



# Neuroprotective effects of nanogold-based *Ayurveda* medicine *Suvarna Bhasma* against rotenone-induced Parkinson's-like model

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## ABSTRACT

**Background:** Neurodegenerative diseases have been one of the major concerns for human health. Genetic and environmental factors are believed to be responsible for neuronal diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease. It is difficult to restore normal nervous function after neurodegeneration; hence, prevention could be the best strategy against these diseases. Ayurved medicines such as *Suvarna Bhasma* (SB) have enormous potential to treat these neurological diseases.

**Aim:** The aim of this study is to examine the protective effect of SB against rotenone-induced Parkinson's-like model in zebrafish.

**Materials and methods:** In this study, we induced Parkinson's-like disease model in zebrafish by inducing it with rotenone (7 µg/L). We examined the behavioural, proteomics and dopamine alterations of rotenone induced zebrafish of SB pre-treated group as compared to the control group.

**Results:** The behavioural experiments showed that due to rotenone exposure, Parkinson's-like behavioural abnormality was induced in zebrafish. However, because of SB treatment, this behavioural abnormality was reduced. The proteomics study of zebrafish brains clearly showed that the SB-treated group was not significantly affected due to rotenone exposure. However, in the SB non-treated group, expression of nine proteins that are linked to Parkinson's disease (gene name: *sncg*, *ywhae1*, *ywhah*, *uchl1*, *ywhaba*, *psma6a*, *ywhabl*, *ywhaqb*, and *ywhabb*) were differentially expressed after rotenone exposure. Finally, prevention of dopamine alteration in SB-treated fish brains confirmed the protective action of SB against rotenone-induced Parkinson's-like model in zebrafish.

**Conclusions:** This study finds that *Suvarna Bhasma* has neuroprotective effects against Parkinson's-like disease model.

## 1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder among the population aged >65 years [1]. Motor impairment due to dopaminergic neuronal loss is the most common symptom of PD. Additionally, many non-motor indications add complexity to the overall disability due to Parkinsonism [2]. The molecular pathogenesis of Parkinson's disease includes  $\alpha$ -synuclein proteostasis, oxidative stress, mitochondrial dysfunction, abnormalities in calcium homeostasis and neuroinflammation. The aggregation of  $\alpha$ -synuclein protein and formation of Lewy body in the central nervous system are

the typical pathological characteristic in PD [3]. Until now, the most common clinical approach to treat PD is by pharmacological substitution of striatal dopamine. Recent treatment against Parkinsonism is also attained by targeting aggregated  $\alpha$ -synuclein. Gene and cell-based approaches for restoring striatal dopamine are also other possibilities to treat PD [4]. However, this is a progressive disease and a permanent cure for this disease is not yet available in modern medicine. In this aspect, Ayurveda could have a potential therapy against PD.

Dr James Parkinson, in early 19<sup>th</sup> century, described PD, although knowledge about this disease existed in India since ancient times. Karpavata (Kampa-tremors) can be considered as an Ayurveda

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analogue to PD and has been described in several Ayurveda scriptures [5]. *Suvarna Bhasma* is one of the prime Ayurveda formulations having anti-aging, immunomodulatory, anxiolytic and intellect promoting actions, [6,7]. *Suvarna Bhasma* is also used in the management of *Kampavata*. Rasatarangini, an authoritative text on *Rasashastra* (Indian alchemy, pharmaceuticals and pharmaco-therapeutics) mentions the use of *Suvarna Bhasma* in *Shirsha Vepathu* (head tremors), which is an important clinical manifestation of PD. Therefore, there could be a therapeutic potential in *Suvarna Bhasma* to treat neurodegenerative disorders like PD. In this study, we have investigated the effect of *Suvarna Bhasma* in rotenone-induced Parkinson's-like model in zebrafish. *Suvarna Bhasma* is a nanogold-based medicine used in various neuronal and immune-related diseases. Furthermore, several recent studies have shown that *Suvarna Bhasma* has anti-depressive, anxiolytic and anti-stress effects in animal models. The detailed insight about the biological action of *Suvarna Bhasma* is still missing in the literature. In this study, a comprehensive behavioural and proteomic study has been conducted to understand the neuroprotective effects of *Suvarna Bhasma* against rotenone-induced Parkinson's-like disease in the zebrafish model.

Rotenone is a common pesticide used in agriculture. It is a mitochondrial complex-I inhibitor that can cause damage to the dopaminergic neurons by ROS generation and altering ATP production [8]. Rotenone is a well-established compound that is known to induce PD in animal models. Several animal models such as *Drosophila*, rat and mice have been investigated for the rotenone-induced PD model. But zebrafish (*Danio rerio*) is comparatively a new animal model in this area. In the last decade, it has been widely used for neurological disease models. Recent studies have found that rotenone can induce PD-like motor and nonmotor alterations in zebrafish, such as increased freezing, decreased exercise capacity (such as reduction of speed) and dysfunction of olfactory behaviour [9]. In this study, we have examined the efficacy of *Suvarna Bhasma* by treating zebrafish with it and then exposing to rotenone (during the rotenone exposure, SB treatment was also continued).

