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Variation in Absorbance of Isoleucine-Lithium Chloride interaction with various Nitric oxide donors.

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ABSTRACT
Branched-chain amino acids (BCAAs: leucine, isoleucine, and valine) are essential amino acids for humans and play an important role as the building blocks of proteins. Isoleucine, as one of the branched chain amino acids, is also critical in physiological functions of the whole body, such as growth, immunity, protein metabolism, fatty acid metabolism and glucose transportation. Isoleucine can improve the immune system, including immune organs, cells and reactive substances. Isoleucine administration can restore the effect of some pathogens on the health of humans and animals via increasing the expression of β-defensins.In present study we have tried to study .
Lithium compounds are used as psychotropic drugs. Numerous lithium salts are used as mood stabilizers, particularly in the treatment of bipolar disorder, where they play a role in the treatment of acute and long-term depression and especially mania. In the present study, we attempted to investigate how lithium affects Isoleucine by examining their interactions.NO* is also a signalling molecule closely linked to the nervous system. Therefore, it will be useful to understand whether NO donors have a direct impact on the interaction of lithium with important biological molecules. In this work we wanted to investigate the role of nitric oxide donors on lithium - Isoleucine binding.
Key words: Lithium, Isoleucine psychotropic,bipolar

I. INTRODUCTION

Isoleucine is an essential amino acid. Isoleucine is the oxygen-carrying pigment inside of red blood cells and helps to make haemoglobin. It is also helpful in controlling blood sugar, boosting energy, and improving endurance. Isoleucine is known to help speed healing of injured muscle and support muscle development and lean body mass. Branched chain amino acids are the essential nutrients for humans and many animals. As functional amino acids, they play important roles in physiological functions, including immune functions. Isoleucine, as one of the branched chain amino acids, is also critical in physiological functions of the whole body, such as growth, immunity, protein metabolism, fatty acid metabolism and glucose transportation. Isoleucine can improve the immune system, including immune organs, cells and reactive substances.

Lithium, a first-line therapy for bipolar disorder, is effective in preventing suicide and new depressive/manic episodes. Yet, how this beguilingly simple monocation with only two electrons could yield such profound therapeutic effects remains unclear. An in-depth understanding of lithium's mechanisms of actions would help one to develop better treatments limiting its adverse side effects and repurpose lithium for treating traumatic brain injury and chronic neurodegenerative diseases.

NO was introduced in the late 1990s as a signaling molecule in osteoclasts and in tooth movement in orthodontics in 2002. The first research area to recognize NO as a signaling molecule was cardiovascular research. The finding that NO influences cell function via sGC has led to further research in various areas, as sGC (soluble guanylate cyclase) can activate various signalling pathways in cells. The discovery of nitric oxide as a signaling molecule began with research into the mechanism by which blood vessels relax and constrict, processes known as vasodilation and vasoconstriction. In addition to understanding the basic biology of these processes, scientists have also recognized their medical importance, as vasodilators can help treat cardiovascular disease. Nitroglycerin, long used to treat angina, is known to promote vasodilation.Nitroglycerin and other nitrogen-containing compounds applied to isolated blood vessels activate a signalling pathway that initially stimulates the production of cyclic guanosine monophosphate (cGMP) and ultimately

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leads to dilation. Nitroglycerin is a "prodrug" that undergoes complex metabolic biotransformation primarily in the intracellular space of smooth muscle. This biotransformation leads to the formation of nitric oxide[2].Nitrates work by releasing nitric oxide (NO), a substance that plays an important role in various organ systems. Nitric oxide has a regulating effect on insulin and carbohydrate metabolism [3]. It plays no regulatory role in the hypothalamic axis, nor in inhibiting prolactin release, nor in regulating steroid and catecholamine secretion [4]. Nitric oxide is a very unstable molecule and nitric oxide synthase only remains active for a few seconds (the half-life varies between 3 and 50 seconds) [5]

In this paper we have made an attempt to study the effect on Isoleucine- LiCl binding due to nitric oxide donors such as Sodium Nitroprusside, Sodium nitrite, Nitro etc. Some positive conclusions was observed in the study.

II. MATERIALS & METHODS

Chemical and Reagents: - All the chemicals used for the work are of A.R. grade of S. D. FineChem. Sisco.

Instruments: - U.V.VISIBLE Spectrophotometer(SHIMADZU MODEL UV-2450) and
Electrical balance (Type Citizen CY 204).

EXPERIMENTAL

All the chemicals used for the work are of A.R. grade of S.D.Fine or Merk .Spectral analysis is carried out with Shimadzu model 2450 U.V. spectrophotometer and for visible Spectrophotometry readings Systronics double beam spectrophotometer is used. All glasswareused were of Borosil or J-sil make. Double distilled water used for analysis was from doubledistillation unit of Borosilmake.Standard procedures were used for measurement of absorbanceand to calculate corrected absorbance.L-Isoleucine solution is prepared in distilled water and is studied at its λ max. 0.01Maqueous solutions of these were used for spectroscopic study.

