Research Article

Ameliorative Effect of Melatonin with Primaquin in Atrazine induced Glucose-6-phosphate Dehydrogenase Deficiency in Brain

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ABSTRACT

Glucose-6-phosphate dehydrogenase is rate limiting enzyme in pentose phosphate pathway (PPP), whose major physiological role is to supply NADPH by conversion of Glucose-6-phosphate to phospogluconate is a major cell reductant and useful to cell survival. Brain is highly sophisticated organ of our body which requires continuous supply of energy in form of glucose. Daily requirement of brain glucose is 120gm. G6PD plays a key role in it. According to WHO 75% of world population have one or two gene for G6PD and 2.9% are G6PD deficient. It is most common enzymatic disorder of cell effecting 200-400 million people. G6PD exist in all cell to oxidative damage and it is responsible for various neurodegenerative disorder like Parkinson's disease, Alzheimer's, Schizophrenia catatonia, Neuronal toxicity etc. The present study was aimed to evaluate the neuroprotective potential of Melatonin with Primaquine and Aspirin (contraindicated to G6PD deficient individuals) in Atrazine induced G6PD deficiency in Albino Rats. Study was carried out for the evaluation of behavioral activity (Elevated-Plus Maze and Morris Water Maze), various biochemical parameters (G6PD, Nitrites, LDH, Glucose), neurotransmitters (Serotonin, Dopamine, Nor-adranaline), anti-oxidant activity (GSH, LPO, SOD and Catalase) and histopathological evaluation. Results suggested that +Melatonin (10mg/kg) along with Atrazine (5mg/kg) induced G6PD deficiency in Albino rats showed statistically more significant improvement in neurodegenerative disorders.

Keywords: Glucose-6-phosphate dehydrogenase, Atrazine, Melatonin, Primaquine, Aspirin.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) is the rate limiting enzyme in Pentose Phosphate Pathway (PPP), whose major physiological role is to supply NADPH by the conversion of Glucose-6-phosphate to 6-phosphogluconate, that is a major cell reductant and useful to cell survival. NADPH is also generated by the conversion of 6 phosphogluconate to ribulose-5-phosphate, in presence of 6-phosphogluconate dehyrogenase. [20] NADPH plays an important role in antioxidant reaction with glutathione (GSH), glutathione-peroxides (GS-Px) and catalase (CAT). G6PD normally acts as "housekeeping" whose expression is constitutive and induced by agents causes oxidative stress.

According to WHO, 75% of world population have one or two gene for G6PD and 2.9% are G6PD deficient.[18] It is most common enzymatic disorder of cell effecting 200-400 million people. G6PD exist in all cell to oxidative damage, also responsible for various neurodegenerative disorder like Parkinson's disease, Alzeimers, Schizophrenia catatonia, Neuronal toxicity etc. Effect of melatonin along with primaquine and aspirin showed significant improvement in G6PD deficient wistar albino rats.[19]

The present study was aimed to evaluate the ameliorative effect of Melatonin along with Primaquine and in Atrazine induced Glucose-6-phosphate dehydrogenase deficiency in brain.

Under the approval of Institutional Animal Ethics Committee approved proposal no. (273/CPCSEA) study were carried out. Animals were divided into five groups and in each group number of animals is n=6. Throughout the experiment, animals were maintained according to the CPCSEA guidelines.

Dosing protocol was followed for 21days according to body weights of the individual animal. During the experimental protocol animals were evaluated for memory exercised using Elevated plus maze test on 7th, 14th and 21th day. 7th day onward animals were trained for swimming to identify hidden platform to check index of acquisition using Morris water maze. On 14th and 21st day transfer memory and retention memory were observed.

Statistical analysis were carried out. All values are expressed as Mean ± SEM. Multiple comparisons between different groups were performed using Analysis of variance (ANOVA) followed by Dennett's multiple comparisons test. Difference level at $P< 0.05$ was considered statistically significant condition.