## 2. Materials and Methods

### 2.1. Chemicals

Rotenone was purchased from Sigma Aldrich (Cat no. R8875). *Suvarna Bhasma* (Batch No: P180300114) was gifted from Shree Dhootapapeshwar Limited, Mumbai, India. All chemicals for LC-MS/MS analysis were purchased from Thermo Fischer. Dopamine ELISA kit was procured from CUSABIO, China.

### 2.2. Zebrafish housing

Adult wild-type zebrafish (age 6 to 8 months) were purchased one month before the experiment and kept in the housing tank. Each housing tank had a water holding capacity of 6 L, which was used to accommodate 10 fish. The water temperature was maintained at 25–27 °C and a 14 h–10 h light-dark cycle was followed. Fish were fed with dry granular food twice a day (Hikari tropical micro wafers). The experiment on zebrafish was carried out as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, India.

### 2.3. Validation of rotenone-induced behavioural alterations

The first objective of this study was to validate the rotenone-induced motor alterations. As Parkinson's disease mainly affects motor behaviour, in this experiment, we focused only on the motor parameters of zebrafish after rotenone treatment. This experiment was done to validate the effect of rotenone on the motor behaviour of zebrafish. To conduct this experiment, the fish were divided into two groups (n = 10/

group), control and rotenone group. The fish from the rotenone group were exposed to rotenone solution with a step-up dose. The concentration of rotenone given with increasing concentration of 4 µg/L, 5 µg/L, 6 µg/L and 7 µg/L respectively for 15 successive days each for every concentration to the rotenone fish group (Fig. 2). After completion of every dose (15 days dose for each concentration), the fish were tracked individually in a novel tank for 5 min. The change in the fish behaviour was compared with the control group.

#### 2.3.1. Drug dose preparation

**SB dose preparation:** 10 mg of SB was mixed with dry food along with 300 µl cod liver oil. Then, a small portion of food was digested in *aqua regia* and subjected to analysis, using ICP-AES to find out gold concentration and therefore SB concentration. Then, a calculated amount of SB mixed food was given to the fish daily (60 mg/kg body weight of fish).

#### 2.3.2. Treatment groups and drug treatment

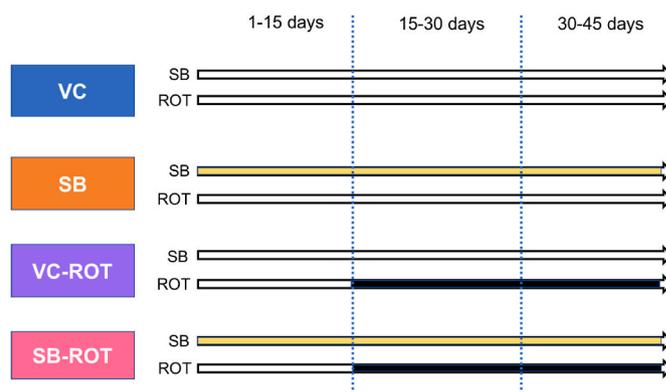
Fish were divided into four groups, namely vehicle control group (VC), VC-Rotenone group (VC-ROT), *Suvarna Bhasma* group (SB) and *Suvarna Bhasma*-Rotenone (SB-ROT) group (n = 25/group). SB treatment was given orally to SB and SB-ROT groups by mixing it in dry granular food. After 15 days of vehicle and *Suvarna Bhasma* treatment, VC-ROT and SB-ROT groups were exposed to 7 µl rotenone (Fig. 1). They were exposed to rotenone continuously for the next 30 days. The rotenone-water mixture was changed for these two groups daily. During the rotenone exposure periods, the *Suvarna Bhasma* dose was continued for the SB-ROT group.

#### 2.3.3. Zebrafish behaviour experiment

Fish behaviour was examined in a novel tank twice: once on the 15<sup>th</sup> day and once on the 30<sup>th</sup> day, after the initiation of rotenone exposure. Fish from each group were individually transferred to the novel tank and the video was recorded for 5 min. Fish tracking and behaviour assessment were done as per our previous studies [7]. Briefly, the videos were further processed with idTracker [10], and the various behavioural parameters were analyzed using indigenously developed MATLAB code.

