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Study of various biochemical parameter on atrazine induced glucose-6-phosphate dehydrogenase deficiency in brain

Pallavi Ghildiyal

Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, Uttarakhand

*Corresponding author email: pallavighldl8@gmail.com

Alok Bhatt

School of Pharmaceutical Sciences, Himgiri Zee University, Dehradun, Uttarakhand

Nidhi Chaudhary

School of Pharmaceutical Sciences, Himgiri Zee University, Dehradun, Uttarakhand

Sonia Narwal

Department of Pharmacy, Panipat Institute of Engineering and Technology, Samalka Panipat Haryana

Palika Sehgal

Department of Pharmacy, Panipat Institute of Engineering and Technology, Samalka Panipat Haryana

Abstract---G6PD is rate limiting enzyme in pentose phosphate pathway (PPP), have effective physiological role in supply of NADPH by converting it into Glucose-6-phosphate to phosphogluconate which acts as 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 more than one gene for G6PD and around 2.9% of population is 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 research work was aimed to check the neuroprotective potential of Melatonin with Primaquine and Melatonin with Aspirin (contraindicated to G6PD deficient individuals) in Atrazine induced G6PD deficiency in Albino Rats. Study was carried

out for the various biochemical parameters (G6PD, Nitrites, LDH, Glucose), neurotransmitters (Serotonin, Dopamine, Nor-adrenaline), 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

G6PD is rate limiting enzyme in pentose phosphate pathway (PPP), have effective physiological role in supply of NADPH by converting it into Glucose-6-phosphate to phosphogluconate which acts as a major cell reductant and useful to cell survival. 20 NADPH plays an effective role in anti-oxidation reaction with glutathione, glutathione peroxides and enzyme catalase. G6PD normally acts as "maintenance department" inside the body. According to WHO, 75% of world population have more than gene for G6PD and around 2.9% are G6PD deficient population.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, Alzheimers, Schizophrenia catatonia, Neuronal toxicity etc. Effect of melatonin along with primaquine and aspirin showed significant improvement in G6PD deficient wistar albino rats.¹⁹ The research work was aimed to evaluate the ameliorative effect of Melatonin along with Primaquine and Melatonin with Aspirin in Atrazine induced G6PD deficiency in brain.

Under the approval of IAEC proposal no. (273/CPCSEA) study were carried out. Experimental Animals are divided into seven different groups (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 the results are expressed as Mean $\pm$ SEM. Various comparisons was done among different groups and performed by using Analysis of variance (ANOVA).

Materials & Methods

Chemicals Atrazine, melatonin, primaquine biphosphate were purchased from Sigma Aldrich. Experimental Animals (Wister albino rats) of around 160-230g body weight and age of 6.5 months (either sex) were procured from the Animal house of institution (SBSPGI, Balawala, Dehradun). The experimental animals were kept in standard animal cages and maintained at room temperature

($24\pm 3^{\circ}\text{C}$), humidity ($52\pm 3\%$) with 12 hour light and 12 hour dark cycle. Animals were feeded with pallet diet and plenty of water with water bottle. The research work conducted after the approval of IAEC, under the approval no. (273/CPCSEA).

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 E (T2; n=6) Atrazine (5mg/kg/orally), Primaquine (10mg/kg/orally) and Melatonin (10mg/kg/orally).

Group F (T3; n=6) Atrazine (5mg/kg/orally) and Aspirin (10mg/kg/orally)

Group G (T4; n=6) Atrazine (5mg/kg/orally) and Aspirin (10mg/kg/orally) and Melatonin (10mg/kg/orally).

Estimation of various biochemical parameters

Biochemical parameters were carried out after the last behavioural activities on 21st day. Brain was removed quickly and washed with saline solution. Tissue was homogenized in 10% w/v of buffer solution (TRIS) and centrifuged at 3000RPM for 10 min and the supernatant liquid was used for various biochemical parameters like G6PD, Nitrite/Nirate, LDH, neurotransmitters like dopamine and nor-adrenaline.

Estimation of G6PD enzyme:

G6PD enzyme is responsible for supply of NADPH in brain for fulfilling the energy requirement and maintain oxidative stress. Activity of G6PD in brain homogenate was estimated as described by (Varley et.al., 1991).

01 ml of brain homogenate supernatant, 2 ml triethanolamine buffer (pH 7.6) and 0.1 ml of NADP was added and mixed well, then allowed to stand for 5 min incubation. To reaction mixture 50 μl of glucose-6-phosphate (9.42 mg/ml) was added and kept for 2 min. OD was measured at 340 nm for every five minutes against a blank without G6P and NADP.

