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Impact of (APZ) Alprazolam on its user in Sidhi District of Madhya Pradesh, India

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Abstract: ***The study aims to evaluate APZ effected on blood metabolites, RBC (Red blood cells) membrane and nitric oxide (NO) levels on long term use by human volunteers. A total of (10) tem volunteers taken for study out of which (5) non APZ users (Gr-I) and Rest (5) is long term APZ user (Gr. II) served as a test sample. Biochemical studies were conducted with plasma and RBC membrane of both two groups (I and II) Result of this study revealed that the concentration of glucose, cholesterol and lipoprotein LDL-C were decrease and in contrast the concentration of triglycerides, lipoprotein (HDL-C & VLDL-C) and Nitrate were increased in plasma of group II when compared with group I.***

Keywords: Alprazolam, benzodiazepine, RBC membrane, nitric oxide lipoprotein

1. Introduction

Presently for the treatment for control of stress and its associated disorder, many anxiolytic drugs are commercially available, one of such drug in (APZ) alprazolam, and is a benzodiazepine (BZD) anti- anxiety agent frequently used for the treatment of generalized anxiety, panic attacks with or without agoraphobia and depression in humans as well as in dogs.

APZ is easily absorbed and extensively metabolized in humans after oral administrations, In order to get desirable effect APZ widely using for recreational of purpose and also with drinks and fruits juices. Presently India Rankes top in APZ manufacture (about 5.1 tone) as well as in consumption the research contribution towards APZ is negligible. So for no information is available on its long term use and their consequences at blood metabolites and RBC membrane. Hence this study is undertaken with a view to evaluate the changes induced by the long term use of APZ in human volunteers for past few years.

2. Materials and Method

Male human volunteers of age group 30 to 35 years old residing in Sidhi town of state Madhya Pradesh were taken for the study. Among all the volunteers 5 were free of sedative and any other anti-depressants or tranquilizers and were considered as group- I and remaining 5 were taken who consumed APZ for the past six month to two years daily dose of 0.25-0.5 mg twice a day as a group-II (As shown in table -1) Both the groups were free from any other chronic disease or illness smoking habit and other psychoactive drugs. All the volunteers were well explained about the experimentation and their written consent was also obtained.

Table 1: Characteristic features of volunteers

SNo	Parameter	Group I (control)	Group II (APZ) users
1	Number(n)	5	5
2	Age and Gender	33±2	33±2
3	APZ mg/day	Nil	0.25-0.5

Blood samples from overnight fasted volunteers were collected, stored at – 4^0c in refrigerator and finally used for conducting the study.

Blood was drawn from each volunteers (from both the group I and II) by vein puncture between 7 to 10 AM into heparinized test tube and immediately chilled to 4^0c later plasma and red blood cells were separated for biochemical analysis. All these work done with the help of technician who work with pathology doctor in pathology clinic in the town.

Plasma was analyzed to determine the biochemical parameters such as concentration of glucose, triglycerides, cholesterol, lipoprotein, (high density lipids HDL, low density lipid-LDL and very low density lipid – VLDL) Nitrites and Nitrates. Plasma glucose was estimate by using monozyme diagnostic kit, which is based on the method of tinder .P.(1969)[1]. Using TRBA diagnostic kit (based on the method of trivelli L.A. (1971)[2] plasma HbAlc (hemoglobin A, protein) was determined.

Plasma total cholesterol and HDL cholesterol were estimated by the enzymatic kit method of Allianpoon c.c. (1974)[3] and LDL and VLDL cholesterol were determined by the formulae of friendwordet.al.(1972)[4]

Plasma triglycerides were estimated by using Qualigens diagnostic kit which developed based on the adaptation of the method fossatiavdpriciple (1982)[5].