#### 2.3.4. Dopamine

The fish were dissected after euthanization in ice cold water after completion of the experiment (after 30 days of rotenone exposure), The fish brains were extracted and homogenated in the phosphate-buffered saline (PBS) at pH 7. The homogenate was centrifuged at 5000 rpm and supernatant was collected. Dopamine in fish brain (n = 4) was analyzed using ELISA kit as per the manufacturer's instructions.



**Fig. 1.** *Suvarna Bhasma* treatment protocol and rotenone exposure to various groups. VC = Vehicle Control group, SB = *Suvarna Bhasma* group, VC-ROT = Vehicle Control exposed with rotenone, SB-ROT = *Suvarna Bhasma* treated fish exposed with rotenone groups. (Yellow colour fill in the arrow represent SB treatment, black colour fill represent rotenone treatment).

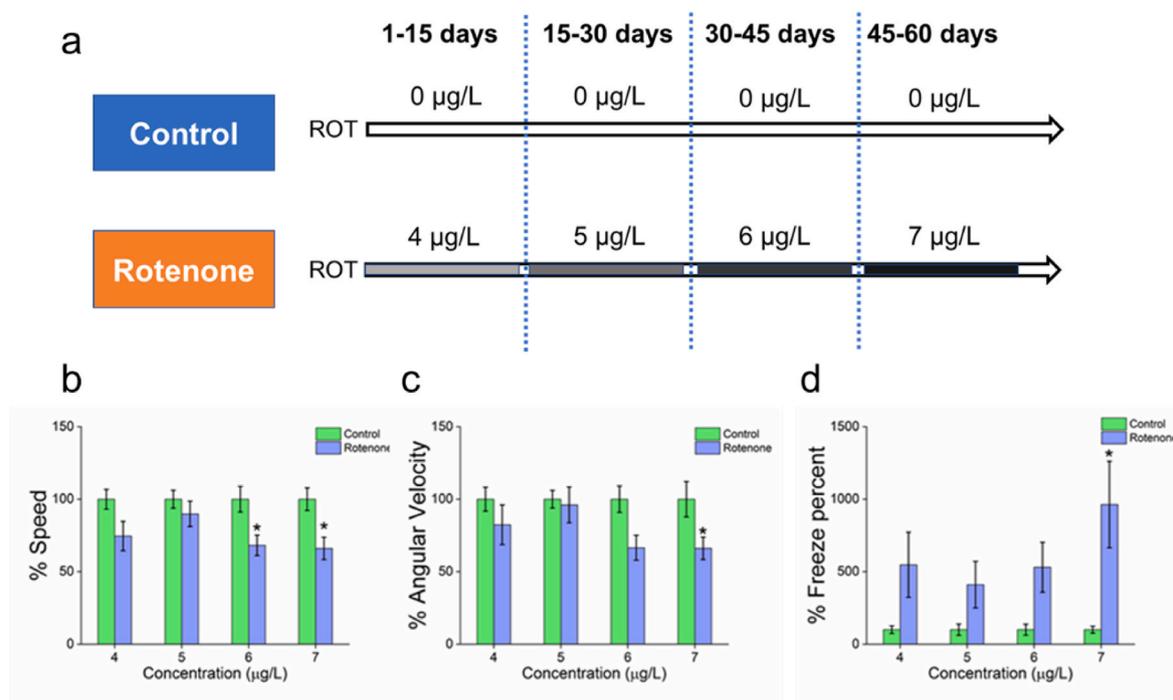


Fig. 2. Variation in motor parameters due to rotenone exposure. A step-up dose was induced to the fish (n = 10/group).

### 2.3.5. Proteomics analysis of zebrafish brain

After the completion of behaviour experiment (after 30 days of rotenone exposure), the fish brains were extracted after euthanize by thermal shock. Fish brains were snap freezing in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until further experimentation. The fish brains were homogenized in urea buffer [8 M urea, 400 mM ammonium bicarbonate and 10 mM dithiothreitol (DTT)]. A pool of three brains was taken and homogenated in the same centrifuge vial as they were considered as one sample. We had taken 3 samples from each group (total 9 brains from one group). After homogenization, the protein was quantified using BCA kit (Thermo Fischer Scientific, USA). Approximately 20 µg of protein was taken from each sample and reduced using DTT for half an hour at  $60^{\circ}\text{C}$ . After that, 25 mM iodoacetamide (alkylation) was added to each sample and kept in the dark for 30 min in room temperature. Then, 50 mM ammonium bicarbonate was added to dilute the sample solution fourfold. The samples were trypsinized (1:20 enzyme to substrate ratio) overnight at  $37^{\circ}\text{C}$  in constant shaking. The samples were dried using vacuum drier and dissolved again in 5 % acetonitrile (with 0.5 % trifluoroacetic acid) solution. A desalination procedure was applied to remove the salt in samples using C18 spin column (Pierce® C18 Spin Columns, Thermo Fischer Scientific, USA). The samples were again reconstituted in 20 µl 0.1 % formic acid. Then, the peptide concentration was analyzed by the UV-Vis spectrophotometers (Implen™ Nano-Photometer, German). The peptide concentration was finally kept at 0.1 µg/ml by diluting with 0.1 % formic acid solution. The peptides were analyzed using liquid chromatography-mass spectroscopy. The chromatographic column used here was C18 reverse phase column (Thermo Fischer Scientific, USA).