1) By Ultraviolet spectroscopy.

The study is carried out with 1.5 cm^3 of L-Isoleucine and 1.5 cm^3 of distilled water + 0.001M LiClis taken.First the wavelength for maximum absorbance is recorded then at ʎ max the readings for all variousvolumes of LiCl is added with total volume keeping fixed as 3.0 cm^3 .

2) By visible spectroscopy using Ninhydrin.

The study is carried out by developing colour with the help of ninhydrin.1.0 cm^3 of L-Isoleucine 0.01M solution taken in boiling tubes to that 4.0 cm^3 of distilled water is added and 4-6 drops of0.01M ninhydrin solution prepared in acetone is added. Tubes are covered with aluminium foiland kept in boiling water bath for ten minutes. Blue colour develops. Tubes are cooled down toroom temperature then by carrying our appropriate dilution, used for recording readings.

1. Effect of NO donors such as Hydroxyurea, Sodium nitroprusside, Sodium nitrite and nitroglycerine on binding in 1 &2 .

III. RESULTS AND DISCUSSIONS

1. To study the interaction of Lithium with Glutamic acids with variable amounts of
Water and LiCl.

a) **By ultraviolet spectroscopy**:

i) **L-Isoleucine**: ʎmax is determined for each combination. Absorbance of solution is determined for Isoleucine and water- LiCl combination in such a way that the total volume is always a constant.

Table 1

Sr no.	Name of compound	Absorbance
1	2 cm^3 Isoleucine + 0.1cm^3 of water scan at 265 ʎ max	0.710
2	2 cm^3 Isoleucine + 0.98cm^3 of water +0.02 cm^3 LiCl	0.735
3	2 cm^3 Isoleucine + 0.96cm^3 of water +0.04 cm^3 LiCl	0.739
4	2 cm^3 Isoleucine + 0.94cm^3 of water +0.06 cm^3 LiCl	0.745
5	2 cm^3 Isoleucine + 0.92cm^3 of water +0.08 cm^3 LiCl	0.773
6	2 cm^3 Isoleucine + 0.90cm^3 of water +0.1 cm^3 LiCl	0.792
7	2 cm^3 Isoleucine + 0.88cm^3 of water +0.12 cm^3LiCl	0.814

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1b) By visible spectroscopy:

i) **L-Isoleucine** : λ max = 583 nm

For working in visible region colour is developed as mentioned in methodology.

Table 2

Solutions cm^3	0.001M LiCl cm^3	Absorbance	Correction factor	Corrected Absorbance
3.0	0.0	0.414	1.0	0.414
3.0	0.02	0.407	1.0067	0.409713
3.0	0.04	0.403	1.01	0.40703
3.0	0.06	0.396	1.01	0.39996
3.0	0.08	0.389	1.01	0.39289
3.0	0.1	0.386	1.01	0.38986
3.0	0.12	0.381	1.01	0.38481
3.0	0.14	0.377	1.01	0.38077
3.0	0.16	0.373	1.01	0.37673
3.0	0.18	0.367	1.01	0.37067
3.0	0.2	0.362	1.01	0.36562
3.0	0.3	0.348	1.045	0.36366
3.0	0.4	0.336	1.043	0.350448
3.0	0.5	0.332	1.042	0.345944

2. Effect of Sodium nitroprusside on Isoleucine–Lithium chloride binding

In each case 2.0 $cm^3$of Isoleucineis taken to that 0.5 cm^3 of lithium chloride is added. To this solution variable volume of sodium nitroprusside is added till 0.5 cm^3.Absorbance is recorded in each case and correction factor is applied for each addition and corrected absorbance is calculated.

Table 3

ISOLEUCINE at 583 nm					
volume of ISOLEUCINE cm^3	Vol. of 0.001M LiCl cm^3	volume of SNP cm^3	Absorbance	correction factor	corrected absorbance
2.0	0.5	0.0	0.448	1.0	0.448
2.0	0.5	0.02	0.437	1.008	0.440496
2.0	0.5	0.04	0.444	1.008	0.447552
2.0	0.5	0.06	0.476	1.008	0.479808
2.0	0.5	0.08	0.416	1.008	0.419328
2.0	0.5	0.1	0.404	1.008	0.407232
2.0	0.5	0.12	0.393	1.008	0.396144
2.0	0.5	0.14	0.375	1.008	0.378
2.0	0.5	0.16	0.363	1.008	0.365904
2.0	0.5	0.18	0.351	1.008	0.353808
2.0	0.5	0.2	0.334	1.008	0.336672
2.0	0.5	0.22	0.323	1.008	0.325584

