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INDUCTION OF DIABETES BY ALLOXAN IN MALE WISTAR ALBINO RATS –A SIMPLIFIED METHODOLOGY

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ABSTRACT
Diabetes mellitus (DM) is burning problem, currently administered therapeutic options are major failure and new therapeutics are required. Globally herbal medicines have been recommended for its treatment, however, proper validation is required before its administration. The best option is to validate in experimental animals.Hence the current investigation is carried in aim to identify the suitable concentration of the alloxan for inducing DM in animal models, in order to avoid unnecessary pancreatic tissue damage and also to develop an experimental animal model to investigate the induction of diabetes mellitus (DM).Thirty eight, Healthy male wistar albino rats were selected andassigned to four groups: Group-II, III and IV, received intraperitoneally with varying concentrations of Alloxan (31.25, 62.5, 87.5 100, 125mg/kg), dissolved in various buffers, that include Citrate buffer, PBS buffer, and NaCl buffer respectively, whereas the experimental rats in Group-I(control) received with water and buffers only. Using a glucometer, fasting blood glucose (FBG) levels were assessed on days 0, 3, 4, and 5 respectively.Out of the 30 rats administered with alloxan, the combination of Alloxan + NaCl buffer worked efficiently, and results exhibit elevated levels of blood glucose which clearly confirms induction of DM.

KEYWORDS: Animal Model, Alloxan, Diabetes Induction.

INTRODUCTION

Diabetes mellitus (DM) consists of a group of disorders characterized by hyperglycaemia, altered metabolism of lipids, carbohydrates, proteins and an increased risk of complications from vascular diseases (Reddy et al., 2013). The present therapeutic options for diabetes include oral hypoglycaemic agents and insulin etc. In spite of advancements in the treatments disease still remains incurable. Hence new age medicines are required. Several studies have shown various efficient drug development technologies and targeting mechanisms, like in silico drug designing and synthesis of numerous molecules for treating various diseases and disorders which include cancer and diabetes etc (Rao and Prasad.,2013;Rao et al., 2012; Rao et al., 2009; Hymavathi et al.,2012;Avinash et al., 2015). Even in traditional medicine many herbal medicines have been recommended for the treatment of various diseases and disorders which include cancer and diabetes etc (Avinash et al.,2015; Gurib-Fakim.,2006; DK Patel et al.,2012; Satyanand et al., 2013; Satyanand et al., 2013; Satyanand et al, 2013; Rao and Prasad 2008). At present, the usage of Medicinal plant/herbal extracts/ formulations for treating various diseases and disorders is rapidly progressing and is presumed to have no side effects. The active components present in plant extracts have been shown to efficiently cure various diseases and disorders in a synergistic manner. (Rao et al, 2009;DK Patel et al.,2012).The active components from medicinal plant/herbal plants encompass polysaccharides, pigments, steroids, terpenoids, flavonoids and alkaloids (Rao et al., 2009;Gurib-Fakim.,2006; DK Patel et al., 2012; Satyanand et al., 2013). Moreover, before administrating in to humans, experimental evidence is needed to validate them. The best way to get better results is to validate these herbal formulations in experimental animals. Therefore diabetes mellitus (DM) is studied using animal models.

Animals are widely used in various experimental research works (Kimwele et al., 2011).The reliability of a study varies on the type animal model used for the concerned experiment. Therefore, it is essential for any researcher to better understand the animal model before designing any experimental study related to animals.Moreover, the current available animal models widely vary from each other. In general various experimental Animal models have been designed with appropriate methodologies which include pancreatectomy, chemical induction, genetic engineering, molecular biology and islet cell transplantation etc.(Frode and Medeiros.,2008; Duncan et al.,2012). Recent advances in the molecular biological techniques like DNA sequencing, genetic engineering,

gene targeting and transgenic methodologies has been shown the new way for the breeding and production of specific animal strains which are vulnerable to development of either type 1 or type 2 DM (Suresh et al., 2016a; 2016b; Singh et al., 2015; Kimwele et al., 2011; Frode and Medeiros., 2008; Duncan et al.,2012).