MATERIALS & METHODS

Chemicals

Atrazine, melatonin, primaquine biphosphate were purchased from Sigma Aldrich.

Animals Models

Wister albino rats of body weight 150-250 g and 6 month of age of either sex, procured in the institutional animal house (Sardar Bhagwan Singh Post Graduate Institute of

Biomedical Sciences and Research) were used for the study. The animals were housed in standard poly propylene cages and maintained under controlled room temperature (22±2ºC) and humidity (55±5%) with 12:12 hour light and dark cycle. All the animals were provided with commercially pallet diet and water *ad libitum*. The study was approved by Institutional Animal Ethics Committee under the approved proposal no. (273/CPCSEA). Throughout the experiment, animals were maintained according to CPCSEA guidelines.

Animal grouping and treatment protocol

Wister albino rats were divided into seven groups (n=6) as follows:

Group A

(Sham control; n=6) normal saline (orally).

Group B

(Control; n=6) Atrazine (5mg/kg/orally).

Group C

(Standard; n=6) Melatonin (10mg/kg/orally).

Group D

(T1; n=6) Atrazine (5mg/kg/orally) and Primaquine (10mg/kg/orally).

Group F

(T3; n=6) Atrazine (5mg/kg/orally), Primaquine (10mg/kg/orally) and Melatonin (10mg/kg/orally).

Estimation of behavioral activity

Elevated Plus-Maze test

Elevated plus-maze was performed for evaluating the memory of animal. The plus maze consisted of two open (16 × 5 cm^2) and closed (16 × 5 × 12 cm) arms, connected by a central platform of (5 × 5 cm^2).[5] The maze was elevated at a height of 25 cm above the floor. A fine line was drawn in the middle of the floor of each closed arm. On the first day (7th day of the treatment) the animal was placed individually for 30 min. The time taken by the animals to move from open to closed arm (transfer latency) was noted on the first day. Transfer latency (TL) is the elapse time (in sec.) between the time of placement of the animals on the open arm and the time at which all four legs were inside the closed arms. The rat was allowed to explore the maze for 2 min. Retention of this learning task (Retention memory) was examined after 24 h of the first trial day (i.e. 24 after last dose). Transfer latency measured on plus maze on first day served as an index of learning and acquisition, whereas transfer latency on 2nd and 3rd day (14th day and 21th day of the treatment) served as an index of retrieval and memory.

Calculation

Inflexion Ratio = L1-L0/L0

L1 = Transfer latency on day 1

L0 = Transfer latency on day 2

Morris Water Maze test

Morris water maze test was performed employed to assess learning and memory of the animal. Morris water maze is a swimming based model where the animals learn to escape on a hidden platform.[17] It consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water maintained at 28±1°C). The water was made opaque with titanium dioxide. The tank was divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (10×10 cm), painted in white was placed inside the target quadrants of this pool, 1 cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive trails on each day with inter-trial gap of 5 min. The mouse was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 sec to locate submerged platform. Then it was allowed to stay on the platform for 20 sec. If it is failed to find the platform within 120 sec, it was guided gently onto platform and allowed to remain there for 20 sec. Day 4 escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition or learning, Animal was subjected to training trials for four consecutive days, the starting position was changed with each exposure as mentioned below and target quadrant (Q4 in the present study) remained constant throughout the training period.

On 5th day, platform was removed and each mouse was allowed to explore the pool for 120 sec. Mean time spent in all four quadrants was noted. The mean time spent by the animal in target quadrants searching for the hidden platform was noted as an index of retrieval or memory. The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory serving, as prominent visual clues were not disturbed during the total duration of study.

