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ORIGINAL ARTICLE

Comparative Biochemical Investigations of Over Doze Diazepam in Albino Rats. An Experimental Forensic Study

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ABSTRACT

Objective: The aims and objectives of current study was to identify the consequences of over doze Diazepam in biological system.

Study Design: It is an experimental Forensic Study in which biological effects of over doze of Diazepam was measured on Albino Rats.

Place and Duration of study: Present study was conducted in animal house of Lahore Medical & Dental College. Experimental Albino Rats were purchase from the University of Lahore. Time of duration of this study was 3 month from 20, March 2022 to June 2022.

Methodology: Total 20 male albino Rats of approximately 100 gm of weight were selected and divided them into four different groups. In Group A, 5 individuals were control while in group B, group C and group D, there were 5 rats in each group and they were treated orally with 0.2mg/kg, 03mg/kg and 0.4mg/kg Diazepam respectively. Blood pH, serum Creatinine, serum Bicarbonate serum ALT and AST levels were measured after 10, 20 and 30 days of intervals of time. Blood sample were collected from tail in container after centrifugation.

Results: Significant (P≤0.05) changes were seen in serum Creatinine, serum Bicarbonate, and serum Alanine transaminase (ALT) and Aspartate aminotransferase (AST) levels of study groups after 30 days than 10 and 20 days. Maximum variations were seen in group-D (1.5±0.01, 42.01±0.02, 47.01±0.04, 49.05±0.01, 7.40±0.02), (1.5±0.01, 47.01±0.02, 101.01±0.04, 139.05±0.01, 7.40±0.02) and (0.6±0.04, 20.10±0.01, 10.02±0.02, 14.01±0.01, 7.35±0.04) as compared with control.

Conclusion: In this study it was concluded that over dose of Diazepam caused hepatic toxicity by increasing serum ALT and AST levels and reduces glucose levels. The hepatic toxicity and hypoglycemia may cause death. In study groups the serum Creatinine, serum Bicarbonate levels also showed a significant (P≤0.05) variation in study groups as compared with control.

Keywords: Diazepam, Aspartate aminotransferase, Alanine transaminase, Creatinine, Bicarbonate, hypoglycemia

INTRODUCTION

Diazepam, which was initially sold under the brand name Valium, is a benzodiazepine medication that has anxiolytic properties. Numerous problems, such as anxiety, seizures, alcohol withdrawal syndrome, muscle spasms, sleeplessness, and restless legs syndrome are frequently treated with it [1,8,19]. 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-2H-1, 4-benzodiazepin-2-one is the chemical name for diazepam. It is an insoluble in water, colorless to light yellow, crystalline substance. Anxiolytic therapy is typically not necessary for anxiety or tension brought on by daily stress[5,9,11]. Diazepam may be helpful in the symptomatic treatment of acute agitation, tremor, imminent or acute delirium tremens, and hallucinosis in acute alcohol withdrawal. To have the greatest possible positive impact, dosage should be customized. While the majority of patients will be satisfied with the typical daily amounts listed below, some may need greater doses [6,24,26].

To prevent negative effects, the dosage should be slowly increased in such circumstances. A benzodiazepine derivative with hypnotic, anticonvulsant, sedative, and anti-anxiety effects is diazepam[12]. Gamma-aminobutyric acid (GABA) has inhibitory properties, and diazepam enhances these effects by attaching to GABA receptors in the limbic system and hypothalamus. Diazepam is a benzodiazepine with amnestic, sedative, muscle-relaxant, anxiolytic, and anticonvulsant properties. The majority of these effects are assumed to be caused by gamma aminobutyric acid (GABA), an inhibitory neurotransmitter in the central nervous system. On arterial pH, pCO2, and pO2 in the rat, the effects of diazepam alone and in conjunction with acute and chronic exposure to methadone were assessed[10-15]. Prior to the drug's administration as well as 15, 30, 60, 120, 180, and 240 minutes afterwards, measurements were taken. The amount of carbon dioxide in arterial or venous blood is measured by the partial pressure of carbon dioxide (PCO2). It frequently acts as a sign of adequate alveolar ventilation in the lungs.[1-10]

The human body uses a variety of physiological adaptations to keep homeostasis. Maintaining an acid-base balance is one of them. Human body pH ranges from 7.35 to 7.45 without pathological conditions, with an average of 7.40. An acidemia is defined as a pH value below 7.35 and an alkalemia as one above 7.45 [15-17]. The human body has compensating mechanisms because maintaining a pH level in the required narrow range is crucial. Bicarbonate levels that are abnormally high or low could indicate that the body is having trouble keeping the acid-base balance, either because it is unable to remove carbon dioxide from the body through the lungs or kidneys, or because of an electrolyte imbalance, particularly a potassium deficiency[18-21]. Creatinine levels that are higher than normal could indicate impaired kidney function. Glomerulonephritis, an inflammation of the kidney's blood-filtering organs, Urinary tract obstruction due to kidney stones and renal failure[5,9,15,18].

