity, mitochondria-derived ROS (mt ROS), cytochrome c release and mitochondrial morphology were assessed in brains from zebrafish with paraquat exposure. In this context a diminished complex-I activity was observed in zebrafish administrated with paraquat (fig. 4) when compared to the control fishes.

In addition mitochondrial ROS was measured using DCF fluorescence as an indicator (fig. 5), we observed a significant increase in the mitochondrial ROS in PQ treated zebrafish as compared control group. Subsequently the cytochrome c was measured in the mitochondria of the PQ treated zebrafish by its characteristic absorption band around 560 nm. As shown in fig. 6-Trace A, a weak signal was observed at 560 nm, shows the cytochrome c release from mitochondria in the PQ treated zebrafish in comparison with untreated fish.

We next examined whether paraquat triggered mt ROS generation and cytochrome c release affects the mitochondrial morphology employing Transmission scanning electron microscopy. In

unexposed control fishes, the morphology of the mitochondria did not change significantly (fig. 7A). However, fishes exposed to paraquat showed a striking disintegration of the mitochondrial morphology (fig. 7B).

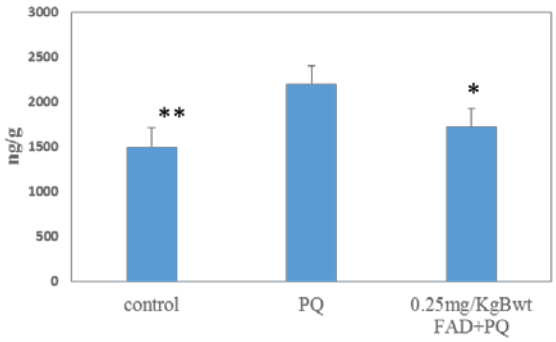


Fig. 3: Effects of FA-D on dopamine levels in the PQ zebra fish brain. Data were shown as mean+SEM. n=6-8, *p<0.05, **p<0.01 vs PQ group

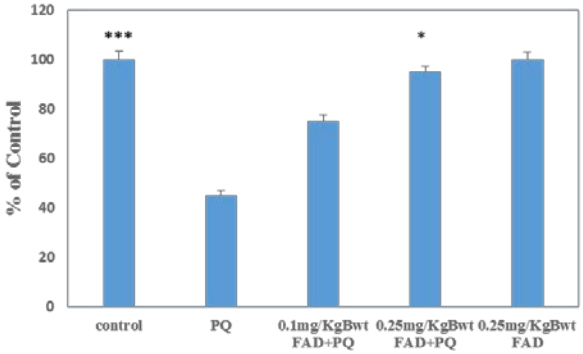


Fig. 4: Effects of FA-D on the activity of complex-I in the PQ zebra fish brain. Data are shown as mean+SEM. n=6-8, *p<0.05, **p<0.01, ***p<0.001 vs PQ group

Proteinaceous Compounds from *Fragaria ananassa* fruit (FA-D) Ameliorated Paraquat-Induced PD-Like alterations in zebrafish

We explored the possibility that FA-D fraction might be a novel regulator of parquat induced Parkinson like symptom i.e., the loss of locomotor activity and alterations in dopamine levels in zebrafish. To test the effect of FA-D *in vivo*, we administered FA-D at 0.1 mg/kgBwt and 0.25 mg/kgBwt once in alternative days for 5 days subsequent to the administration of PQ alternatively for a period of 7days. The fish survival and locomotor activity were monitored; 0.25 mg/kgBwt FA-D significantly improved fish survival and prevented locomotor impairment induced by paraquat (fig. 2 and inset). Increase of FA-D concentration to more than 0.25 mg/kgBwt did not further enhance protective effect (data not shown). Also, 0.25 mg/kg Bwt FA-D substantially blocked the hyperactivity of dopaminergic mechanism in PQ treated fish (fig. 3).

FA-D Reduced Paraquat-Induced mitochondrial damage in zebrafish

To further assess whether FA-D reduces paraquat induced mitochondrial damage, fishes were exposed to 0.25 mg/kgBwt FA-D. FA-D fraction markedly prevented the decrease in complex-I activity induced by 55 mM paraquat (fig. 4). The same concentration was effective in normalizing the increment in mt ROS levels induced by paraquat (fig. 5). Exposure of fish to 0.25 mg/kgBwt FA-D effectively lowered the release of cytochrome c induced by paraquat (fig. 6, Trace B). At this particular concentration a strong absorption peak was observed at 560 nm indicating that the release of cytochrome c from mitochondria induced by paraquat administration was abolished. Furthermore, specific restoration of complex-I activity, cytochrome c release and mt ROS levels by FA-D effectively preserved the morphology of mitochondria following PQ induced neurotoxity in zebrafish (fig. 7C).