RESULTS

Effect of drugs on transfer latency and retention transfer latency on Elevated Plus -Maze

In control group, Atrazine (5mg/kg) results increase in the transfer latency and retention transfer latency on 7th, 14th and 21th day that indicates impairment of learning, memory and anxiety. In treatment groups (T1&T2), administration of Primaquine (10mg/kg) and Aspirin (25mg/kg) alone with Atrazine (5mg/kg) shown almost similar results as to control group. Further, co-administration of Melatonin (10mg/kg) with Aspirin (25mg/kg) than Primaquine (10mg/kg) results statistically significant decrease in transfer latency and retention latency on 7th, 14th and 21th day as compared to standard group. Moreover, treatment with standard Melatonin (10mg/kg) results statistically more significant

decrease in transfer latency and retention transfer latency on 7th, 14th and 21th day as compared to control group. **(Table 1, 2, 3 and 4)**

Effect of drugs on escape latency of water maze

In control group, Atrazine (5mg/kg) produced impaired acquisition by impairing gradual reduction in escape latency time. In treatment group (T1&T2), administration of Primaquine (10mg/kg) and Aspirin (25mg/kg) with Atrazine (5mg/kg) shown almost similar results in escape latency time with successive acquisition trails on day1-6 as compared to control group. Further co-administration of Melatonin (10mg/kg) with Aspirin (25mg/kg) than Primaquine (10mg/kg) resulted statistically significant decrease in escape latency time from 1st to 6th trails as compared to standard group. Moreover treatment with standard Melatonin (10mg/kg) showed statistically more significant decrease in escape latency as compared control group. **(Table 5)**

RESULTS

Table 1: Body weight of animals on respective days 7th, 14th and 21th.

Treatment (oral)	Body weight (g)		
	Day 7	Day 14	Day 21
Normal	202.3±1.109	214.8±1.434	218.3±1.548
Atrazine (Control)(5mg/kg)	189±4.301	186.3**±2.780	171.8***±1.250
Atrazine (5mg/kg) + Melatonin (10mg/kg, orally)	194±2.799	202.3*±1.702	209.8±1.250
Atrazine (5mg/kg) + Primaquine (10mg/kg)	199±0.7071	203.3*±2.287	204.5*±1.708
Atrazine (5mg/kg) + Melotonin (10mg/kg) + Primaquine (10mg/kg)	210.5±2.901	211±3.189	222.5±4.481

Values are expressed as Mean ± SEM, *P< 0.05, **P < 0.001, ***P> 0.001, n=6 each group.

Table 2: Ameliorative effect of Melatonin on transfer latency on day 7th, 14th and 21th in Atrazine induced Glucose-6-phosphate deficiency in Rats using Elevated Plus Maze

Treatment (oral)	Transfer latency (sec)		
	Day 7	Day 14	Day 21
Normal	25±1.140	26.8±1.855	29.80±1.655
Atrazine (Control)(5mg/kg)	13.20±1.715***	7.400±0.5099***	3.800±0.6633***
Atrazine (5mg/kg) + Melatonin (10mg/kg, orally)	19±2.915**	151.761**	23.80±2.818
Atrazine (5mg/kg) + Primaquine (10mg/kg)	4.800±1.068***	5.400±1.030***	4.600±0.8124***
Atrazine (5mg/kg) + Melotonin (10mg/kg) + Primaquine (10mg/kg)	8.00±1.414***	14.20±1.562***	16.80±2.345***

Values are expressed as Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, n=6 in each group.

Table 3: Ameliorative effect of Melatonin on retention transfer latency on day 7th, 14th and 21th in Atrazine induced Glucose-6-phosphate deficiency in Rats by using Elevated Plus Maze

Treatment (oral)	Retention transfer latency (sec)		
	Day 7	Day 14	Day 21
Normal	179.4±5.836	179.2±5.843	177.8±5.324
Atrazine (control) (5mg/kg)	104.4±6.153***	60.40±3.982***	46.20±2.782***
Atrazine (5mg/kg) + Melatonin (10mg/kg)	117.6±3.614***	151.2±4.116***	155.4±7.840*
Atrazine (5mg/kg) + Primaquine (10mg/kg)	69.60±6.947***	51.80±4.684***	98.40±3.894***
Atrazine (5mg/kg) + Melotonin (10mg/kg)+ Primaquine (10mg/kg)	82.60±4.802***	98.20±4.684***	100±4.336***

Values are expressed as Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, n=6 in each group.