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2.0	0.5	0.24	0.305	1.008	0.30744
2.0	0.5	0.26	0.291	1.008	0.293328
2.0	0.5	0.28	0.28	1.008	0.28224
2.0	0.5	0.3	0.26	1.008	0.26208
2.0	0.5	0.32	0.249	1.008	0.250992
2.0	0.5	0.34	0.234	1.008	0.235872
2.0	0.5	0.36	0.225	1.008	0.2268
2.0	0.5	0.38	0.207	1.008	0.208656
2.0	0.5	0.4	0.195	1.008	0.19656
2.0	0.5	0.42	0.182	1.008	0.183456
2.0	0.5	0.44	0.164	1.008	0.165312
2.0	0.5	0.46	0.152	1.008	0.153216
2.0	0.5	0.48	0.144	1.008	0.145152
2.0	0.5	0.5	0.137	1.008	0.138096

3. Effect of Sodium nitrite on Isoleucine–Lithium chloride binding .

In each case 2.0 $cm^3$of Isoleucineis taken to that 0.5 cm^3 of lithium chloride is added .To this solution variable volume of sodium nitrite is added till 0.5 cm^3.Absorbance is recorded in each case and correction factor is applied for each addition and corrected absorbance is calculated.

Table 4

Isoleucine at 583 nm					
Vol. of SERINE cm^3	**Vol. of 0.001M LiCl cm^3**	**Vol. of 0.001M $NaNO_2$ cm^3**	**Absorbance**	**correction factor**	**corrected absorbance**
2.0	0.5	0.0	0.475	1.0	0.475
2.0	0.5	0.05	0.464	1.02	0.47328
2.0	0.5	0.1	0.454	1.02	0.46308
2.0	0.5	0.15	0.443	1.02	0.45186
2.0	0.5	0.2	0.436	1.02	0.44472
2.0	0.5	0.25	0.425	1.02	0.4335
2.0	0.5	0.3	0.419	1.02	0.42738
2.0	0.5	0.35	0.412	1.02	0.42024
2.0	0.5	0.4	0.405	1.02	0.4131
2.0	0.5	0.45	0.398	1.02	0.40596
2.0	0.5	0.5	0.389	1.02	0.39678