MaxQuant software (<https://maxquant.net>, v1.6.10.43) was used to analyse peptides. UniProt sequence database was used to identify proteins. Quantification of proteins was carried out using Label-free quantification technique (LFQ). Perseus software (<https://maxquant.net/perseus>) was used for data analysis using LFQ intensity of various proteins. In this LFQ proteomics study, three biological replicates from each of the four groups were analyzed. Those proteins that were present in all 12 samples were further processed for statistical analysis using Perseus software (<https://maxquant.net/perseus>). Statistical analysis of differentially expressed proteins in SB, VC-ROT and SB-ROT groups were

carried out using Student's t-test ( $p < 0.05$ ) as compared to the VC group. PANTHER (Protein Analysis Through Evolutionary Relationships) database version 15.0 (<http://www.pantherdb.org>) was used to identify various PANTHER pathways of differentially expressed (DFE) proteins of those treatment groups [11].

### 2.3.6. Accumulation of gold in different forms

Accumulation of gold in whole body zebrafish was investigated in the SB-treated group. After SB oral ingestion, gold could be accumulated in the fish in two forms: either in the particle form or in the ionic form (released from SB particles in the gastrointestinal system). To investigate the accumulation concentration of both gold forms, we used two different methods for analysis. To investigate ionic gold concentration (released from SB particles surface), fish were digested in ultrapure nitric acid. On the other hand, to find out the gold concentration in particle form, *aqua regia* was used to digest accumulated gold particles in the fish.

The investigation of gold concentration was carried out 24 h after the last dose of SB (after 45 days of SB treatment). The fish were first euthanized in ice cold bath. Then, the whole body was digested in the respective acids. The concentration of gold in both cases was measured using ICP-MS technique. In both ICP-MS experiments n = 3 fish were taken.

### 2.3.7. Statistical analysis

All the data are represented as mean  $\pm$  standard error of the mean (mean  $\pm$  SEM). Statistical differences between the data set were carried out by one-way-ANOVA. Fisher's least significant difference (LSD) test for post hoc comparisons was used with \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ .

## 3. Results

### 3.1. Validation of rotenone-induced motor behaviour changes

Fig. 2 shows the motor behaviour changes due to rotenone exposure. The results show that the motor parameters such as speed, angular velocity and freeze% significantly changed as the dose concentration

increases (as well as the exposure time). At 7 µg/L concentration, rotenone exposure shows the maximum variations in speed (34 % decrease), angular velocity (34 % decrease) and freeze% (836 % increase) as compared to the control group. Therefore, the study validates the alteration of motor behaviour due to rotenone exposure.

### 3.2. Effect of SB against rotenone-induced toxicity

#### 3.2.1. Survival rate

To investigate the protective effect of SB against rotenone toxicity, fish from the VC-ROT and SB-ROT groups were exposed to 7 µg/L rotenone for 30 days. The exposure to rotenone (7 µg/L) caused mortality in zebrafish. In the SB-ROT group, out of 25 fish, 6 fish died due to rotenone exposure, while in the VC-ROT group, 10 fish died after rotenone exposure.

#### 3.2.2. Motor behaviour

The motor behaviours were measured at two-time points: after 15 days and after 30 days of rotenone exposure. In this experiment, fish were exposed to rotenone at a concentration of 7 µg/L. The results (Fig. 3) shows the behaviour of the four groups. Here, we found the alteration in speed (Fig. 3a), angular velocity (Fig. 3b) and freeze% (Fig. 3c) for VC-ROT and SB-ROT groups after 15 days of rotenone treatment. After 30 days of rotenone exposure also, similar type of alteration was observed for VC-ROT and SB-ROT groups. For VC-ROT and SB-ROT groups, decrease in speed (Fig. 3d), decrease in angular velocity (Fig. 3e) and increase in freeze % (Fig. 3f) was observed. Interestingly, the results show that SB-treated fish (SB-ROT) prevented the alteration of speed, angular velocity and freeze % at both time points. The 15 days timepoint showed significant variations in speed (Fig. 3a) and angular velocity (Fig. 3b) among SB-ROT and VC-ROT groups. Whereas, after 30 days of rotenone treatment, a significant variation was observed in angular velocity among SB-ROT and VC-ROT groups.

