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Research Article

Estimation of the Appropriate Alloxan Dose for Induction of Diabetes in Albino Mice

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Abstract: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to an absolute or relative deficit in insulin production or action. Diabetic animal models are important tools to understand the pathogenesis and complication of disease. In addition to study the effect and action of many anti diabetic agents. Alloxan has been used as diabetic inducer in experimental animal with different protocols and doses. The purpose of this study is to estimate the Alloxan diabetic dose appropriately according to body weight of animal. In this study 12 male albino mice body weight 30-37.7gm were divided into 3 groups "A, B, C" each group with 4 animals. Alloxan injected intraperitoneally with 3 different protocols to each group. In group A the dose was (150mg-100mg-150mg)/kg, (100mg-150mg-150mg)/kg for group B and (150mg-150mg)/kg in group C in two days interval respectively to induce and maintain diabetes. .Blood glucose and body weight was measured at baseline before injection then 48, 72, 96h after injection .Results in group A showed that blood sugar increased gradually after first and second injection, and decreased blood sugar after third dose. In group B the blood sugar increased after first dose and started gradual decreased after second dose then increased again after third dose. On other hand group C showed that the blood sugar gradual elevated after each injection. These results indicate that injection of Alloxan at doses (150-150mg)/kg is more practical as diabetogenic dose in albino mice.

Keywords: Alloxan dose, Diabetes mellitus, albino mice.

INTRODUCTION:

Diabetes mellitus is chronic metabolic disorder characterized by hyperglycemia due to an absolute or relative deficit in insulin production or action. Diabetes mellitus is subdivided in to diabetes type 1 and type 2 according to pathogenesis of disease .Diabetes mellitus has strong association with genetic predisposition, obesity and stress life style (Song, I. *et al.*, 2015). Recently, diabetes mellitus become high prevalence worldwide, and many serious complication occurs due to chronic hyperglycemia, including retinopathy,nephropathy,neuropathy and cardiovascular complication (3).Many laboratory researches of diabetes established various animal models to study the pathogenesis ,complication of the disease and in the same time study the effects of anti-diabetic agents.

Alloxan is one of chemical agents that used to induce diabetes mellitus in albino mice. The diabetogenic dose of Alloxan depends mainly on the age, specie and weight of mice. Moreover, many experimental researches were designed to estimate the appropriate dose of Alloxan for induction of diabetes. However the results were controversial. Therefore this study was performed for this purpose (Macdonald Ighodaro, O. *et al.*, 2017).

MATERIAL AND METHODS

Animal

Healthy Albino mice male weighted 30-37.7 g were used and has been kept under observation for 4 wk in animal house under controlled condition in standered cages with free access to food and water. Samples

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collected for blood glucose and body weight at zero time, 48, 72, and 96 hour after injection.

Chemicals:

Alloxan monohydrates were obtained from SIGMA-ALDRICH from Tunisia, other chemical and glucose strips from research center in Zawia Libya.

Experimental design

Twelve albino mice divided in 3 groups each group with 4 mice were exposed to overnight fasting.

Group A was injected with (150mg-100mg-150mg)kg of alloxan, group B was injected with (100mg-150mg-150mg)kg of Alloxan, group C was injected with(150mg-150mg)kg of Alloxan with two days interval respectively to induce and maintain diabetes. Blood glucose and body weight were estimated before injection and then 48, 72,96h. And injected after 48, 72, 96 hour. Alloxan was prepared with 0.5 ml of citrate buffer at pH 6.8. Also, the groups of mice were injected intraperitoneal with alloxan.

Statistical Analysis:

The data was presented as Mean ± S.E.M. One Way Analysis of variance (ANOVA) was performed on means to determine the significant ($p < 0.05$) difference among the groups.

RESULT:

During the experiment the dose of Alloxan based mainly on weight of mice. Most of mice became diabetic after first injection in all groups at dose 150 in group A, dose 100mg in group B and 150mg in group C.

In group A the blood sugar increased gradually except the mouse No 4 the blood sugar was very high Bs=410mg and this may explain genetic variability to response to Alloxan. Blood sugar getting higher after second injection 100mg but decreased after third dose 150mg.The result indicate that there is no statistically significance(p value >0.05).

In group B the blood sugar increased after first dose(100mg) and then decreased after second dose 150mg and increased again after third dose (150mg).The result indicate that there was no statistically significance (p value >0.05).