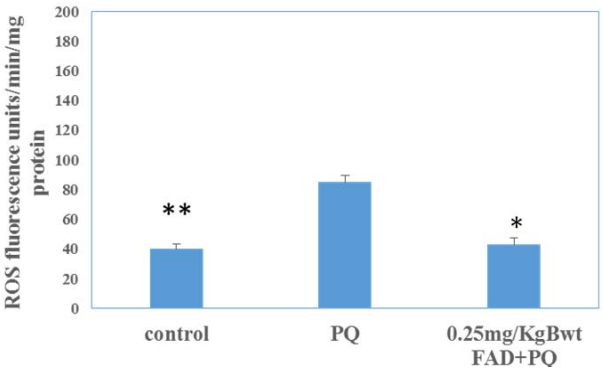
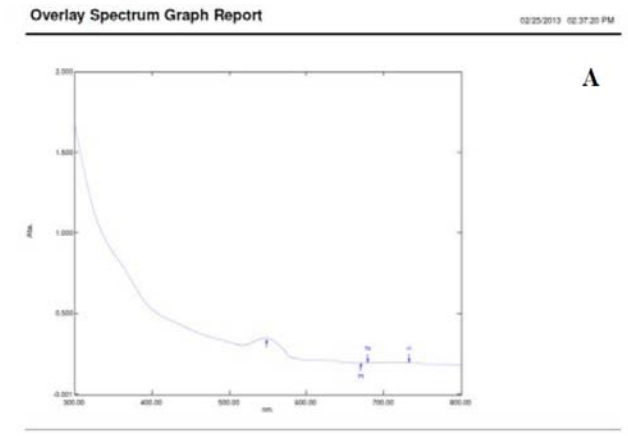


Fig. 5: Effects of FA-D on mitochondrial ROS levels in the PQ zebra fish brain. Data are shown as mean+SEM. n=6-8, *p<0.05, **p<0.01 vs PQ group



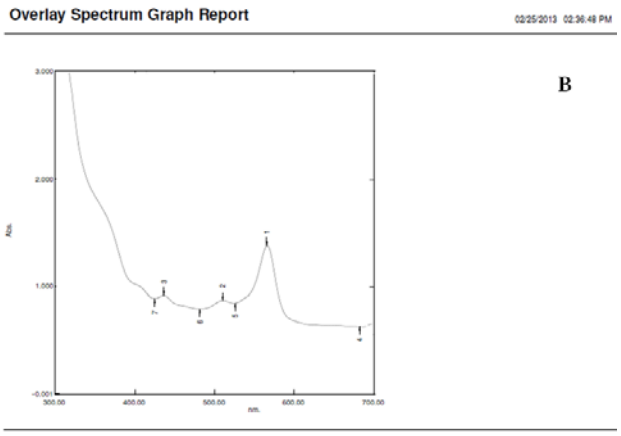


Fig. 6: Cytochrome C release from zebrafish brain mitochondria. Traces A and B represent the difference spectrum of the mitochondrial suspension treated with PQ and PQ+FA-D fraction respectively

DISCUSSION

The main finding of this study is that intraperitoneal (i. p.), administration of 55 mM paraquat alternatively for a period of 7 days caused Parkinson like locomotor and mitochondrial alterations

in zebrafish and proteinaceous compounds from *Fragaria ananassa* (FA-D) fruits protect against Paraquat induced neurotoxicity in zebrafish. Proteinaceous compounds from fruits of *Fragaria ananassa* (FA-D) attenuates paraquat-induced neurotoxicity in zebrafish via restoring dopamine levels, complex-I activity, and inhibiting cytochrome C release. FA-D increased survival time of zebrafish in the presence of paraquat, alleviated their locomotor impairment and mitochondrial degeneration. These results suggest that FA-D may be a potential compound for PD intervention.

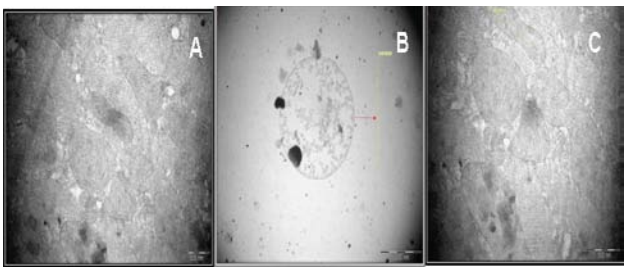


Fig. 7: Effect on morphology of mitochondria by TEM analysis A) Control B) PQ treated group C) PQ+FA-D treated group

An experimental model mimicking the major pathological and phenotypic features is crucial for a full understanding of the PD pathogenesis, which lead to searching for potential therapeutics. The herbicide paraquat is also able to induce behavioral and neurological disorders such as Parkinsonism [24-26]. Recent investigations have shown that paraquat induces liver toxicity through mitochondria perturbation including cytochrome C release, subsequent caspase-3 and poly (ADP-ribose) polymerase cleavage [27, 28]. There is a time dependency of paraquat effects such that mitochondrial complex I activities in rat brain decrease progressively within a few hours of exposure [29]. A number of paraquat-based toxin cell and rodent models have been used to study the molecular mechanisms of cell death and potential therapeutics for PD [30]. Here, we demonstrated that chronic exposure of adult zebrafish to Paraquat recapitulated some key features of Parkinsonism, including early mortality, locomotor impairment and increased dopamine levels. Many studies have suggested that paraquat is a potent inducer of intracellular ROS, which have a critical role in paraquat induced cell death in PD [31]. Recent studies have reported that paraquat treatment led to apoptosis, which was mediated with ROS generation and cytochrome C release in paraquat treated NIH3T3 cells [32]. Our results showed that paraquat increases mitochondrial ROS levels, reduces complex-I activity and cytochrome c release in zebrafish brain. These results indicate that paraquat based zebrafish is a good model to evaluate the therapeutic effect by candidate drug.

