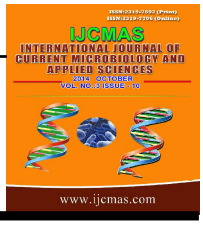
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 97-106**
http://www.ijcmas.com



Original Research Article

Studying the effect of anti-amylase inhibitor extracted from white kidney bean (*Phaseolus vulgaris)* in treat diabetes and obesity in an affected mice

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A B S T R A C T

In this study twelve healthy male mice (Swiss albino aged about five weeks) were used, and divided into four groups. This study was designed to measure the levels of glucose in the blood of mice and also the histological effect that may be happen after the use of amylase inhibitor. Type II diabetes developed in three groups of these mice using alloxan. Treatment with the anti - amylase inhibitor started using a dose of 50 mg per ml after 72hr of the infection with alloxan at which the glucose, concentration reached 333mg/dL. The dose of amylase inhibitor increased to be 100 mg per ml after five days of treatment. The other infected group used as positive control. The results show that the anti-amylase inhibitor was very powerful to reduce the level of glucose in the blood of the treated mice to the normal comparison with the negative controls. The weight (g) of mice treated with anti-amylase reduced compared with controls. The amount of food that they consumed daily was increase gradually. Mice in all the groups were then sacrificed and slides from the tissues of kidney, liver and spleen had been made.

Keywords

anti-amylase inhibitor, white kidney bean *Phaseolus vulgaris* alloxan

Introduction

Diabetes mellitus is a chronic disease that occurs when there is some problem with the pancreas that makes it does not produce enough insulin, and / or when the body loses it effectively to use the produce insulin. The high blood sugar is a common effect of uncontrolled diabetes and it makes a serious damage to many of the systems in the body, especially the kidney, nerves and blood vessels (WHO, 2011). The number of diabetic people is expected to rise from present estimate of 150 million to 230 million in 2025.

For a long time, diabetes has been treated with several medicinal plants or their extract based on the folklore medicine (Malpani and Manjunath, 2013). The use of dietary plants and herbal preparations as alternative medicine has recently received considerable attention in the United States and Europe (Ang-Lee *et al.*, 2001). At the present time, many, research has been focused on scientific evaluation of dietary plants and preparations of plant origin for the control and treatment of several diseases. Pumpkin is one such plant that has been frequently

used as functional food or medicine. *C.maxima* reported to have anti-diabetic (Ang-Lee *et al.,* 2001) hepatoprotective (Sharma *et al.,* 2013), anthelmintic (Saha *et al.,* 2011a, b). Microbial drug resistance and the growing need for useful therapeutic compounds with high potency and low toxicity has attracted interest towards the endophytic fungi research (Strobel *et al.,* 2005). Endophytes are microorganisms that inhabit the healthy tissues of living plants without causing any apparent symptoms of disease (Strobel and Daisy, 2003).

A majority of endophytes is fungi (Kharwar *et al.,* 2008). There are a large number of plants and natural biomolecules that have been discussed in the literature for their ant diabetic effects. For example, plants have been used since ancient times to prevent conditions associated with diabetes. The mechanism is most often not completely understood, but more and more studies are being conducted to elucidate the mechanisms of action of different plants and natural compounds (Cristina *et al.,* 2012).

Inhibition of α-amylase, an enzyme that plays a role in digestion of starch and glycogen, is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity, as well as, dental caries and periodontal diseases. Plants are an important source of chemical constituents with potential for inhibition of α-amylase and can be used as therapeutic or functional food sources. A review about crude extracts and isolated compounds from plant source that have been tested for α-amylase inhibitory activity has been done. Disorders of carbohydrate uptake may cause severe health problems such as diabetes, obesity, and oral diseases, all of which threaten an increasing worldwide population. Diabetes mellitus is a metabolic disorder resulting from a deficiency in insulin secretion, insulin action, or both, promoting disturbance of carbohydrate, fat and protein metabolism. Long term complications of diabetes mellitus include retinopathy, nephropathy, neuropathy, microangiopathy and increased risk of cardiovascular disease (de Sale *et al.,* 2012).

One of the therapeutic approaches to treat Type II Diabetes is to lower the postprandial blood glucose level by inhibition of carbohydrate hydrolyzing enzyme such as alpha-amylase (Gopinath et la., 2013). Alpha-Amylase enzyme catalyses the initial step in hydrolysis of starch to the mixture of oligosaccharides consisting of maltose, maltotriose, and branched oligosaccharides of 6– 8 glucose units that contain both α-1,4 and α-1,6 linkages. These are further degraded to glucose by Alpha-Glycosidase which on absorption enters bloodstream. Rapid degradation of starch by Alpha-Amylase enzyme leads to elevated postprandial hyperglycemia (PPHG). Thus decreasing the degradation of starch to reducing sugar by inhibition of Alpha-Amylase enzyme plays a key role in the control of diabetes. Inhibitors of pancreatic Alpha-Amylase prevent starch breakdown and absorption thereby lowering postprandial glucose levels and also weight loss in humans (Tarling *et al.,* 2008; Bailey, 2003). Leguminous plants are a rich source of proteins and peptides that are involved in plant defense, including proteinaceous amylase inhibitors (Khan, 2011). α-Amylase inhibitors from several cereals and legumes have been well characterized, with respect to their structures and inhibitory potential (Rekha *et al.,* 2004).

