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Effect of Aluminium Acetyl Acetonate on the Chemical Status of Glutathione by Influential Parameters in Aqueous Medium

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Abstract – Aluminium is the most abundant metal and the third most abundant element, after oxygen and silicon, in the earth's crust. It is widely distributed and constitutes approximately 8 percent of the earth's surface layer. However, Aluminium is a very reactive element and is never found as the free metal in nature. Ellman's method was used to check the concentration, pH, and temperature and time dependent effect of Aluminium- acetyle acetonate on GSH status in aqueous medium. Aluminium- acetyle acetonate reduced the GSH level with concentration, pH, temperature and time. GSH reduction is due to oxidation of reduced GSH by Aluminium- acetyl acetonate by changing it to GSSG or by forming GS-Aluminium conjugate. Present study is in vitro condition which may present the model for in vivo condition.

Keywords – Aluminium acetyl acetonate, Ruduced Glutathione (GSH), Ellman's method, 5, 5-Dithiobis, 2-Nitrobenzoic Acid (DTNB)

1. Introduction

The widespread distribution of glutathione and its role in protection against oxidative stress, detoxification of xenobiotics and modulation of enzyme activity by disulfide interchange (Meister et al 1989) have aroused continuous interest in analytical methods for this intracellular low-molecular-weight thiol, which is involved in a multitude of cellular functions. In cells, oxidation of reduced glutathione (GSH), the predominant form, leads to the formation of glutathione disulfide (oxidized glutathione, GSSG). Intracellular glutathione is effectively maintained in the reduced state by a ubiquitous and crucial flavoenzyme, GSSG reductase (GR), linked to the NADPH/NADP+ system (Akerboom. and Sies 1981). As a thiol redox couple, glutathione disulfide (GSSG) and GSH also function in gene regulation and intracellular signal transduction (Dalton et al., 1999). Depletion of GSH in cell is the suicide of cell and this phenomenon is called apoptosis (Khan et al., 2010).

Exposed to increased oxidative stress, the ratio of GSH/GSSG decreases due to the accumulation of GSSG (Baskin and Salem 1997). On this basis, the reduced glutathione/oxidized glutathione ratio (GSH/GSSG ratio) is used to evaluate oxidative stress status in biological systems. Oxidative stress has been recognized in different chronic diseases and has been assessed by blood GSSG levels (Mills et al 1994). A decrease in GSH concentrations has been implicated in several pathological conditions, such as diabetes, alcoholic liver disease, AIDS, cataractogenesis, rheumatoid arthritis, muscular dystrophy, amyotrophic lateral sclerosis, Alzheimer disease, respiratory distress syndrome, acute hemorrhagic gastric erosions, xenobiotic-induced oxidative stress and toxicity and aging (Pastore et al 2003). Aluminium Acetylacetonate is an Aluminium source that is soluble in organic solvents as an organometallic compound (also known as metalorganic, organo-inorganic and metallo-organic compounds). The high purity acetylacetonate anion complexes by bonding each oxygen atom to the metallic cation to form a chelate ring.Because of this property, Aluminium Acetylacetonate is commonly used in various catalysts and catalytic reagents for organic synthesis

2. Materials and methods

2.1. Materials

L.Glutathione (GSH)(Fluka), DTNB (Sigma), Sodium Hydroxide (Fluka HG), Potassium Dihydrogen Phosphate (Merck), HCl 35% (Kolchlight) Aluminium Acetylacetonate (BDH, Germany), Sodium Chloride (Merck), Disodium Edetate (Riedel Dehean HG Sleeze Hannover). Distilled Water (Double Distilled). U.V 1601 spectrophotometer (Shimadzu). pH Meter : Model NOV-210, Nova Scientific Company Ltd. Korea, Oven: Memmert Model U-30,854

Schwabach (Germany). Megnetic Stirrer, hot plate 400(England) .Micropipettes 200 µl, 500 µl, 1000 µl were used of Socorex Swiss (Finaland), Sortorius Balance, , Disposable Rubber Gloves, were used in this research work

2.2. Effect of Different Concentrations of Aluminium Acetylacetonate on the Chemical Status of Glutathione (GSH) In Aqueous Medium

To 0.8ml (800µl) of 1mM Glutathione (GSH) taken in five separate test tubes,1ml (1000µl) of different concentrations of 50µM,100µM,150µM, 200µM, 25µM,300µ 350µM,400µM,450µM and 500µM solution of Aluminium Acetylacetonate were added separately, shaked and further diluted to 2ml with phosphate buffer pH 7.6.The final concentration of Glutathione (GSH) in each five tubes were 0.4mM (400µM). Five separate tubes were prepared with 0.2ml (200µl) Aluminium Acetylacetonate plus GSH mixture from each previously made five tubes diluted with 2.3ml(2300µl) of phosphate Buffer pH 7.6 and added 0.5ml(500µl) of 1mM 5,5-Dithiobis,2-Nitrobenzoic Acid (DTNB) stock solution. The final concentration of Glutathione (GSH) in each of these five tubes was 0.02666mM (26.66µM).The Aluminium Acetylacetonate on the chemical status of Glutathione (GSH) was studied using influencial parameters by a well known Ellman's method, as mentioned in standard curve for GSH. A control for Glutathione (GSH) was also prepared by taking 0.8ml (800µl) of 1mM Glutathione (GSH) stock solution in a test tube and diluted with 1.2ml (1200µl) of phosphate buffer pH 7.6 with final concentration of 0.4mM (400µM) of Glutathione (GSH).