Calculation

$\Delta\text{E}_{340}/\text{min}$ was converted by multiplying with 500.

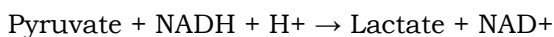
Estimation of Nitrite/Nitrate

Brain nitric oxide plays crucial role such as in neuromodulation, neurotransmission, synaptic plasticity, neurodegeneration and neuroinflammation. This method expresses the nitrous oxide concentrations by enzymatic conversion in presence of nitrate reductase by nitrate to nitrite. The reaction is was observed in colorimetric detection of nitrite at 540-570nm.

The 750 µl of sample (brain homogenate) and Sodium nitrite, 750 µl of Griess reagent was added. Mixture was kept in incubation in dark for 15 min at normal conditions. Blank were prepared. Absorbance was taken at 540 nm against blank. Triplicate estimation of sample was done.

Lactate dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is extensively present in animal body tissues such as heart, brain blood cells, etc. LDH has receive significance medical attentions as it release during the tissue damage and serve as an important marker of injuries and disease such as cancer, hemolysis, meningitis, encephalitis etc. The conversion of pyruvate to lactate take place due to LDH catalyse, NAD to NADH. The activity of tissue sample is directly proportional to rate of decrease in absorbance (340 nm).



Working solution was prepared with reconstitution of reagents 1 and reagent 1A (240µl of reagent 1 in 3ml of reagent 1A). About 20µl sample was added to the 1 ml of working solution. Mix and read immediately at 340nm against blank. Rate of decrease in absorbance per minute were observed.

Results

Table 1.1. Ameliorative effect of drugs on the level of brain G6PD, Nitric oxide and LDH

Treatment	G6PD (NADPH/min)	Nitric oxide (µmol/L)	Lactate Dehydrogenase (U/L)
Sham control	85.60 ±4.273	40.540 ± 4.810	341.4 ±28.97
Control	21.80 ±2.354***	151.400 ± 32.820***	546.7 ±18.05***
Standard	64.40 ±2.159***	69.110 ± 6.963	389.9 ±26.60
T1	5.800 ±1.158***	346.900 ± 24.170***	552.8 ±13.60***

T2	28.40 ±1.364***	321.800 ± 14.690***	507.4 ±23.91***
T3	46.40 ±2.421***	132.600 ± 3.627**	389.4 ±10.63
T4	53.00 ±2.775***	84.740 ± 3.738	328.4 ±17.23

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

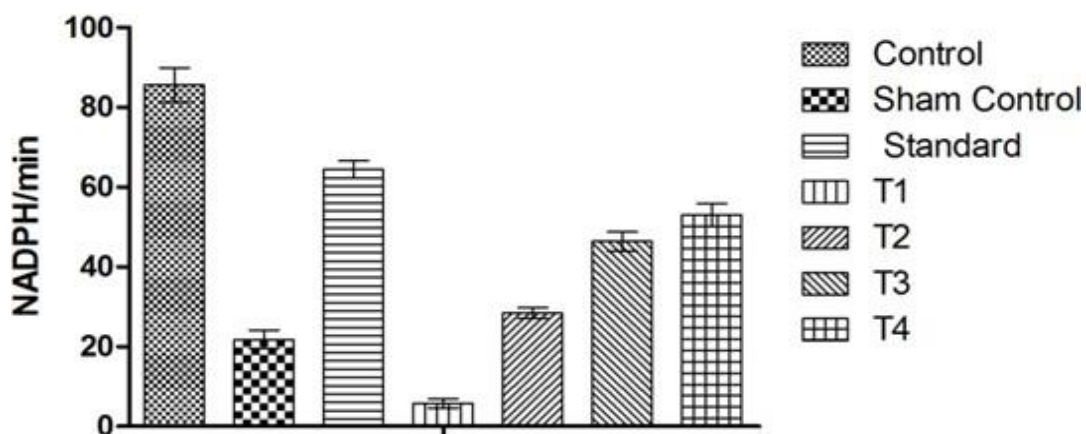


Figure 7.5 Ameliorative effects at brain (Level of Glucose-6-phosphate dehydrogenase)