A growing body of evidences shows that mitochondria constitute a popular target in efforts to protect dopaminergic neurons, thereby arresting degeneration and maintaining the functional capacity of patients. However, available antioxidants have not proven to be particularly effective against these disorders. Possibility is that some of the antioxidants do not reach their relevant sites of free radical generation, especially, if mitochondria are the primary sources of ROS (reactive oxidative species) generation. Therefore, development of new approaches for treatment of neurodegenerative disorders that can protect mitochondria is desirable.

Recently there has been a growing interest in the search for natural antioxidants [33] as numerous clinical and epidemiological studies have demonstrated that consumption of fruits and vegetables is associated with reduced risks of developing neurodegenerative diseases such as Parkinson's disease; and due to the society's perception that natural and dietary antioxidants are safer than synthetic analogues. In recent years, there has been a considerable interest in plant proteins with antioxidant activity which might have beneficial effects on human health. So far there are no reports on antioxidant proteins obtained from strawberry fruit.

In this study, proteins from strawberry fruit were extracted using an aqueous extraction buffer followed by ammonium sulfate precipitation and dialysis, which are general methods for the isolation of biologically active hydrophilic proteins. Ammonium Sulfate precipitation is the commonly used salt precipitation because this technique stabilizes most proteins in solution and reduces the lipid content resulting in a better protein profile on the gel [34]. The 80% ammonium Sulphate precipitated protein (FA-D) showed 50% antioxidant activity when used at 450µg/ml concentration which, is consistent with recent reports where proteins isolated from plant sources such as *Curcuma comosa* rhizomes [35], *Cicerarietium* seeds [36,37], *Cajanusindicus* leaves [38], wheat germ [39], *Murrayakoenigii*[40], *Solanumtorvum* seeds [41],*Curcuma longa* waste grits [42], *Ginkgo biloba* seeds [43] and *Ficusdeltoidea* fruit show 50% antioxidant activity *in vitro* at 650µg/ml concentration by DPPH assay.

After *in vitro* antioxidant activity confirmation, the FA-D was analyzed by SDS-PAGE in order to determine the apparent molecular mass. The protein profiles showed three bands of 20.0 kDa, 17.0 kDa and 14.4 kDa respectively by silver staining. Similar results were previously reported where strawberry proteins separated by SDS-PAGE showed many low molecular weight polypeptide bands (10–21.5 kDa) [44].

A recent study by [34] has asserted that presence of low molecular mass proteins in the active water fractions of *F. deltoidea* fruits were polar in nature and were involved in the effective antioxidant and antidiabetic properties. Consistent with this finding, our results demonstrated that low molecular mass proteinaceous compounds from *Fragaria ananassa* fruit (FA-D) ameliorated paraquat induced PD-like symptoms in zebrafish. Using Paraquat-zebrafish model, we found that FA-D at 0.25 mg/kgBwt concentration improved fish survival and prevented locomotor impairment induced by paraquat. Moreover, we found that FA-D significantly reduces paraquat induced mitochondrial ROS levels in zebrafish brain. Furthermore, the dopamine levels are increased in paraquat exposed zebrafish brain which is consistent with previous reports [45, 46].

We also found that paraquat induced increased dopamine levels, reduced complex-I activity, cytochrome C release and mitochondrial damage is alleviated by decreased mitochondrial ROS levels in FA-D treatment. The shielding effect of the protein (FA-D) may be due to its potent antioxidant activity, and/or its ability to scavenge free radicals and inhibit cytochrome C release. Several investigators demonstrated that the antioxidant activity of the protein isolated from *Puerarialobata, Ficusdeltoidea* fruit and other plant sources may be due to the presence of hydrophobic amino acids, sulfur containing amino acids, and aromatic amino acids. There is a need for further research to purify and identify sequence of protein from *Fragaria ananassa* fruit in order to understand how this protein inhibits or prevents paraquat induced neurotoxicity which, may lead to the better therapeutic utility of proteinaceous compounds from *Fragaria ananassa* fruit for PD intervention.

CONCLUSION

The paraquat based zebrafish model can provide a valuable system for preclinical investigation on PD therapeutics. We demonstrate that low molecular mass proteinaceous compounds from *Fragaria ananassa* fruit (FA-D) can protect against paraquat-induced Parkinson like locomotor and mitochondrial alterations in zebrafish via reducing ROS level and inhibiting cytochrome C release. To our knowledge, this is the first report to show that low molecular mass proteinaceous compounds from *Fragaria ananassa* fruit (FA-D) has a protective effect in paraquat-based PD models, suggesting that low molecular mass proteinaceous compounds from *Fragaria ananassa* fruit (FA-D) can be a promising therapeutic compound for PD.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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