#### 3.2.3. Dopamine analysis

The dopamine analysis results in brain tissue showed a substantial

decrease of dopamine in both VC-ROT and SB-ROT groups due to rotenone exposure (Fig. 4). Interestingly, the VC-ROT group showed maximum dopamine alteration (42 % decrease), whereas in the SB-ROT group, a 25 % decrease of dopamine was observed as compared to VC group.

#### 3.2.4. Proteomics study

A total of 2333 proteins were found in all 12 different fish brain samples from four treatment groups (n = 3/group). Out of these, 405 proteins were common in all groups (present at least in all 12 samples). These 405 proteins were further processed for statistical analysis. Statistical analysis was carried out using Student's t-test (p < 0.05 was consider as significant) for SB, VC-ROT and SB-ROT groups as compared

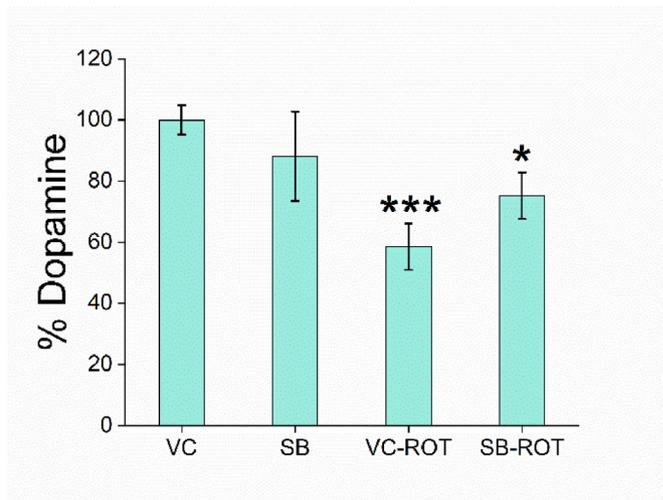


Fig. 4. Dopamine of various groups after 30 days of rotenone exposure (Exposure of rotenone was done for VC-ROT and SB-ROT groups). Values are reported in mean ± SEM with \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 against the VC group.

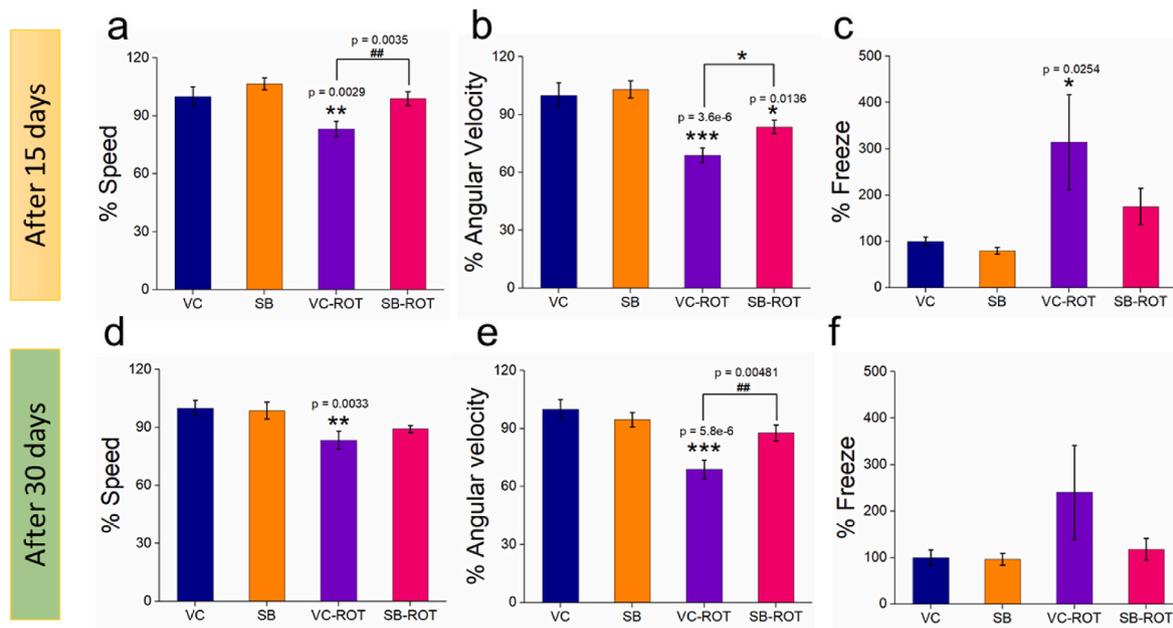


Fig. 3. Neuroprotective effect of *Suvarna Bhasma*. a) speed b) angular velocity c) freeze percent, after 15 days of rotenone treatment. d) Speed e) angular velocity f) freeze percent, after 30 days of rotenone treatment. VC = Vehicle Control, SB = *Suvarna Bhasma* treated, VC-ROT = VC group that was treated with rotenone and SB-ROT = SB group that was treated with rotenone. Values are reported in mean ± SEM with \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 against the VC group, whereas #p < 0.05 and ##p < 0.01 is between VC-ROT and SB-ROT group.