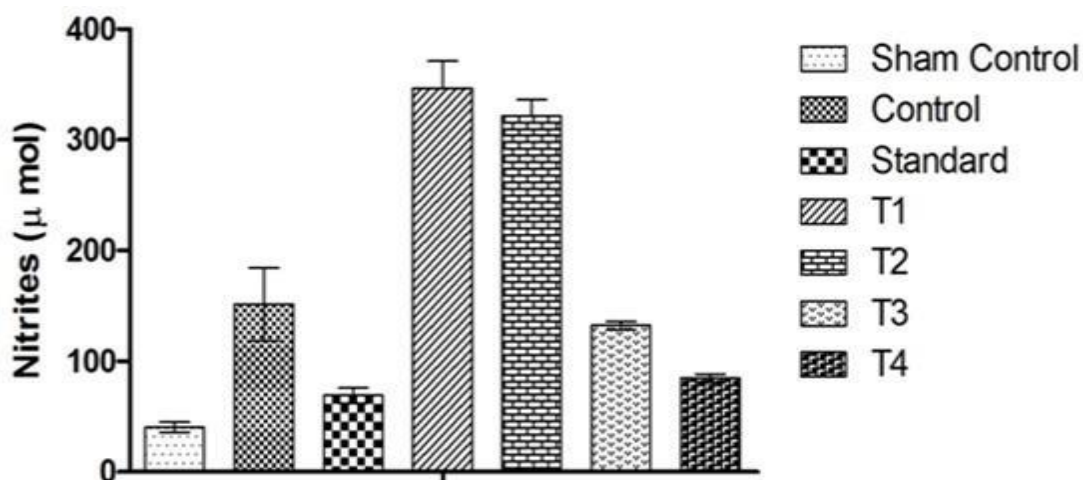


Figure 7.6 Ameliorative effect at brain (Level of Nitric oxide)

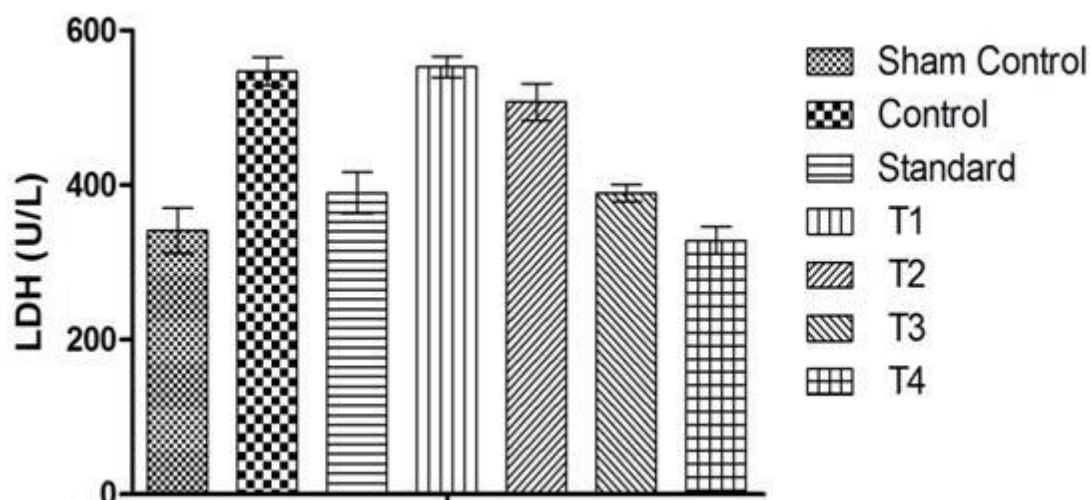


Figure 7.7 Ameliorative effect at brain (Level of Lactate dehydrogenase)

Discussion

From the Table 1.1 was observed that the T2 and T4 group have significant improvement in G6PD level as compared to control group, whereas the level of T4 treatment is much effective then T2. The T4 treatment group significantly shows reduction in nitric oxide level as compared to Standard group, whereas the T2 treatment group also has significant reduction as compared to T1 group. The Lactate Dehydrogenase level of T1 group is higher as compared to Control group. There is significantly reduction in LDH level is seen in T2 and T4 treatment group as compared to T1 and T3 group. Overall observation reflects that the level of G6PD, Nitric oxide and LDH shows significant improvement in Treatment group T4 and T2 as compared to T3 and T1 group.

Conclusion

G6PD is rate limiting enzyme in pentose phosphate pathway (PPP), have effective physiological role in supply of NADPH by converting it into Glucose-6-phosphate to phospho-gluconate which acts as 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 dehydrogenase. According to WHO 75% of world population have more then one gene for G6PD deficiency and around 2.9% are G6PD deficient population. More prevalent is G6PD (A-) deficient variants. Mediterraneans (population) was identified with less oxygen carrying capacity red blood cells. While in India 60% of population was suffering from various degree of G6PD deficiency.

In the present study protocol was followed for 21days to evaluate various biochemical parameters, in brain homogenate tissues. Therefore, according to the above shown results it was concluded that treatment group (T4) results statistically significant improvement in biochemical parameters. Moreover, treatment with standard Melatonin (10mg/kg) results statistically more significant effect on above said studies.

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