Nitrite and Nitrate level in plasma were estimated as described by the method of sastry et.al.(2002)[6]

For Biochemical analysis of erythrocyte membrane, blood was diluted with saline and then passed through a cellulose column, the filtrate collected was centrifuged at 1000 rpm for 10 minutes. The erythrocytes settled as pellet were separate by removing the upper supernatant carefully. This washing step was repeated and the finally obtained erythrocyte were used for estimating the lipid peroxidation

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and preparation of erythrocytes ghost were prepared by using the method of Dodge et.al. (1963)[7]

Total lipid was extracted from different cellular function and was done by the method of ways and hanhan (1964)[8]The phospholipids and cholesterol of the membrane were respectively estimated by connerty et. Al. (1961)[9] and Zlatkisea at.(1953)[10]methods.

The test of osmotic fragility was determined with the concentration of solute inside the red cells by placing the cells in different concentrations of Nacl 0.9% to 0.1% and also to determined the hemolysis in hypotonic solutions. The intracellular solute concentration was reflected by the red cells fragility can be helpful in establishing the pathologic state of the red cells.

Nacl in the concentration range of 0.1% to 0.7% was taken in 7 different centrifuge tube with a volume of 10 m.l. and 1 m.l. of 50% 1:1 diluted red cells suspensions was added to each tube and mixed immediately by gentle swirling allowed to stand at room temp. for 30 minutes. After 30 minutes of incubation, tubes were remixed and centrifuged at 2000 xg for 5 minutes. And absorbance of the supernatant was read at 540 nm against blank (tube containing 0.9% Nacl with no hemolysis). The percentage of hemolysis of each tube is calculated as follows

$$\% \text{ of hemolysis} = \frac{\text{O.D. of individual tube x 100}}{\text{O.D. of 100\% hemolysis (0.1\%)}}$$

Date was analyzed for significant difference ($p \leq 0.05$) among value of control and chronic APZ users using STUDENT t. tesr.

3. Result with Discussion

In the present study, an attempt has been made to evaluate the biochemical changes induced in the blood erythrocyte membrane of long term use of APZ in human volunteers. The result of this study presented in Table-2 and 3

Table 2: Osmotic fragility in Red blood cells

SNo.	Concentration of Nacl	Osmotic fragility	
		Control	APZ Users
1	0.1	0.39	0.33
2	0.2	0.35	0.30
3	0.3	0.31	0.29
4	0.4	0.10	0.17
5	0.5	0.14	0.16
6	0.6	0.08	0.09
7	0.7	0.06	0.08

Table 3

Parameter	Group	
	Gr.- I control	Gr. II APZ Users
Plasma		
Glucose	93.12±2.78	80.20±2.1
HbA,C	5.741±2.0	5.0832±0.2
Lipid		
Cholesterol mg/dL	220.4±4.15	198.87±4.89
Triglycerides mg/dL	146.40±6.01	170.30±4.99
Lipoprotein		
HDL-C (mg/dL)	48.92±2.96	59.01±2.33
LDL-C (mg/dL)	65.96±3.64	55.12±3.01
VLDL-C (mg/dL)	13.02±1.79	31.01±2.00
Nitric oxide (NO)		
Nitrate (µ moles/L)	3.12±0.08	3.36±0.05
Nitrate (µ mols/L)	31.98±0.12	36.87±0.41
RBC membrane		
Cholesterol (mg/dL)	101.21±1.2	82.19±1.16
Phospholipids (mg/dL)	107.19±1.8	83.98±2.10
c/p ratio	0.91	0.96

A significant decrease in the plasma glucose (93.12 vs 80.20 mg/dl) and HbAC (5.74 vs 5.08) concentration were observed in group II when compared with Gr-I observed decrease in plasma glucose and HbA1c are within normal range.

A significant decrease in plasma cholesterol moiety in gr II (220.4 vs 198.8) indicated hypocholesterolemic effect of APZ in human volunteers using APZ for the past few year. Studies of shioriet. Al (1996)[11] demonstrated that significant reduction of cholesterol by APZ treatment.

Increase in plasma triglycerides (146.4 vs 170.3), HDL-c (48.9 vs 59.0) VLDL-C (13.02 vs 31.01) with a decrease in LDL-c (65.9 vs 55.1) are recorded in group II of the present study suggesting the unique action of the tri azolobenzodiazepine, APZ, on lipids and lipoprotein pattern where a mixed affect with a risk and benefit was observed.