However the induction of DM in animals is mainly depends on the type of the experimental model used and also the type of chemical used, along with other environmental factors. Most studies clearly indicate that chemical-induced diabetes models have been effectively used (Frode and Medeiros., 2008). The commonly used DM inducing agents in various animal models include alloxan, and streptozotocin (Duncan et al., 2012; Lenzen 2008). Moreover, cost wise Alloxan is found to be cheaper when compared to streptozotocin. Interestingly, in one study conducted in 2010, has shown that streptozotocin had been used in 69% of chemical-induced diabetes animal models, when compared to alloxan used in 31% of the studies (Etuk., 2010). Alloxan is a urea derivative, widely used to induce type 2 DM in animals such as rabbits, rats, mice and dogs. It causes selective necrosis of the pancreatic islet β-cells (Etuk, 2010). Several studies reported its usage in inducing DM in experimental animal models but the dose that applied in those studies varied drastically (Dinesh and Raj Kumar, 2011). Hence it is essential to evaluate and identify the suitable dose concentration of the drug for inducing DM in experimental animal models, in order to avoid unnecessary pancreatic tissue damage.

The commonly administered intravenous dose of alloxan in rats is 65 mg/kg, but its dose may vary when it is administered intraperitoneally or subcutaneously (Antia et al, 2005). Hence, the current study is aimed to evaluate and identify the suitable dose concentration of the alloxan for inducing DM in experimental animal models, in order to avoid unnecessary pancreatic tissue damage and also develop an experimental animal model to investigate the induction of diabetes mellitus (DM).

METHODOLOGY

2.1 Study animals

Animals (Healthy male wistar albino rats) were obtained from National Institute of Nutrition, Hyderabad, India. The experiments with animals were in accordance with CPCSEA guidelines and as well as with the approval of Institutional Animal Ethical Committee. Thirty eight (Shanti et al, 1994), six month old Healthy male wistar albino rats weighing between 200-250g were selected for the study. These animals were housed in plastic cages and transported to the diabetic experimental lab of the animal house facility. A twelve hour light/dark cycle was maintained, with an air-conditioned room along with controlled temperature and humidity, and the experimental animals fed with standard diet that was supplied by NIN. Water was provided *ad libitum*.

The animals were allowed a 15 day acclimatization period after which baseline blood glucose and body weights were taken.These 38 rats were separated in to Four groups: Group-I named as control group (n=8), Group-II as Alloxan + Citrate buffer group (n=10), Group-III, as Alloxan + PBS buffer group (n=10) and Group-III as Alloxan + NaCl buffer group (n=10). Animals that underwent fasting and were deprived of food for 17 hr, but they were allowed free access to water.

Induction of Experimental Diabetes

To induce diabetes in experimental rats various procedures has been followed. In the present study alloxan has been used as diabetic inducing agent. The Chemical alloxan was obtained from the sigma Aldrich chemicals and stored at -4°C. The experimental rats were fasted for 17 hours before inducing DM.

Initially various doses of Alloxan ranging from 30-125 mg/kg was dissolved with various buffers and administered intra-peritoneally to the experimental group (Group-II-iV). In all the experiments various doses of Alloxan solution was prepared freshly just prior to injection and administered to the rats as quickly as possible.In Control group(Group-I), equal volumes of the respective buffers were administered intra-peritoneally to the rats which includes, Only water (Control), Citrate buffer, PBS buffer, and NaCl buffer. After the treatment, the animals were provided free access to drinking water and pellet diet.