Amylase inhibitors present in many cereals such as wheat (*Triticum aestivum*), barley *(Hordeum distichum*) and white kidney bean (*Phaseolus vulgaris*). These substances have different benefits. They can be used as

pesticides to control the insects and also drug-design targets for treatment of diabetes and digestion disorders. The Proper extraction method for amylase inhibitor from different cereals had been established (Shamkhy *et al.,* 2011). In a study an amylase inhibitor was purified by anion exchange chromatography on DEAE-cellulose and gel filtration chromatography on Sephadex G-75 from white kidney bean (*Phaseolus vulgaris*) extract. The molecular weight of the inhibitor was estimated to be 30000 Daltons. The inhibitor showed maximum stability to heat at 60˚C for 30 min incubation and it was stable under a broad pH range (Ali *et al.,*).

Materials and Methods

White kidney bean (*Phaseolus vulgaris*) obtained from the local Iraqi market and extracted in the previous work by ammonium sulfate (Shamki *ET AL.,* 2012). Mice were obtained from the Department of medicines and medical equipment.

Assay of Amylase Inhibitor Activity

Amylase inhibitory activity was measured according to Richardson (1991).

The total hydrolytic activity assay used to determinate the reduction in amylase activity when the amylase inhibitor was extracted and added to the reaction mixture. A 3.5-Di Nitro salicylic acid used as an alkaline color reagent 1 ml of the incubation mixture (3ml soluble starch 2% and 3 ml extracted sample) after 30 min incubation in 30°C was added to an equal volume of alkaline color reagent, mixed thoroughly and heated for 5 min in boiling water bath. Samples (with their replication) including:

1) Alpha-amylase standard without inhibitor as a blank

2) Alpha-amylase

Mixed with same volume (1:1) of alpha amylase inhibitor extracted and purified from Iraqi *Phaseolus vulgaris* samples, then cooled to room temperature and stored for at least 30min. absorbance at 546nm was measured using Aquarius 7000 Series spectrophotometer against a reference and blank. One unit of inhibitor is the amount that suppressed the amylase activity under the assay conditions.

Protein was estimated by the method of Bradford (1976) using bovine serum albumin as standard.

Experimentally induce diabetes in mice

Alloxan 100 mg/kg of body weight was used to induce diabetes. Mice were injected intraperitoneally. The dose was prepared by dissolving o.2ml alloxan in 0.9 NaCl solutions. A solution of 5% glucose was administered orally to combat could occur. Blood glucose was observed 20 hours after alloxanistion (Sushruta *ET AL.,* 2006)

The treated of mice with amylase inhibitor

The solutions used in the treatments were prepared as flow:

1- Solution number one was prepared by dissolving 50 mg of extract in 5o ml distill water. The final concentration was 50mg/ml. This solution used to treat group one by giving them 2ml orally twice a day. It was used for the first three days.

2- Solution number two was the same, but added some drops of olive oil as coated to prevent the effect of the extract on the stomach of mice. It was used in the same way to group two and continues for three days.

3- solution number three was prepared by

mixing 100mg of extract with 50 ml of distill water, the final concentration was 2mg/ml. This solution was used to treat group one till the end of the experiment.
4- Solution number four was prepared in the same concentration (2mg/ml) with the addition of some drops of olive oil for the same reasons mention be for. This solution used to treat group two till the end of the experiment.

Testing the glucose level in the blood of mice

Blood glucose monitoring system uses Bayer contour blood glucose test strips.

The animal used in the experiment

Twelve healthy male mice were used. Mice have been divided into four groups, each total of three male mice. Aloxan was used to induce diabetes in three groups of mice by injection under the skin. The dose was 100mg/kg. After 72 hours, the level of glucose has been developed to be about 333 as an average of the three treated groups. At this time the treatment was started. The crude extract solutions were prepared and used as mentioned before