to VC group. Our findings suggested that 137 proteins were differentially expressed in VC-ROT groups as compared to VC group (Fig. 5b, the blue dot represent the differentially expressed proteins). For SB-ROT group also, the alteration of various proteins out of 405 proteins was observed (Fig. 5c); however, the alteration was not statistically significant as compared to VC group. For the VC-ROT group, 99 proteins were downregulated, whereas 38 proteins were upregulated (significant alteration). Interestingly, for SB-ROT group, statistically significant difference in protein expression was not observed as compared to VC group. Thus, the proteomics study inferred that the effect of rotenone was more prominent for VC-ROT group as compared to SB-ROT group (concluded by statistical analysis as compared to the VC group). Therefore, the proteomic study suggested that *Suvarna Bhasma* has a protective effect against rotenone-induced toxicity.

The PANTHER pathway analysis of differentially expressed proteins of VC-ROT group was examined (Fig. 6). We found that several PANTHER pathways were enriched due to rotenone exposure related to dopamine, Parkinson's disease, receptor mediated signalling, GABA-B receptor signalling, glutamine-glutamate conversion and many more. The expression of nine proteins that are related to Parkinson's disease (gene name: *sncgb*, *ywhae1*, *ywhah*, *uchl1*, *ywhaba*, *psma6a*, *ywhabl*, *ywhaqb*, and *ywhabb*) were expressed differentially in VC-ROT group (Table 1). It was further observed that the another two proteins (gene name: *atp5f1b* and *cyc1*) related to ATP synthesis were differentially expressed due to rotenone exposure in VC-ROT group.

### 3.2.5. Gold concentration

The quantification of gold concentration was represented per gram body weight of fish. The ionic gold concentration was  $101.5 \pm 21.09$  ng/g bw, whereas the gold accumulation in the gold particle form was  $2.03 \pm 0.19$   $\mu$ g/g bw after 45 days of SB treatment.

## 4. Discussion

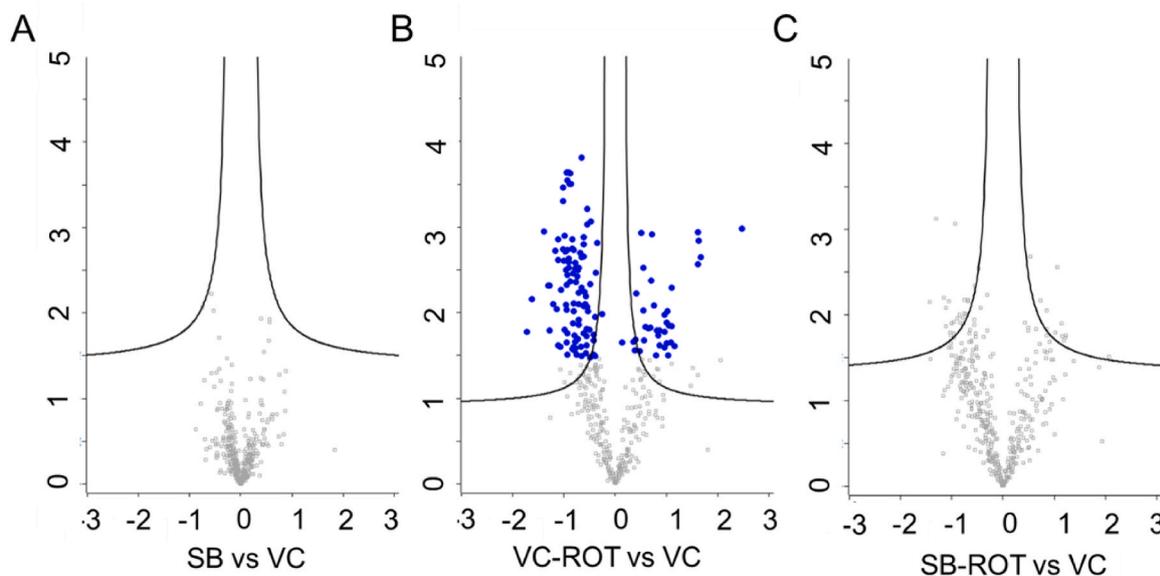
PD is a progressive and incurable condition. PD is not fatal, but it affects the quality of life of the patient severely [12]. There are several hypotheses about its occurrence; however, the exact etiology of PD is yet to be elucidated. Most of the PD cases are not inherited (only 5–10 % of cases are inherited). It is inevitable that environmental factors could play a major role in this disease and it appears to be restricted to the human species. No animal species has been found to have PD naturally.