Confirmation of diabetes

Blood samples were collected before and after the administration of Alloxan to know the status of diabetes. The blood sample was obtained by sequential snipping of the tail (Fluttert et al., 2000). A glucometer (USA) was used to measure the blood glucose levels. After two days, diabetes was confirmed by testing blood glucose level using glucometer and they were further maintained for four days for well establishment of diabetes. Regular blood glucose levels were assessed on days 0, 3 and 6 following administration of alloxan. Blood glucose level 200 mg/dl is considered as moderate diabetes. The experimental animals who crossed this value has been selected for the experiment. Thereafter, the animals were followed up for 3 weeks with a weekly assessment of the blood glucose levels.

Statistical analysis

The statistical analysis will be performed using Sigmaplot-11 software as described (Singh et al, 2015;Suresh et al, 2014;Chetan et al, 2013). Values were expressed as the mean ± SD of three independent experiments and statistical significance will be determined by one-way analysis of variance (ANOVA).

RESULTS

Various methods were followed to induce DM in the experimental rats using alloxan as a diabetic agent. The

experimental rats were categorized in to four different groups out of which Group-I severe as control and the other starting from Group-II to IV were treated ones. All the experimental rats were fasted for 17 hours before inducing DM. The fasting blood glucose levels were found to be in between 75 ± 3.72 to 87 ± 6.36 respectively which was presented in table-1.

Group-I: Group-I represents the control group of the experimental rats as demonstrated in the table-1. Various buffers were tried as effective controls in which later varying concentrations of alloxan will be dissolved. The experimental rats in this group received only buffers without alloxan are as follows.

Control-1 (only water)
The experimental rats in this group received only water without alloxan. It has been named as Control-1. The results clearly indicate that blood glucose levels were found to be normal after the administration of water and the findings were reported in table-1.

Control-2 (only Citrate buffer)
The experimental rats in this group received only citrate buffer without alloxan. It has been named as Control-2. The results clearly indicate that blood glucose levels were found to be normal after the administration of citrate buffer and the findings were reported in table-1.

Control-3 (only PBS buffer)
The experimental rats in this group received only PBS buffer (Phosphate-buffered saline) without alloxan. It has been named as Control-3. The results clearly indicate that blood glucose levels were found to be normal after the administration of citrate buffer and the findings were reported in table-1.

Control-4 (only NaCl buffer)
The experimental rats in this group received only NaCl buffer (sodium chloride) without alloxan. It has been named as Control-4. The results clearly indicate that blood glucose levels were found to be normal after the administration of NaCl buffer and the findings were reported in table-1. Later the diabetes inducing agent alloxan was dissolved in various buffers and tried its efficacy in experimental rats.

Group-II (*Alloxan + Citrate buffer)*
The experimental rats in this group received citrate buffer along with varying concentrations of alloxan (31.25, 62.5, 87.5 100, 125mg/kg). The results clearly indicate that blood glucose levels were found to be normal after the administration of Alloxan in varying concentrations which was dissolved in citrate buffer and the findings were reported in table-1.

Group-III (*Alloxan + PBS buffer)*
The experimental rats in this group received PBS buffer along with varying concentrations of alloxan (31.25, 62.5, 87.5 100, 125mg/kg). The findings clearly reveal that the blood glucose levels were found to be normal after the administration of Alloxan in varying concentrations which was dissolved in PBS buffer and the findings were reported in table-1.

Group-IV (*Alloxan + NaCl buffer)*
The experimental rats in this group received NaCl buffer along with varying concentrations of alloxan (31.25, 62.5, 87.5 100, 125mg/kg). The results clearly indicate that blood glucose levels were found to be normal only in the lower doses, but tends to increase in the higher doses after the administration of Alloxan in varying concentrations which was dissolved in NaCl buffer. The blood glucose levels were found to be normal, particularly in the doses 31.25 and 62.5 respectively. The remaining doses 87.5, 100 and 125mg/kg exhibited an increasing trend in the elevation of blood glucose levels, which indicates a positive response for the induction of diabetes. These findings were depicted in table-1.

Table 1: Effect of alloxan in the induction of diabetes in male wistar rats.