The histology study

After the end of the experiment, the animals had been sacrificed for the purpose of collecting samples for the liver, spleen, and kidney. Those organs put in a container containing physiological saline solution for the purpose of cleared from the remnants of the fatty textile surrounding it, and then kept in test tubes containing bone solution for a period ranging between 160-180 hours for the purpose of stabilization. Then transferred to test pipelines containing alcohol by 70 percent for the keeping of until the time of their use. Then transferred to test pipelines containing alcohol by 70 percent for the keeping of until the time of their use, and then took to laboratories in Baghdad, as health education have been transferred to a container tube containing ethanol by 90% for 6 hours. Then transferred to pipelines containing 99 percent of alcohol for 6 hours. Then the substance of the Xylol for two hours, then poured into a wax container, cut off to thick chips to about 5 microns by using the manual orbit microtome. These segments have been put in a warm water bath (45 degree Celsius) and heat for some second, then plastered with glass chip at an angle on uptrend to start the stage of the use of natural day of Eosin and Hematotoxilin. The pictures of the textile chip were taken using a digital camera under microscope strongly Zooming 400x 100 xs. Contained

Results and Discussion

From this table we can find that the percentage of inhibition is about 36%. And that gives as an induction about our crude extracted that it is working as amylase inhibitor.

The results in table two show that there're decreases gradually with the time of treatment in the glucose level. On the other hand, we can't find a difference in both treatments (with oil or without), and that give an education about our extract. It is not harmful and its very power, fuel agents, diabetes if we compare with the both control.

The result in table three indicates the amount of feed consumed daily during the experimental time. It is very clearly shows that the treated mice consume more feed than those in control groups and non-treated. These results may support the theory of the effect of amylase inhibitor on the end chain of starch and affect its digestibility by amylase [12, 13]

Table.1 The activity of amylase inhibitors extracted from bean as it was measured at absorbance at 546nm

Rep.	Standard (O.D)	Amylase with inhibitor (O.D)
1	0.93	0.32
2	0.99	0.49
3	0.99	0.29
4	0.97	0.35
5	0.99	0.30

Table.2 The value of glucose in the blood of the mice treated with the curd extract *in vitro*. During the experimental compared with the positive and negative control

Type of Treatment / Days	mg/Ld _ control	mg/Ld Treated with amylase inhibitor with oil	mg/Ld Treated with amylase inhibitor without oil	mg/Ld + control	mg/Ld Mean
1	113.8	332.3	289	324.2	264.825
2	123.7	345	299.7	340.8	277.3
3	125.1	316.5	321.6	377.7	285.225
4	121.1	271.7	288.8	377.7	264.825
5	112.5	191.1	200.1	388.4	223.025
6	117.8	198.3	192.7	411.1	229.975
7	116.3	186.9	199.3	410.1	228.15
8	128.7	180.5	189.6	396.4	223.8
9	112.9	178.4	174.3	424.1	222.425
10	111.8	177.1	178.5	419.6	221.75
11	116.5	169.9	161.5	431.5	219.85
12	110.7	167.9	169.9	429.5	219.5
13	115.9	166.5	166.8	416	216.3
14	121.1	153.9	161.9	497	233.475
15	126.5	156.7	152.4	512	236.9
Mean	118.2933	212.8467	209.74	410.4067	
LSD 0.05	Day= 18.42; treatment type= 10.22; Day * treatment type= 36.83				

Table.3 The effect of treated mice with crude extract on the consuming of diet daily in grams during the experiment

Type of treatment / Days	_ control	Treated with amylase inhibitor with oil	Treated with amylase inhibitor without oil	+ control	Mean
1	2.767	2.467	2.433	2.333	2.5
2	2.7	2.6	2.433	2.45	2.54575
3	2.633	2.467	2.507	2.5	2.52675
4	2.733	2.733	2.9	2.3	2.6665
5	2.6	3.333	3.267	2.467	2.91675
6	2.867	3.533	3.667	2.4	3.11675
7	2.733	3.533	3.433	2.367	3.0165
8	2.733	3.933	4.133	2.433	3.308
9	2.567	4.167	4.267	2.633	3.4085
10	2.933	4.633	4.01	2.467	3.51075
11	2.7	4.8	4.433	2.267	3.6
12	2.467	5.267	5.2	2.133	3.91675
13	2.967	5.5	6.067	1.833	4.24175
14	2.833	6.533	6.533	1.533	4.608
15	3.033	7.3	7.7	1.367	5.175
Mean	2.751067	4.1866	4.198867	2.478867	
LSD 0.05	Day= 0.4730; treatment type= 0.2442; Day * treatment type = 0.9459				

Table.4 The effect of crude extract on the weight (g) of the experimental animals *in vitro*.