However, several animals such as rat and mouse can be modelled for Parkinsonism using genetic alterations or chemical exposure. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), methyl-phenyltetrahydropyridinium (MPP<sup>+</sup>) and rotenone are some of the examples that can chemically induce Parkinson's disease in animal models. In this study, we have validated that zebrafish can be modelled for Parkinson's-like disease model. The behavioural, proteomics and dopamine studies suggested Parkinson's-like symptoms in zebrafish.

The proteomics study suggested that the expression of nine proteins which are closely related to Parkinson's pathway altered significantly with rotenone treatment. Two proteins related to dopamine receptor mediated signalling, showed altered expression with rotenone exposure. Additionally, the dopamine level of zebrafish after rotenone exposure was decreased, which is typical of Parkinsonism. Lastly, the behaviour experiment showed a decrease of speed, angular velocity and increase of the freezing behaviour. Taking into account all these parameters, we suggest that the zebrafish can be a screening model for PD

The main aim of the study was to find a prevention for this disease. In this view, we used the Ayurveda formulation, *Suvarna Bhasma*, for preventing rotenone induced Parkinson's disease. Our previous study found that *Suvarna Bhasma* has anxiolytic effect in zebrafish [7]. Ayurveda scriptures also advocate the use of *Suvarna Bhasma* in neurodegenerative disorders like *Kampavata* which bears resemblance to PD. The overall referencing and support data led to our hypothesis that *Suvarna Bhasma* would be potential candidate against neurodegenerative diseases.

This study found that the alterations in motor behaviour was less for *Suvarna Bhasma* treated fish, as compared to *Suvarna Bhasma* non-treated fish. Behavioural alteration is the most common symptom of PD, especially motor behaviour. Due to dysfunction of neuronal activity, motor behaviour was altered. Several neurotransmitters are involved in the proper motor functioning. The dopaminergic neurotransmitter system is responsible for controlling motor behaviour. It can be inferred that the protection of dopamine neurotransmitters by *Suvarna Bhasma* is one of the reasons for the lesser behavioural alteration in SB-ROT group. It was further observed that differentially expressed proteins of VC-ROT group involved in neurotransmitter receptor-related pathways such as dopamine, acetylcholine and glutamate where as in the case of SB-ROT group the proteins were not expressed differentially. Overall, the protection of biomolecules and protein expression by *Suvarna Bhasma* resulted in less behavioural alteration in zebrafish.



**Fig. 5.** Volcano plot of differentially expressed proteins (A) SB vs VC (B) VC-ROT vs VC, and (C) SB-ROT vs VC. Blue dots represent the differentially expressed proteins as compared to VC group. Statistical significance was carried out using student-t-test ( $p < 0.05$ ) using Perseus software.

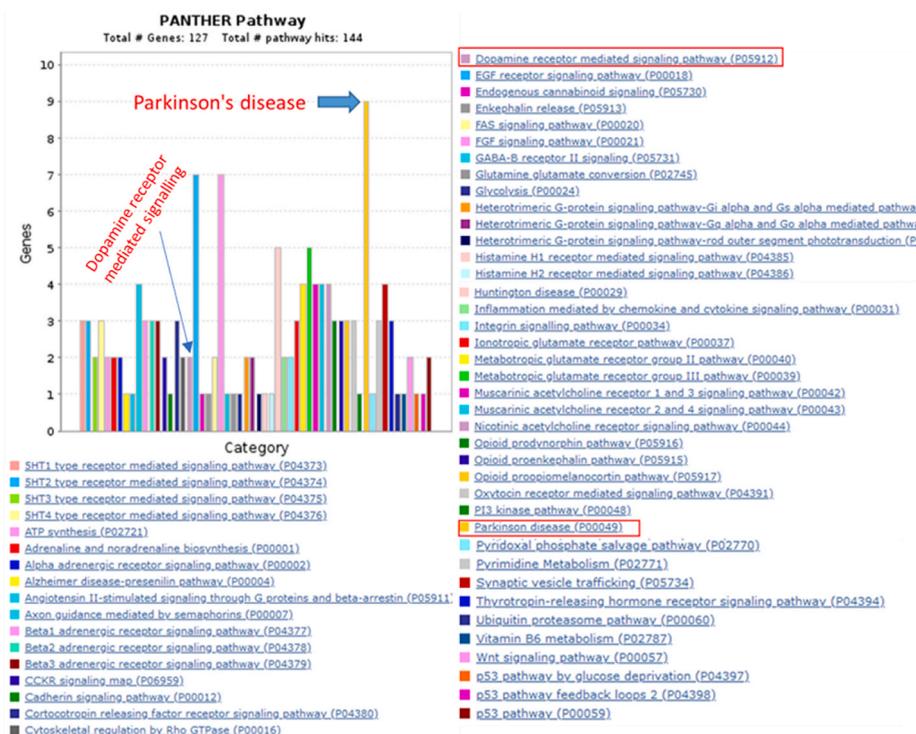


Fig. 6. Functional classification of Panther pathway of differentially expressed proteins of VC-ROT group as compared VC group.