Table 4: Ameliorative effect of Melatonin on retention transfer latency on day 7th, 14th and 21th in Atrazine induced Glucose-6-phosphate deficiency in Rats by using Elevated Plus Maze

Treatment (oral)	Inflexion ratio (sec)	
	Day 7-14	Day 14-21
Normal	14.14±1.524	15.13±1.282
Atrazine (control) (5mg/kg)	8.59±1.317***	89.65±2.587***
Atrazine (5mg/kg) + Melatonin (10mg/kg)	26.61±1.866**	24.60±1.983
Atrazine (5mg/kg) + Primaquine (10mg/kg)	75.07±1.755***	76.24±2.014***
Atrazine (5mg/kg) + Melotonin (10mg/kg)+ Primaquine (10mg/kg)	29.95±2.925***	29.95±2.954**

Values are expressed as Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, n=6 in each group.

Table 5: Ameliorative effect of Melatonin on escape latency in Atrazine induced Glucose-6-phosphate deficiency in Rats using Morris Water Maze

Treatment (oral)	Escape latency (secs)		
	Day 7	Day 14	Day 21
Normal	18±1.049	14±1.612	23±2.915
Atrazine (Control) (5mg/kg)	41.20±1.158***	52.20±1.393***	54.80±1.685***
Atrazine (5mg/kg) + Melatonin (10mg/kg)	37.60±0.7483***	32.60±1.435***	25±0.9487
Atrazine (5mg/kg) + Primaquine (10mg/kg)	44.20±0.9695***	48.20±1.881***	49.60±2.676***
Atrazine (5mg/kg) + Melotonin (10mg/kg) + Primaquine (10mg/kg)	27±0.7071***	23.40±1.208***	20±0.8367

Values are expressed as Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, n=6 in each group.

DISCUSSION

Glucose-6-phosphate is a house-keeping enzyme acts as potent scavenger of free radicals, whose secretions is governed by endocrine system in pentose phosphate pathway (PPP) which is fast-rate limiting reaction. An impression of G6PD is present in different parts of body in various percentage. The presence was observed in brain, heart, lungs, kidney, liver, pituitary, hypothalamus, placenta, retina, prostrate, trachea, thyroid, thalamus, tongue, testis, thymus, small intestine, uterus, smooth muscles, skeletal muscles, salivary glands, pancreas etc. Likewise deficiency of G6PD is responsible for various neurodegenerative disorder like Parkinson's, alzeimers disorders etc.

The study was aimed to evaluate the neuroprotective role of melatonin with Primaquine in Atrazine induced G6PD deficiency in Wister albino rats. The animals of body weight (150-200gm) were housed in standard poly propylene cages and maintained under controlled room temperature (22±2ºC) and humidity (55±5%) with 12:12 hour light and dark cycle. All the animals were provided with commercially pallet diet and water *ad libitum*. The study was approved by Institutional Animal Ethics Committee under the approved proposal no. (273/CPCSEA). Throughout the experiment, animals were maintained according to the CPCSEA guidelines. Animals were divided into seven groups and observed under treatment protocol.

Atrazine were used for induction of glucose-6-phosphate dehydrogenase deficiency in animals. Atrazine was dissolve in DMSO, Melatonin in propylene glycol, Primaquine in distilled water. Dosing protocol was followed for 21days according to body weights of the individual animal. During the experimental protocol, animals were evaluated for memory exercised using Elevated plus maze test on 7th, 14th and 21th day. 7th day onward animals were trained for swimming to identify hidden platform to check index of acquisition using Morris water maze. On 14th and 21st day, transfer memory and retention memory were observed and results showed significant improvement in it.

The statistical analysis was carried out using Graph Pad Prism 5.0 software. All values are presented as Mean ± SEM. Multiple comparisons between different groups were performed using Analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Difference level at P< 0.05 was considered statistically significant condition.

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