2.3. Effect of Different Concentrations of Aluminium Acetylacetonate on the Chemical Status of Glutathione (GSH) With Time

To 0.8ml (800µl) of 1mM Glutathione (GSH) taken in five separate test tubes ,1ml (1000µl) of different concentrations of 50µM,100µM,150µM, 200µM, 25µM,300µ 350µM,400µM,450µM and 500µM solution of Aluminium Acetylacetonate were added separately, shaked and further diluted to 2ml with phosphate buffer pH 7.6.The final concentration of Glutathione (GSH) in each five tubes were 0.4mM (400µM). Five separate tubes were prepared with 0.2ml (200µl) Aluminium Acetylacetonate plus GSH mixture from each previously made five tubes diluted with 2.3ml(2300µl) of phosphate Buffer pH 7.6 and added 0.5ml(500µl) of 1mM 5,5-Dithiobis,2-Nitrobenzoic Acid (DTNB) stock solution. The final concentration of Glutathione (GSH) in each of these five tubes was 0.02666mM (26.66µM).The effect of Aluminium Acetylacetonate on the chemical status of Glutathione (GSH) was studied in terms of determination of concentration of GSH in mixtures by a well known Ellman's method, as mentioned in standard curve for GSH. The observation is shown in the table#2

A control for Glutathione (GSH) was also prepared by taking 0.8ml (800µl) of 1mM Glutathione (GSH) stock solution in a test tube and diluted with 1.2ml (1200µl) of phosphate buffer pH 7.6 with final concentration of 0.4mM (400µM) of Glutathione (GSH).

3. Results

3.1. Effect of Aluminium Acetylacetonate on the Chemical Status of Glutathione (GSH) In Aqueous Solution

Effect of Aluminium Acetylacetonate on the Chemical status of Glutathione was studied in term of determination of Concentration of Glutathione (GSH).

Aluminium Acetylacetonate caused a decrease in the concentration of Glutathione (GSH). Different concentrations of Aluminium Acetylacetonate cause a gradual decrease in the concentration of Glutathione (GSH) in aqueous medium. The observations are shown in the table 1and in graphical form in figure 1.

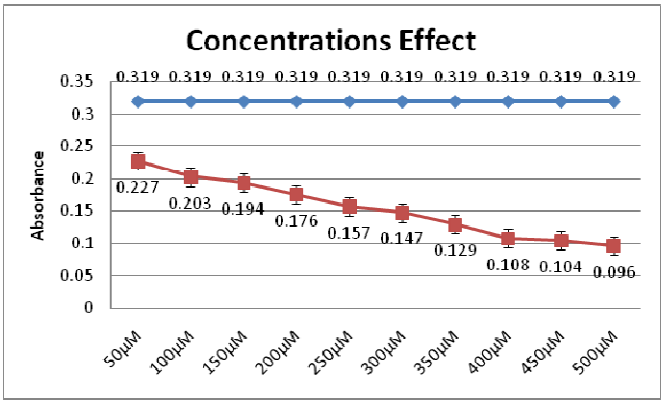

Figure 1: Concentration effect

3.2. Effect of Aluminium Acetylacetonate on the Chemical Status of Glutathione (GSH) with time

Effect of Aluminium Acetylacetonate on the chemical status of Glutathione (GSH) was also studied for the time dependency and noted that the concentration of Glutathione (GSH) was gradually decreased as the time passes from 0 minute interval of time to 90 minutes. The observations are shown in the table1 and in graphical form in figure2.

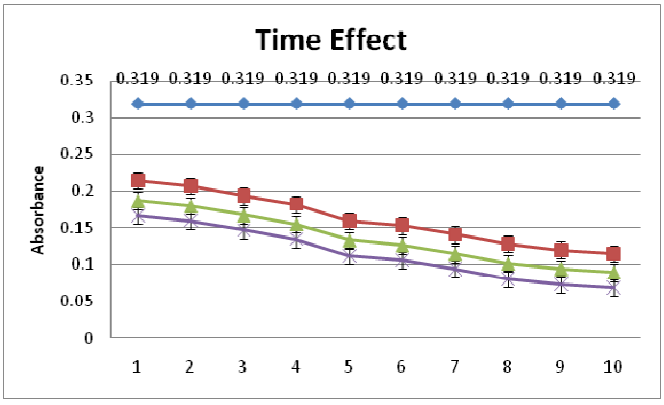

Figure 2: Time effect

Effect of Aluminium Acetylacetonate on the Chemical Status of Glutathione (GSH) withPH, and Effect of Aluminium Acetylacetonate on the Chemical Status of Glutathione (GSH) with temperature are shown in figure 3 and figure 4 respectively.