Experimental Groups	Body weight of the rats	Glucose levels after 17 hrs of fasting	Concentration (mg/kg)	Day-3	Day-4	Day-5
Group-I	**Only water (Control)**					
	207 ± 2.83	75 ± 3.72	0	108± 2.69	105.7 ± 3.46	112±6.32
	Only Citrate Buffer					
	210 ± 2.83	80 ± 4.24	0	110.5 ± 3.54	107 ±5.46	111.5 ± 7.78
	Only PBS Buffer					
	203.5 ± 4.95	77.5 ± 2.12	0	111.5 ± 6.36	103.5 ± 7.61	105.5 ± 3.54
	Only NaCl Buffer					
	205 ± 3.21	79 ± 1.24	0	109 ± 2.33	105 ± 7.61	107 ± 4.94
Group-II	**Alloxan + Citrate buffer**					
	205 ± 1.41	78.5 ± 3.54	31.25	108.5 ± 7.78	118.5 ± 6.36	113.5 ± 7.78
	201.5 ± 0.71	77 ± 4.24	62.5	103 ± 4.24	98 ± 5.66	112 ± 11.31
	200 ± 0.9	76 ± 1.41	87.5	104.5 ± 3.54	111.5 ± 13.44	122.5 ± 2.12
	210.25 ± 0.35	80.25 ± 3.18	100	109.2 ± 4.60	116.75 ± 12.37	123.7 ± 13.7
	206.75 ± 2.48	87 ± 6.36	125	109.7 ± 3.89	123.25 ± 0.35	118 ± 2.83
Group-III	**Alloxan + PBS buffer**					
	202.5 ± 3.54	81 ± 1.41	31.25	118.5 ± 9.19	147.5 ± 27.58	129 ± 25.46
	204 ± 5.66	71.5 ± 2.12	62.5	107.5 ± 6.36	105 ± 9.9	108.5 ± 13.44
	207.5 ± 3.54	74 ± 2.83	87.5	101 ± 4.24	114.5 ± 12.02	110 ± 5.66
	206.25 ± 1.77	76 ± 2.83	100	103.25 ± 4.6	118.5 ± 14.85	110.25 ± 8.13
	204 ± 2.83	76.25 ± 2.47	125	103 ± 15.56	115 ± 2.12	108 ± 4.95
Group-IV	**Alloxan + NaCl buffer**					
	207.5 ± 2.12	82 ± 2.83	31.25	120 ± 5.66	114 ± 2.83	105 ± 4.24
	209.5 ± 2.12	81 ± 2.83	62.5	130.5± 26.1	108.5 ± 2.12	114.5 ± 3.54
	204 ± 4.24	85 ± 1.41	87.5	199.5 ± 89.8	256 ± 182.43	249 ± 52.33
	204 ± 2.83	82.5 ± 0.71	100	372.5 ± 74.2	375 ± 63.64	402 ± 5.66
	211 ± 1.41	79 ± 2.83	125	421 ± 11.7	471 ± 12.72	491 ± 14.72

DISCUSSION

As evidenced by earlier studies Induction of DM in experimental animal models is a huge task. Most studies revealed that so many factors play a role for the lack of uniformity in the induction of DM.Alloxan and streptozotocin is widely used for inducing DM in experimental animals, however alloxan is found to be cost effective when compared to streptozotocin (Duncan et al., 2012; Lenzen.,2008). Alloxan (2,4,5,6-tetraoxypyrimidine;2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative, which is derived by combing two words Allantoin and Oxaluric acid, which exists in the form of alloxan hydrate in aqueous solution. Interestingly the product of uric acid is Allantoin, which was excreted by the foetus in the allantois. Similarly the formation of oxaluric acid will takes place in combination of oxalic acid and urea which was present in the urine.Uric acid oxidation by nitric acid leads to formation of alloxan. Similarly the monohydrate is also prepared by the oxidation of barbituric acid by chromium trioxide (Ankur and Shahjad., 2012).