Group C was injected with 2 doses 150 mg-150 mg. The results showed that blood sugar increased gradually at end of 96h after injection. The p value was (0.074) significant as compared with other groups.

Group A

Descriptives

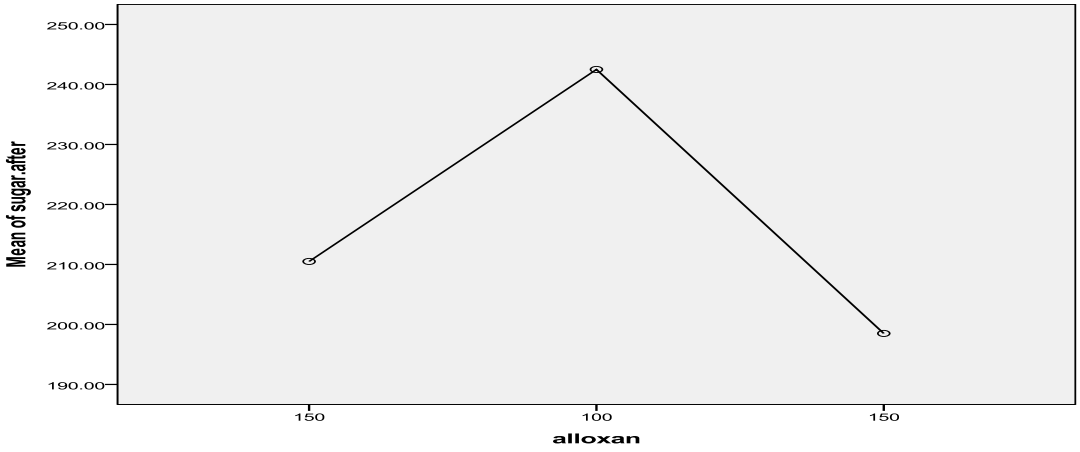
sugar.after

4	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
150	4	210.5000	133.99627	66.99813	-2.7180-	423.7180	131.00	410.00
100	4	242.5000	181.77367	90.88683	-46.7425-	531.7425	145.00	515.00
150	4	198.5000	123.40313	61.70157	2.1381	394.8619	115.00	382.00
Total	12	217.1667	135.78515	39.19780	130.8929	303.4404	115.00	515.00

ANOVA

Sugar. after

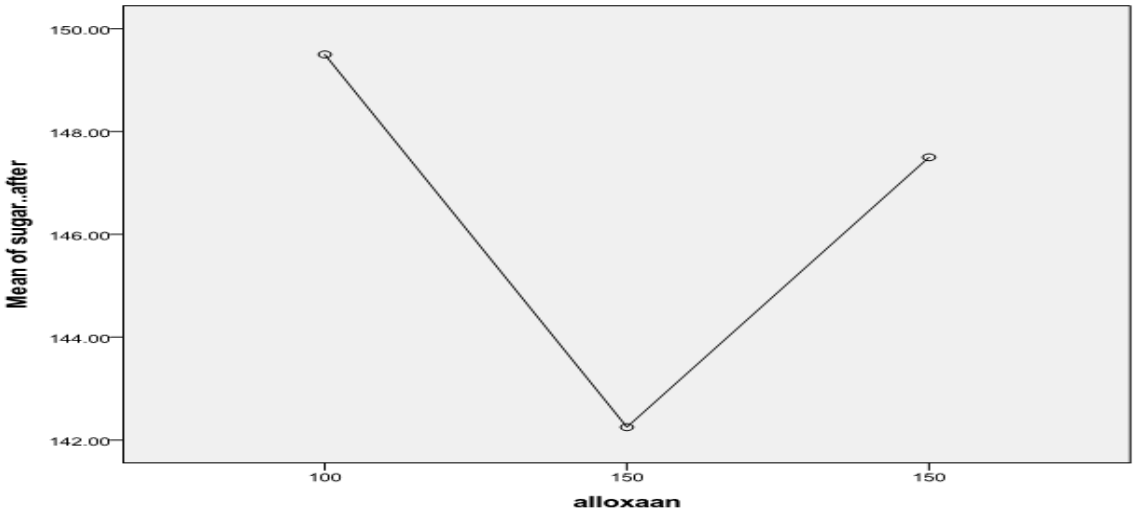
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4138.667	2	2069.333	.094	.911
Within Groups	198675.000	9	22075.000		
Total	202813.667	11			