Rationale of Study: This study was conducted for identification of the consequences of over doze Diazepam in biological system and results of different dozes are very informative for human life because this compound used in many medical indications.

MATERIALS AND METHODS

Study Design: It is an experimental Forensic Study in which biological effects of over doze of Diazepam was measured on Albino Rats.

Sample collection method: Blood sample were collected from tail blood vessels with disposable syringe and stored in blood tubes after centrifugation

Exclusion and Inclusion Criteria: In the present study total 20 male albino Rats of approximately 100 gm of weight were selected and divided them into four different groups. Group A was control while group B, group C and group D, were study and 5 male rats placed in each group and they treated with 0.2mg/kg, 0.3mg/kg and 0.4mg/kg Diazepam per body weight orally respectively. Diazepam were dissolved in distilled water and given to rats of each study group orally in nasal bottles.

Biochemical analysis: Blood pH, serum Creatinine, serum Bicarbonate serum Alanine transaminase (ALT) and Aspartate aminotransferase (AST) levels were measured with duration of time. Three time blood sample were collected from treated individuals.

Data processing: The raw data were presented with the help of SPSS version 21 and significant (P≤0.05) value were considered for mean standard deviation of different biomarkers.

RESULTS

When 0.2 mg, 0.3mg and 0.4mg diazepam were administrated orally to the albino rates of study groups A, B and C for 30 days and three-time blood samples were collected from treated individuals. Remarkable changes were seen in all study groups as compared than control group shown in table 1, 2, 3 and 4 respectively. In group-A serum Creatinine, serum Bicarbonate, serum Alanine transaminase (ALT), Aspartate aminotransferase (AST) and Blood pH levels were measured in three time after intervals of 10 days collectively. The serum Creatinine, serum Bicarbonate, serum Alanine transaminase (ALT), Aspartate aminotransferase (AST) and Blood pH levels in three intervals shown in Table-2 i.e. (0.7±0.01, 23.05±0.04, 20.02±0.01, 24.01±0.03, 7.37±0.04), (0.9±0.01, 27.05±0.04, 24.02±0.01, 28.01±0.03, 7.37±0.04) and (0.9±0.01, 29.05±0.04, 70.02±0.01, 81.01±0.03, 7.37±0.04).

In Table-3 when 0.3mg diazepam were given to the albino rates the serum Creatinine, serum Bicarbonate, serum Alanine transaminase (ALT), Aspartate aminotransferase (AST) and Blood pH levels (1.01±0.03, 39.04±0.03, 37.01±0.02, 30.05±0.01, 7.39±0.02), (1.01±0.03, 40.04±0.03, 67.01±0.02, 80.05±0.01, 7.39±0.02) and (1.01±0.03, 49.04±0.03, 100.01±0.02, 130.05±0.01, 7.39±0.02). It has seen a remarkable changes in ALT and AST levels after 30 days than the 10 and 20 days sampling.

Table-1: Group-A: Normal group i.e. Control (n=5)

Parameters	Units	10 days sampling Mean ± SD	20 days sampling Mean ± SD	30 days sampling Mean ± SD
Serum Creatinine	mg/dl	0.6±0.04	0.6±0.04	0.6±0.04
Serum Bicarbonate	M Eq/L.	20.10±0.01	20.10±0.01	20.10±0.01
Serum ALT	U/L	10.02±0.02	10.02±0.02	10.02±0.02
Serum AST	U/L	14.01±0.01	14.01±0.01	14.01±0.01
Blood pH	-	7.35±0.04	7.35±0.04	7.35±0.04

Table-2: Group-B: Albino Rats treated with 0.2mg/kg Diazepam orally, (n=5).

Parameters	Units	10 days sampling Mean ± SD	20 days sampling Mean ± SD	30 days sampling Mean ± SD
Serum Creatinine	mg/dl	0.7±0.01	0.9±0.01	0.9±0.01
Serum Bicarbonate	M Eq/L.	23.05±0.04	27.05±0.04	29.05±0.04
Serum ALT	U/L	20.02±0.01	24.02±0.01	70.02±0.01
Serum AST	U/L	24.01±0.03	28.01±0.03	81.01±0.03
Blood pH	-	7.37±0.04	7.37±0.04	7.37±0.04

Table-3: Group-C: Albino Rats treated with 0.3mg/kg Diazepam orally, (n=5)

Parameters	Units	10 days sampling Mean ± SD	20 days sampling Mean ± SD	30 days sampling Mean ± SD
Serum Creatinine	mg/dl	1.01±0.03	1.01±0.03	1.01±0.03
Serum Bicarbonate	M Eq/L.	39.04±0.03	40.04±0.03	49.04±0.03
Serum ALT	U/L	37.01±0.02	67.01±0.02	100.01±0.02
Serum AST	U/L	30.05±0.01	80.05±0.01	130.05±0.01
Blood pH	-	7.39±0.02	7.39±0.02	7.39±0.02

While in Table-4 forth group 0.4mg diazepam were given orally to the experimental animals and their blood samples were collected three time for measurement of serum Creatinine, serum Bicarbonate, serum Alanine transaminase (ALT), Aspartate aminotransferase (AST) and Blood pH levels (1.5±0.01, 42.01±0.02, 47.01±0.04, 49.05±0.01, 7.40±0.02), (1.5±0.01, 47.01±0.02, 101.01±0.04, 139.05±0.01, 7.40±0.02) and (0.6±0.04, 20.10±0.01, 10.02±0.02, 14.01±0.01, 7.35±0.04) respectively. Significant (P≤0.05) changes were seen in Creatinine, serum Bicarbonate, serum Alanine transaminase (ALT), Aspartate aminotransferase (AST) samples collected after 30 days as compared with the samples collected after 10 and 20 days.