Alloxan is also termed as strong oxidizing agent which forms a hemiacetal with its reduced reaction product; dialuric acid, in which a carbonyl group is reduced to a hydroxyl group, which is called alloxantin. Its administration either intravenously or intraperitoneally or subcutaneously induces diabetogenic action.The actual dose for the induction of diabetes mainly depends on the type of animal species, how it is administered? And status of nutrition (Federiuk et al, 2004). Interestingly, in some studies it has been demonstrated that the alloxan will be non-toxic to the human beta-cells, either from low dose to high doses, the reason of which may be attributed to the differing glucose uptake mechanisms in humans and rodents (Ankur and Shahjad., 2012).

The mechanism and phases of action of alloxan is quite complex (Ankur and Shahjad, 2012). Alloxan induces diabetes by destructing the insulin-producing pancreatic beta-islets. In other words when it is administered into the animal a multiphasic blood glucose response will be achieved, for instance inverse changes in the plasma insulin concentration will be noticed, followed by sequential changes in the features of ultrastructural beta cells, further leading to necrotic cell death. The first phase is transient hypoglycemic phase, which stimulates the secretion of insulin, and therefore results in the increase of the plasma insulin concentration that lasts maximally for 30 minutes after alloxan injection. The mechanism of this phase may be attributed to a short-term increase in ATP availability due to inhibition of glucose phosphorylation through glucokinase inhibition. In the second phase there will be rise in the levels of blood glucose where as, the levels of plasma insulin tend to decrease at the same time after one hour after administration of alloxan. This hyperglycemic phase may lasts for 2-4 hours which is accompanied with low levels

of plasma insulin after the first contact of the pancreatic beta cells with the alloxan. These sudden changes may be due to inhibition of insulin secretion from the pancreatic beta cells or destruction of beta cells due to alloxan.

The third phase is the hypoglycemic phase which lasts for several hours that is noticed 4-8 hrs after the alloxan injection. The flooding of insulin through circulation occurs as a result of the alloxan-induced secretory granule and rupture in the cell membrane leads to severe transitional hypoglycemia (Ankur and Shahjad., 2012). Apart from this, various subcellular organelles are also ruptured which include cisternae of rough endoplasmic reticulum and the golgi complex. Mitochondria is also loses its structural integrity. These alterations are irreversible, which are hallmark features for a necrotic cell death of pancreatic islets. The final 4^{th} phase is the permanent diabetic hyperglycemic phase, where complete degranulation and loss of the integrity of the beta cells will be noticed within 24-48 hrs after the injection of alloxan (Ankur and Shahjad., 2012; Mythili et al.,2004). However, few sub-cellular organelles remain intact which include non-beta cells, other endocrine and non-endocrine islet cell types along with extrapancreatic parenchyma providing the proof of selective toxic action of alloxan (Jorns et al., 1997; Lenzen et al., 1996).

The alloxan mechanism has been studied quite well from decades (Ankur and Shahjad., 2012; Mythili et al.,2004). Several studies have been clearly demonstrated that after injecting alloxan, there will be an immediate rise in insulin secretion either in the presence or absence of glucose (Ankur and Shahjad., 2012; Mythili et al.,2004; Tasaka et al.,1988). This type of alloxan-induced insulin release will stay only for shorter period; further complete suppression of the islet response to glucose has been noticed even at higher doses of glucose.

Alloxan induced diabetogenicity will be determined by its immediate uptake by pancreatic beta islet cells followed by series of metabolic reactions that takes place in the pancreas. The reduction reaction will takes place in the pancreatic beta cells in the presence of various reducing agents that include reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups (Ankur and Shahjad., 2012; West et al., 1996). The sugar binding site of glucokinase will have two -SH groups, where alloxan will react and forms the disulfide bond, which leads to inactivation of the enzyme. The reduction reaction of alloxan, leads to formation of dialuric acid which is further re-oxidized to alloxan, clearly demonstrates a redox cycle, where generation of reactive oxygen species (ROS) and superoxide radicals will takes place. Further, these superoxide radicals release ferric ions from ferritin and reduce them to ferrous and ferric ions. Along with this, superoxide radicals also undergo dismutation in the presence of superoxide dismutase to yield hydrogen peroxide (H_2O_2). Consequently, highly reactive hydroxyl radicals will be generated according to the Fenton reaction in the presence of ferrous and H_2O_2.