**Table 1**  
Protein related to Parkinson's disease altered due to rotenone exposure.

Sl No	Protein name	Gene	Name
1	A0A2R8RP85	sncgb	Synuclein, gamma b
2	Q7ZW20	ywhae1	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide 1
3	Q7T3G2	ywhah	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
4	Q6Y149	uchl1	Ubiquitin carboxyl-terminal hydrolase
5	Q5PRD0	ywhaba	14-3-3 protein beta/alpha-A
6	Q7SXN6	psma6a	Proteasome subunit alpha type
7	Q6P102	ywhabl	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide-like
8	Q803M8	ywhaqb	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide
9	Q7T356	ywhabb	14-3-3 protein beta/alpha-B

The mechanism of *Suvarna Bhasma* against Parkinson's disease can be explained by the presence of gold nanoparticles (starting from ~10 nm) in it [13]. In our previous study [13], we found that a portion of *Suvarna Bhasma* particles is in nano sizes encapsulated with a coating of Si, Na, and Ca. The presence of nanogold particles could have neuroprotective effects against Parkinson's disease. Gao et al. [14] proved that gold in nano-size has protective effects against Parkinson's disease. They showed that gold nanocluster inhibits the aggregation and fibrosis of  $\alpha$ -synuclein in cell culture study. Also, in mouse, gold nanocluster exhibits neuroprotective effects against MPP<sup>+</sup> induced Parkinson's model. Furthermore, gold nanoparticles have anti-inflammatory activity [15], which could be one of the reasons for its protective effects against PD.

Another possible mechanism of neuroprotection by SB could be due to the release of ionic Au into the gastrointestinal system. The small amount of ionic gold could be released from the SB particles that can bind with numerous proteins and biomolecules forming gold complexes.

The gold complex could induce protective effects on the neuronal cell, similar to auranofin. Auranofin is a gold complex that is used to treat rheumatoid arthritis. It acts as an anti-inflammatory agent in the brain cells [16]. Neuroinflammation is a common condition in PD. Auranofin alters the excretion of cytokines: increasing the secretion of IL-8 and reducing IL-6 secretion [17]. The anti-inflammatory intervention of Auranofin has the potential to slow down the neuronal loss due to PD [17]. It also regulates the protective enzyme heme oxygenase-1 (HOX-1). HOX-1 induction could have a preventive action against PD model [18]. The main advantage of Auranofin is that it can cross the blood-brain barrier. Similar to Auranofin, ionic gold released from *Suvarna Bhasma* could protect various pathways to prevent neurodegeneration.

However it is important to note that the zebrafish as an animal model for Parkinson's disease has some limitation. The chemical models often do not cause PD but instead employ different mechanism to produce phenotypes that are comparable. These pathways may not accurately capture every facet of the disease.

## 5. Conclusion

This study found that the expression of nine proteins that are related to Parkinson's disease were differentially expressed by rotenone induction (for VC-ROT group) whereas for the SB pretreated group (SB-ROT) those proteins were not differentially expressed. From behavioural, dopamine and proteomics findings, it was observed that *Suvarna Bhasma* treatment has neuroprotective effects against rotenone-induced toxicity. However, to validate the efficacy of *Suvarna Bhasma* against Parkinsonism, further experiments in a higher animal model is required. This study indicates that *Suvarna Bhasma* could be a potential candidate against neurodegeneration.

## CRediT author statement

**Snehasis Biswas:** Conceptualization, Methodology, Formal analysis, Data Curation, and Writing- Original draft preparation.

**Mukesh Chawda:** Project administration, Writing - Review &

## Editing.

**Ramacharya Gudi:** Writing - Review & Editing.

**Jayesh Bellare:** Conceptualization, Supervision, and Writing - Review & Editing.

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## Authors' contributions

Conceptualization: Snehasis Biswas (SB), Mukesh Chowda (MC), Jayesh Bellare (JB), Ramacharya Gudi (RG). Experimentation: SB. Project administration: SB, MC, RG, JB. Supervision: JB. Writing: original draft: SB. Writing, review and editing: SB, MC, RG, and JB.

## Declaration of competing interest

The other two authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Two of the co-authors are from Shree Dhootapapeshwar Limited, which has also funded the study. The other authors declare no conflict of interest.

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