Group B

Descriptives								
Sugar. after								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
100	4	149.5000	11.09054	5.54527	131.8525	167.1475	141.00	165.00
150	4	142.2500	14.45395	7.22697	119.2505	165.2495	127.00	159.00
150	4	147.5000	15.80084	7.90042	122.3573	172.6427	124.00	158.00
Total	12	146.4167	12.99271	3.75067	138.1615	154.6718	124.00	165.00

ANOVA					
Sugar. .after					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	112.167	2	56.083	.289	.755
Within Groups	1744.750	9	193.861		
Total	1856.917	11			

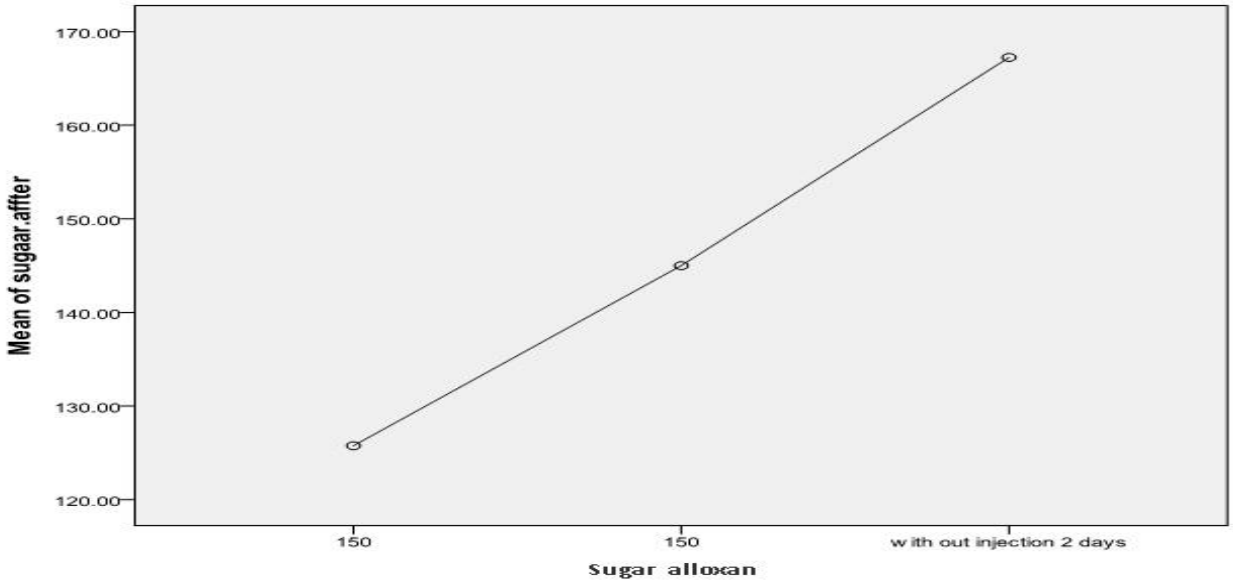


Group C

Descriptives

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
150	4	125.7500	9.63933	4.81966	113.00	135.00
150	4	145.0000	7.61577	3.80789	138.00	153.00
with out injection 2 days	4	167.2500	36.22499	18.11250	143.00	221.00
Total	12	146.0000	26.69695	7.70675	113.00	221.00

ANOVA					
Sugar. after					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3450.500	2	1725.250	3.537	.074
Within Groups	4389.500	9	487.722		
Total	7840.000	11			



DISCUSSION:

Alloxan is one of the effective diabetogenic inducer in diabetic researches. The dose depends mainly on age, species and weight of models and route of administration (Macdonald Ighodaro, O. *et al.*, 2017) There are many route of administration e.g. subcutaneous (Akhtar, N. *et al.*, 2018), intraperitoneal or intravenous in one dose or multiple doses .In this study 3 different protocols the Alloxan injected intraperitoneal in 12 albino mice and most of mice became diabetic after first injection that proof the effect of Alloxan as diabetogenic (Akhtar, N. *et al.*, 2018) this may be explained by accumulation of Alloxan in beta cells and necrosis of cells. Maintenance of blood sugar elevation in animal model is the most important factor to estimate the appropriate dose for Alloxan to induce diabetes (Macdonald Ighodaro, O. *et al.*, 2017). In our study we showed that multiphase response of hyperglycemia this may be due to the one of most important struggling of Alloxan is auto-reverse of hyperglycemia and animal model become non diabetic. In protocol C at dose 150mg twice is sufficient to induce and maintain diabetes in albino mice (Macdonald Ighodaro, O. *et al.*, 2017).

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