Few studies reported another mechanism on ROS, where the effect of ROS on the DNA of pancreatic islets has demonstrated.If once Alloxan is exposed to beta cells, fragmentation of DNA will takes place, leading to DNA damage, which activates DNA repair mechanism through the process of poly ADP-ribosylation. Apart from these, antioxidants like superoxide dismutase, catalase and the non- enzymatic scavengers of hydroxyl radicals were also known to protect against alloxan induced toxicity.Some studies revealed that the diabetogenic action of alloxan depends on the intracellular calcium homeostasis. It has been reported that alloxan induces high levels of cytosolic free Ca2+ in the beta cells of pancreatic islets (Ankur and Shahjad., 2012; Mythili et al.,2004). Depolarization of pancreatic beta cells by alloxan leads to opening of voltage dependent calcium channels, which enhances calcium entry into pancreatic cells there by maintaining the calcium influx. The elevated levels of Ca2+ ions further contributes for the release of supra physiological insulin. Later this released Insulin along with ROS causes ultimate damage to the beta cells of pancreatic islets.

Several studies reported the extensive usage alloxan in inducing DM in experimental animal models but the dose that applied in those studies varied drastically. Moreover administration of such doses to animals may be lethal to the animals. Hence it is essential to evaluate and identify the suitable dose concentration of the drug for inducing DM in experimental animal models, in order to avoid unnecessary pancreatic tissue damage and as well as safety of the animals. The commonly administered intravenous dose of alloxan in rats is 65 mg/kg, but its dose may vary when it is administered intraperitoneally or subcutaneously (Antia et al.,2005). Dinesh et al., highlighted the effect of alloxan models exhibit inconsistencies in doses of drugs, routes of administration, duration and severity of diabetes and methodology (Dinesh and Raj Kumar., 2011). Recent studies showed that Moreover inconsistencies in doses of drugs, routes of administration, duration and severity of diabetes and methodology in alloxan-induced diabetic models, which make its accuracy controversial. The pharmacokinetic and pharmaco-dynamic profile of alloxan is ignored by various researchers working on these aspects. We wish to make the following observations in this regard. Hence in the present study, Diabetes mellitus has been induced in male Wister rats using alloxan as a diabetic inducible agent.

As mentioned above various methodologies were tried to induce DM in the experimental rats using Alloxan as a diabetic agent. The experimental rats were categorized in to four different groups out of which Group-I severe as control and the other starting from Group-II to IV were treated ones. All the experimental rats were fasted for 17

hours before inducing DM. The fasting blood glucose levels were found to be in between 75 ± 3.72 to 87 ± 6.36 respectively which was presented in table-1. Similar findings were reported in other studies too. Group-I represents the control group of the experimental rats and comprised of Control-1 (only water), Control-2 (only Citrate buffer),Control-3 (only PBS buffer), and Control-4 (only NaCl buffer) which was demonstrated in the table-1. Various buffers were tried as effective controls in which later varying concentrations of alloxan will be dissolved. The results clearly indicate that blood glucose levels were found to be normal in all the controls that include Control-1 (only water), Control-2 (only Citrate buffer), Control-3 (only PBS buffer), and Control-4 (only NaCl buffer) and the findings were reported in table-1.