Hike in VLDL-C and T.G. (triglycerides) increase the susceptibility of the volunteers for cardiovascular risk.

LDL deposits cholesterol in artery wall forming plasma whereas HDL removes cholesterol from plaue and form the blood stream. Therefore increased level of LDL and small dense LDL are associated. with the risk of coronary artery diseases. Lower level of LDL-C have been observed in Gr. II when compared to Gr I (65.9 vs 55.1) indicating favor the Gr. II volunteers from the risk of above said diseases in this study. However this benefit may not be applicable to the volunteers of who consume APZ more than 2 years.

A decrease in tendency of hemolysis at various concentration of Nacl (As shown in table -2) solution which indicating the protective effect or tolerance related to red cell membrane. Significant decrease in red cell membrane cholesterol (101.2 vs 82.1) and phospholipids (107.1 vs 83.9) moieties as well as the consequent CP ratio (0.91 vs 0.96) in APZ users indicated decrease in membrane fluidity leading alteration in physiochemical properties in APZ users. As shown in table-3.

Increase of Nitrite (3.12 vs 3.36) and Nitrate (31.98 vs 36.87) levels in plasma of group II volunteers of this study indicates an increase NO production in APZ users, which plays an important role in APZ induced events. Its versatile role in beneficiary one.

4. Conclusion

In the present study an attempt was made to evaluate its effects an long term use by human volunteers. Finding of this work revealed that APZ induced alteration in blood

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glucose homeostatic, decrease in plasma glucose level as well as alteration in plasma lipid, lipoproteins.

Significant decrease in red cell membrane cholesterol and phospholipid moieties as well as the consequent C:P ratio in APZ Users indicated decrease in membrane fluidity leading to alterations in physicochemical properties in APZ users. Increased NO in the blood and erythrocyte membrane of APZ users is an evident for NO action.

Furthermore APZ appears to be rendering tolerance to erythrocyte membrane and thereby preventing RBC hemolysis to some extent. The actual mechanism of APZ include alterations in blood and erythrocyte membrane is not known and is needed to be elucidated.

References

[1] Tinder P. (1969) Determination of blood glucose, "annals of clinical biochemistry " 6,(24)
[2] Trivelli L.A., Ranney H.M. Lai H.T. (1971) hemoglobin components in patients with diabetes mellitus, " New England Journal of medicine," 284,353-537.
[3] AllianPoonC.C., Chan L.S. Richmand C.S.G., fulp W. (1974) Enzymatic determination of total cholesterol, clinchem 20, 470-475.
[4] Friedewald W.T. Levy R.I. Fredrickson,DS., (1972) Estimation of the concentration of lowdensity lipoprotein cholesterol in plasma, without the use of preparative centrifuge, Clinchem ,18:499-502.
[5] Fossati P. Priciple L.(1982) serum triglyceride determined calorimetrically with an enzyme that produces hydrogen peroxides clinical chemistry, 28, 2077-2080.
[6] Sastry K.V.H., moudgal R.P., (2002) spectrophotometry determination of serum nitrite and nitrate by copper cadmium alloy Analytical biochemistry 306, 79-82.
[7] Dodge J.T. Mitchell C., Hanahan D.J. (1963) The preparation and chemical characteristics of hemoglobin free ghost of human erythrocytes Archives of biochemistry and Biophysics 100, 119-130.
[8] Ways P., Hanahan D.J. (1964) "characterization and qualifications of red cell lipid in normal man Journal of lipid, 5,318.
[9] Connerty H.V. Briggs A.R., Jv Eaton E.H.,(1961) simplified determination of the lipid components of blood serum clinical chemistry journal 7, 37-53.
[10] Zlatkis A, Zak B., Boyle A.J. (1953) "A new method for direct determination of serum cholesterol, Journal of laboratory and clinical medicine 4: 486-492.

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