Table-4: Group-D: Albino Rats treated with 0.4mg/kg Diazepam orally, (n=5)

Parameters	Units	10 days sampling Mean ± SD	20 days sampling Mean ± SD	30 days sampling Mean ± SD
Serum Creatinine	mg/dl	1.5±0.01	1.5±0.01	1.5±0.01
Serum Bicarbonate	M Eq/L.	42.01±0.02	47.01±0.02	49.01±0.02
Serum ALT	U/L	47.01±0.04	101.01±0.04	137.01±0.04
Serum AST	U/L	49.05±0.01	139.05±0.01	149.05±0.01
Blood pH	-	7.40±0.02	7.40±0.02	7.40±0.02

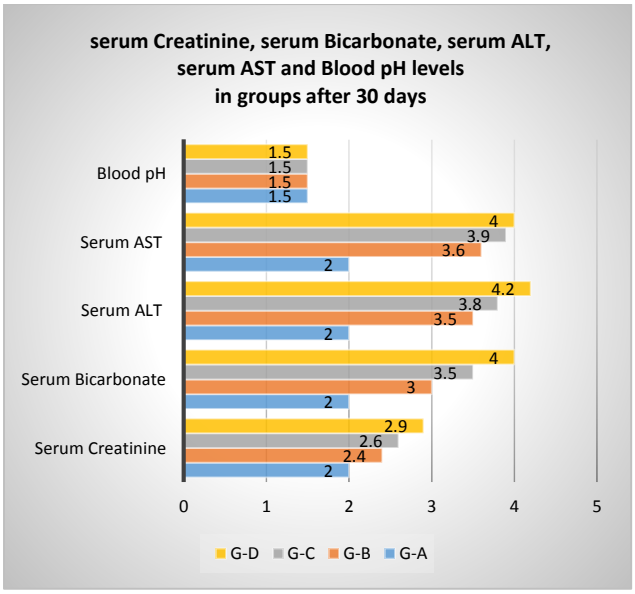

Fig-1: Demographic display of serum Creatinine, serum Bicarbonate, serum ALT, serum AST and Blood pH levels in groups after 30 days

DISCUSSION

Current study showed that diazepam overdose does not affect on blood pH levels whereas a significant (P≤0.05) changes were seen in serum creatinine, serum Bicarbonate, serum Alanine transaminase (ALT), Aspartate aminotransferase (AST) samples collected after 30 days as compared with the samples collected after 10 and 20 days[1-10]. On the other side conflicting results have been reported in many studies[12]. Few studies were reported a significant increase in serum Alanine transaminase (ALT), Aspartate aminotransferase (AST) levels with over doses of diazepam while some studies showed very slight changes in serum creatinine, serum bicarbonate levels [2,12,13,19].

Contrarily, several research claim that diazepam raises blood biochemistry levels in both people and animals. For instance, diazepam (13.3 mg/kg) given to mice led to a rise in serum LFT levels at various points following injection, according to the study's findings. Additionally, a different Albino rats study revealed that diazepam could raise serum ALT and AST levels in a dose-dependent way. Their study revealed that diazepam's potential effects on peripheral receptors and caused hyperglycemia associated with benzodiazepines[20,24]. The 2-

adrenergic receptor antagonist was able to stop diazepam from inducing hyperglycemia in an experimental animal [5,8,12,21].

Similar another study claimed that diazepam significantly (P≤0.05) changes in serum ALT and AST levels in most of the groups a prominent increase in these two enzymes were occurred but very rare liver damage reported. In particular parenterally administered diazepam has not been known to generate serum enzyme increases [12-15]. In a case history when a 33-year-old lady started using diazepam (2 mg, three times per day) for anxiety, she started experiencing jaundice and stomach pain four months later [13,17,19]. Alkaline phosphatase was twice the normal level, bilirubin was 4.2 mg/dL, and serum ALT was 306 U/L. The gallbladder and biliary tree were normal despite the cholecystectomy, which was performed due to suspicions of cholelithiasis [20,26].

CONCLUSION

In this study it was concluded that over dose of Diazepam caused hepatic toxicity by increasing serum Alanine transaminase (ALT) and Aspartate aminotransferase (AST) levels and reduces glucose levels. The hepatic toxicity and hypoglycemia may cause death. In study groups the serum Creatinine, serum Bicarbonate levels also showed a significant (P≤0.05) variations in study groups as compared with control.

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