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Graphical Representation -

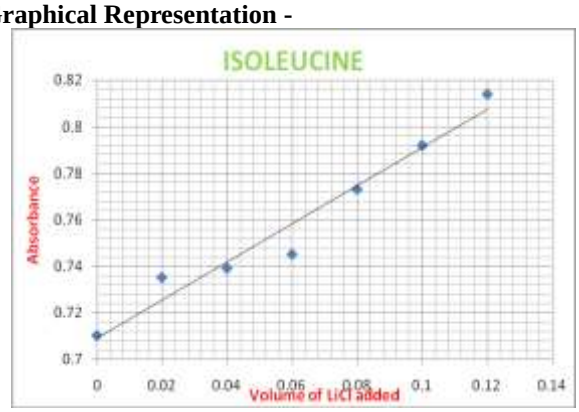

Fig.**1 Effect of Lithium with Isoleucine acids -UV Spectroscopy**

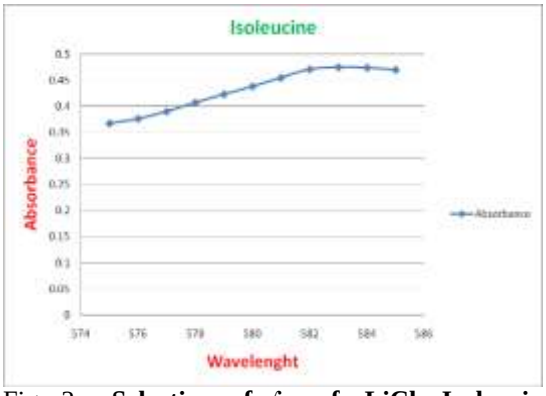

Fig. 2a- **Selection of ʎmaxforLiCl- Isoleucine Visible spectroscopy**

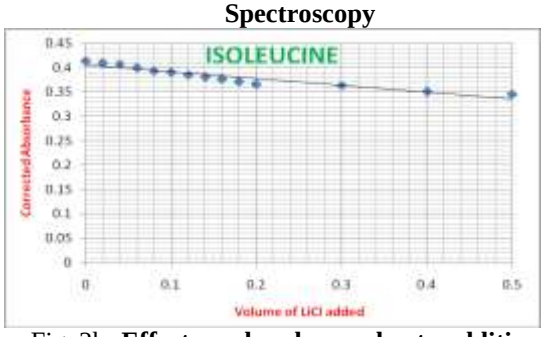

Fig. 2b- **Effect on absorbance due to addition of LiCl in visible region**

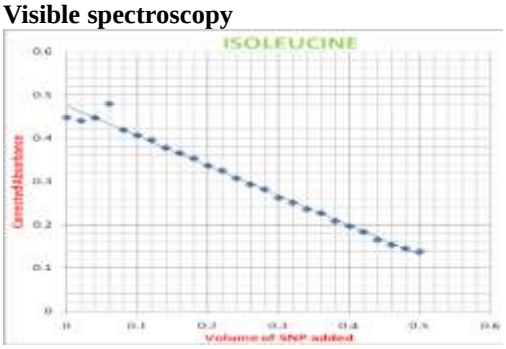

Fig.3- **Effect of Sodium nitroprusside on Isolucine –Lithium chloride binding**

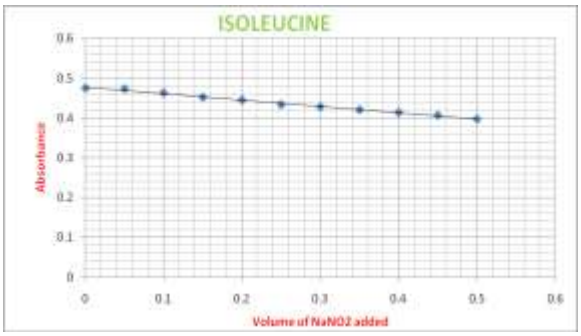

Fig. 4**Effect of Sodium nitrite on Isoleucine – Lithium chloride binding**

Fig. 1 shows effect of addition of various amount of LiCl to Isoleucine. On addition of lithium chloride the absorbance values increases gradually at their respective **ʎmax. , indicating positive binding .i.e. the addition of lithium chloride does show a regular trend in absorbance values.**

Fig.2a- shows selection of ʎmaxvalues for Isoleucine only within the accuracy range is shown.

Fig. 2b- shows the effect of addition of lithium chloride on amino acids. It has been observed that the addition of lithium chloride decreases the colour intensity of the solution decreasing the absorbance values.

Fig.3 &4 shows effect on absorbance due to addition of Sodium Nitroprusside and sodium nitrite, in both cases it is observed that the absorbance of the solution decreases. This shows positive effect on the binding between amino acid and lithium chloride. But this decrease is gradual in case of sodium nitrite.

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IV. CONCLUSIONS

During the 1980s, the free radical, nitric oxide (NO), was discovered to be a crucial signalling molecule, with wide-ranging functions in the cardiovascular, nervous and immune systems.

The actions of organic nitrates and sodium nitroprusside that have long been used in the treatment of angina and hypertensive crises respectively, the discovery generated great hopes for new NO-based treatments for a wide variety of ailments.

It is important to recognize that the concentrations of NO required to mediate the primarily protective effects described above are extremely low (picomolar to nanomolar). An important feature of NO is that its properties and cellular targets at higher concentrations are profoundly different, particularly under conditions of oxidative stress, where it rapidly reacts with superoxide to form peroxynitirite ($ONOO^-$).

Lithium chloride (LiCl) is an essential trace element and the smallest alkali metal with an atomic weight of 6.9 that is found in grains, vegetables and in supplemented drinking water sources that seems to have profound effects on human well-being, susceptibility to several diseases and stem cell growth kinetics. Much remains to be learned and understood about the implications of LiCl in nutrition and stem cell culture. Although there is anecdotal evidence in ancient literature thatingesting inorganic lithium salts can cause weakness, tremors, and blurred vision, this solutionwas marketed by dealers who assured that it was “completely safe,” but to use it only undersupervision recommended by a doctor. Therefore, it will be useful to understand whether NO donors have a direct impact on theinteraction of lithium with an important biological molecule. In this context, we conducted a study using the chemicallithium chloride and an in vitro study to learn how lithium affects amino acids by studying theirinteractions.NO is also a signalling molecule that is strongly linked to the nervous system.

Therefore, it will be useful to understand whether NO donors have a direct impact on the

interaction of lithium with an important biological molecule. In the co-authorslaboratory, work is also being done on various sulphur, iron and glutathione compounds as well ason the influence of various nitrogen oxide donors. The researcher also examined the effects ofsome nitric oxide donors (sodium nitroprusside and sodium nitrite) on amino acids and proteins.

The influence of nitric oxide on the spectral properties of Isoleucine is studied. We obtained interesting results, which is why we think it isimportant to study the role of lithium in proteins and amino acids. The study will help us betterunderstand the physicochemical factors associated with the effects of lithium as a psychotropicdrug and the importance of chemical parameters in its involvement in various physiologicalfunctions. From absorption studies, we observed that lithium influences the spectral behaviour ofamino acids. In this paper we have just focused on Isoleucine. The study was carried outusing nitric oxide donors such as sodium nitroprusside and sodium nitrite, the results are quiteinteresting. Now we need to study the binding effect using the binding parameters. These valuesallow us to determine the magnitude of the effect, which will be the next part of our work.

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