The treated group starts from Group-II to IV all the experimental rats were received with varying concentration of alloxan dissolved in various buffers are as follows. Group-II (Alloxan + Citrate buffer), Group-(Alloxan + PBS buffer), Group-IV (Alloxan + NaCl buffer) Group-IV (Alloxan + NaCl buffer). The experimental rats in the Group-II, III and IV received with varying concentrations of Alloxan (31.25, 62.5, 87.5 100, 125mg/kg) dissolved in various buffers which include Citrate buffer, PBS buffer, and NaCl buffer respectively. The results data clearly reveal that the blood glucose levels were found to be normal after the administration of Alloxan in varying concentrations which was dissolved in Citrate buffer and PBS buffer and the findings were reported in table-1.In case of NaCl buffer the blood glucose levels were found to be normal only in the lower doses, but tends to increase in the higher doses. The levels of glucose in the blood remain to be normal, especially in the doses 31.25 and 62.5 respectively, whereas an increasing trend in the elevation of blood glucose levels has been noticed in the doses 87.5, 100 and 125mg/kg respectively, which indicates a positive response for the induction of diabetes. These findings were depicted in table-1.

Thus, the effect of alloxan (75, 100 and 125mg/kg) in NaCl buffer in experimental animals was clearly noticed with the elevation of blood glucose levels which ultimately leads to induction of DM. This variation in response to the same dose of alloxan (125 mg/kg) with other buffers, demonstrates that there is an inter-individual dissimilarity among the rats with respect to resistance to the diabetogenic effect of alloxan. This type of dissimilarity within a species may reveal variation in cytological features especially in number, along with the other properties of the cells. Moreover it may partly reveal the diverse response to similar environmental conditions in the induction of DM among the common population. In addition to the varied response, the findings obtained in present study corroborate with the previous findings on the role of alloxan in the destruction of β-cells, thus reducing pancreatic ability to secrete insulin (Lenzen., 2008).

Previous studies has been proposed various mechanisms in demonstrating the diabetogenic effect of alloxan through ROS (superoxide radicals, hydrogen peroxide and hydroxyl radicals) generation, which leads to cellular damage (Lenzen., 2008),which in turnevokes auto-immune reactions against the β-cells. Alloxan disrupts the formation of microtubules, and also destroys current exiting ones (Schmidt et al., 1990). It inhibits the enzyme O-linked Nacetyl glucosamine transferase which is very abundant in the β-cells and catalyzes protein O-glycosylation (Konrad et al., 2002). Thus, Alloxan induces diabetes mellitus by selectively destroying pancreatic β-cells (Rakieten et al., 1963; Wilson et al., 1984).

Earlier studies clearly revealed that after administration of alloxan in experimental animals, it is concentrated in the islets of Langerhans and in the liver where it is reduced to dialuric acid, which is unstable in aqueous solutions and undergoes oxidation back to alloxan, leading to the generation of O_2, H_2O_2 and OH radicals by fenton type reaction (Uchigata et al.,1982;Alam et al., 2005). Recent evidences suggest that alloxan is efficiently used as effective pro-oxidant and also found cytotoxic to ß cells of the pancreatic islets of langerhans (Alam et al.,2005; Shanti et al., 1994). Moreover the Hydroxy radicals generated could also cause DNA fragmentation in the islets cell DNA. Along with this there is a two-fold increase in lipid conjugated dienes, which are the primary products of lipid peroxidation (Alam et al.,2005; Shanti et al., 1994). The enzymatic activities like glutathione and catalase which can scavange these free radicals are found to high in the liver, whereas very low in the beta islet cells of the pancreas and are extremely vulnerable to free radical injury (Alam et al.,2005; Halliwell andGutteridge., 1989). Few studies mentioned that Alloxan induced diabetes in experimental animals is found to be reduction in the anti oxidant enzyme superoxide dismutase activity in islets cells. In antioxidant enzyme superoxide dismutase activity (Alam et al., 2005; Halliwell and Gutteridge., 1989).

CONCLUSION

Thus, the above experimental study concludes that the effect of alloxan (75, 100 and 125mg/kg) in NaCl buffer in experimental animals was clearly noticed with the elevation of blood glucose levels which ultimately leads to induction of DM.

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