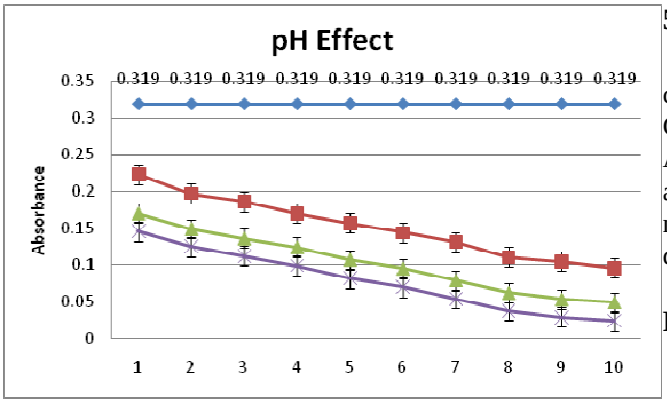

Figure 3: pH effect

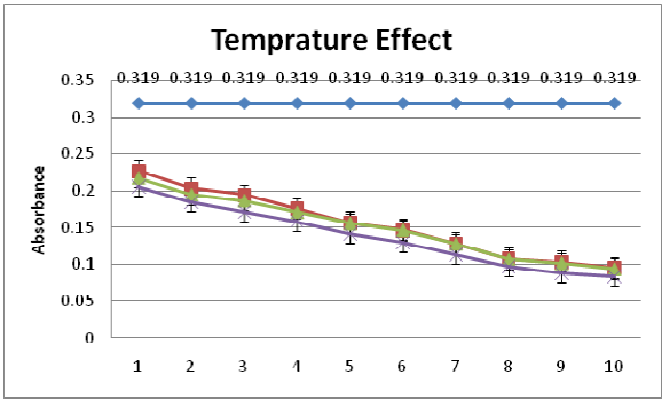

Figure 4: Temperature effect

4. Discussion

Different parameters were discussed in this research which is concentration, time, pH and temperature. Aluminium Acetylacetonate plays no known natural biological role in humans, and possible health effects of Aluminium Acetylacetonate are a subject of dispute. Aluminium Acetylacetonate itself is not toxic but most Aluminium Acetylacetonate are, and some may be carcinogenic, Thus it was of interest to study the interaction of these metals in vitro to establish further scientific data.Aluminium Acetylacetonate on the chemical modulation of GSH will enable us to understand further the role of Aluminium Acetylacetonate and GSH and strengthen our Knowledge about their therapeutic uses in many diseases and the treatment for their toxic nature to health.In the same manner the effect of Aluminium Acetylacetonate was also studied for the concentration dependent and time dependent on the chemical status of Glutathione (GSH) and the concentration of reduced Glutathione (GSH) was decreased with increasing concentration of Aluminium Acetylacetonate in solution and with the passage of time ,respectively. The results also suggested that there was a possibility of formation of intermediate or conjugate between Aluminium Acetylacetonate and GSH. However it was not possible to estimate or determine those conjugates under those conditions .Since both GSH and Aluminium Acetylacetonate, is biological active compounds. It was of interest to study the possible interaction of this metal in vitro as a model of in vivo interaction.

5. Conclusion

Different concentration of Aluminium Acetylacetonate caused a gradual decreased in the concentration of Glutathione (GSH) in aqueous.Effect of Aluminium Acetylacetonate on the chemical status of Glutathione was also studied for the time, pH and temperature effect and noted that the concentration of Glutathione (GSH) gradually decreased by influential parameters in aqueous solution.

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Concentration,time,pH and temperature effect of Aluminium Acetylacetonate on GSH													
	Time	50µM		100µM	150µM	200µM	250µM	300µM	350µM	400µM	450µM	500µM	Control
1	**Avg ABS at 0 Min.**	0.272		0.261	0.252	0.235	0.220	0.206	0.192	0.174	0.173	0.164	0.317
		PH	6.5	0.267	0.255	0.244	0.229	0.220	0.203	0.194	0.177	0.173	0.164	0.317
			7.6	0.215	0.208	0.194	0.183	0.170	0.154	0.142	0.129	0.122	0.117	0.317
			8.5	0.191	0.183	0.170	0.158	0.145	0.129	0.117	0.104	0.097	0.092	0.317
		Temp	25C	0.272	0.261	0.252	0.235	0.220	0.206	0.192	0.174	0.173	0.164	0.317
			37C	0.263	0.254	0.245	0.230	0.219	0.206	0.191	0.173	0.170	0.162	0.317
			45C	0.250	0.243	0.230	0.218	0.186	0.189	0.177	0.164	0.157	0.152	0.317
2	**Avg ABS at 30 Min.**	0.214		0.207	0.193	0.182	0.149	0.153	0.141	0.128	0.120	0.115	0.317
3	**Avg ABS at 60 Min.**	0.187		0.180	0.167	0.155	0.123	0.126	0.114	0.101	0.094	0.089	0.317
4	**Avg ABS at 90 Min.**	0.167		0.160	0.147	0.135	0.103	0.106	0.094	0.081	0.074	0.069	0.317

Table.1.Concentration,time,pH and temperature effect of Aluminium Acetylacetonate on GSH.