Type of treatment / Days	_ control	Treated with amylase inhibitor with oil	Treated with amylase inhibitor without oil	+ control	Mean
1	28.411	27.989	28.222	28.078	28.15
2	28.556	28.389	28.111	28.044	28.275
3	28.433	28.044	27.922	28.056	28.11375
4	28.489	28.011	27.9	28.011	28.10275
5	28.511	28.011	27.778	27.856	28.039
6	28.556	27.9	27.778	27.6	27.9585
7	28.689	27.767	27.878	27.944	28.0695
8	28.667	28.044	27.644	27.9	28.06375
9	28.656	27.178	27.389	27.9	27.78075
10	28.811	27.522	27.089	27.811	27.80825
11	28.789	23.86	23.889	27.567	26.02625
12	29.011	23.116	23.722	27.8	25.91225
13	29.122	23.591	23.256	27.067	25.759
14	29.056	23.311	23.144	27.767	25.8195
15	29.389	23.08	22.9	27.311	25.67
Mean	28.74307	26.38087	26.30813	27.7808	
LSD 0.05	Day= 0.5036 ; treatment type= 0.2601 ; Day * treatment type = 1.0072				

The results of the histological test in figures (1-4) shows that there was no effect of the crude extract of amylase inhibitor on any of the organ of the treated mice.

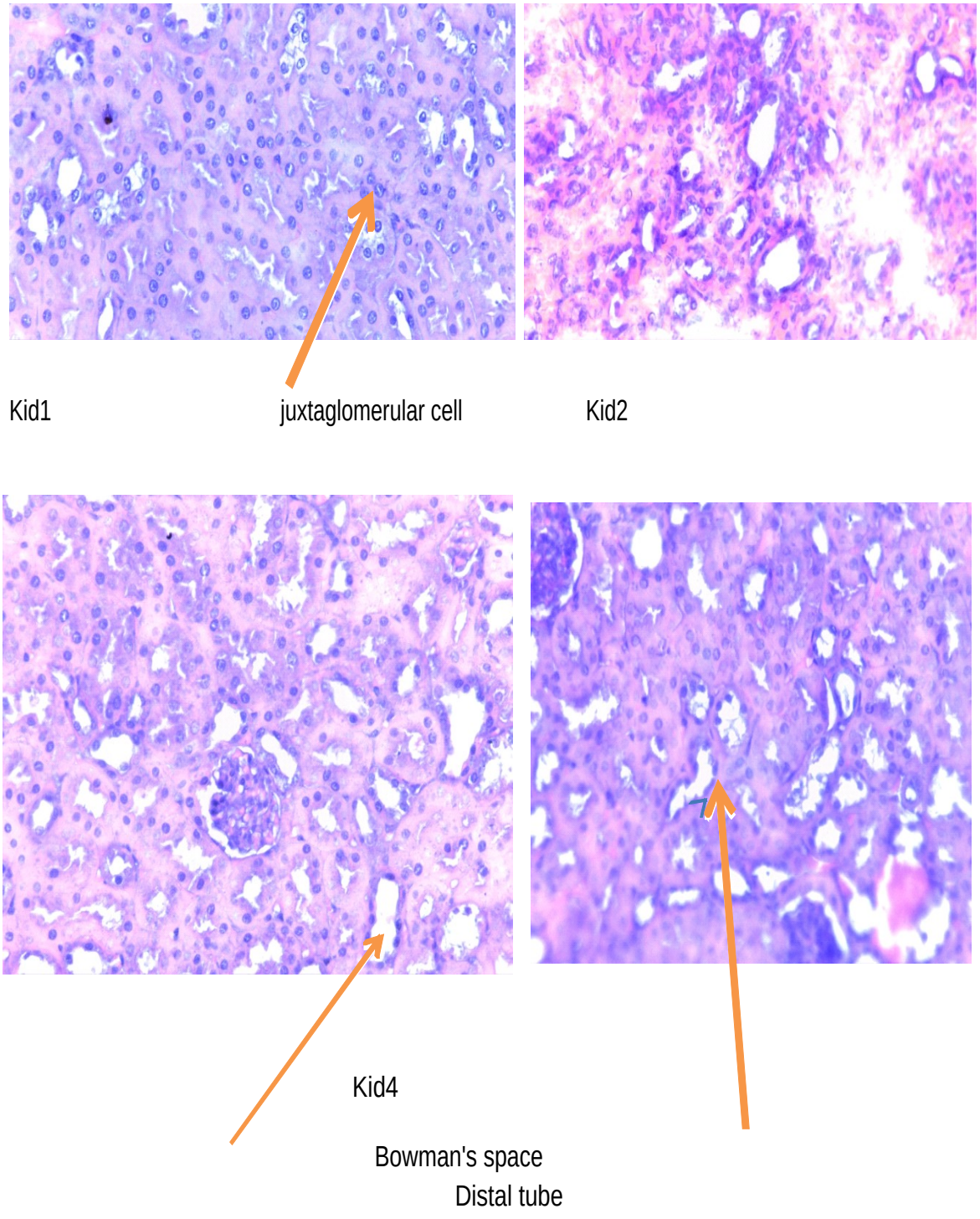

Figure (1) the slides of kidney for both control and treated mice

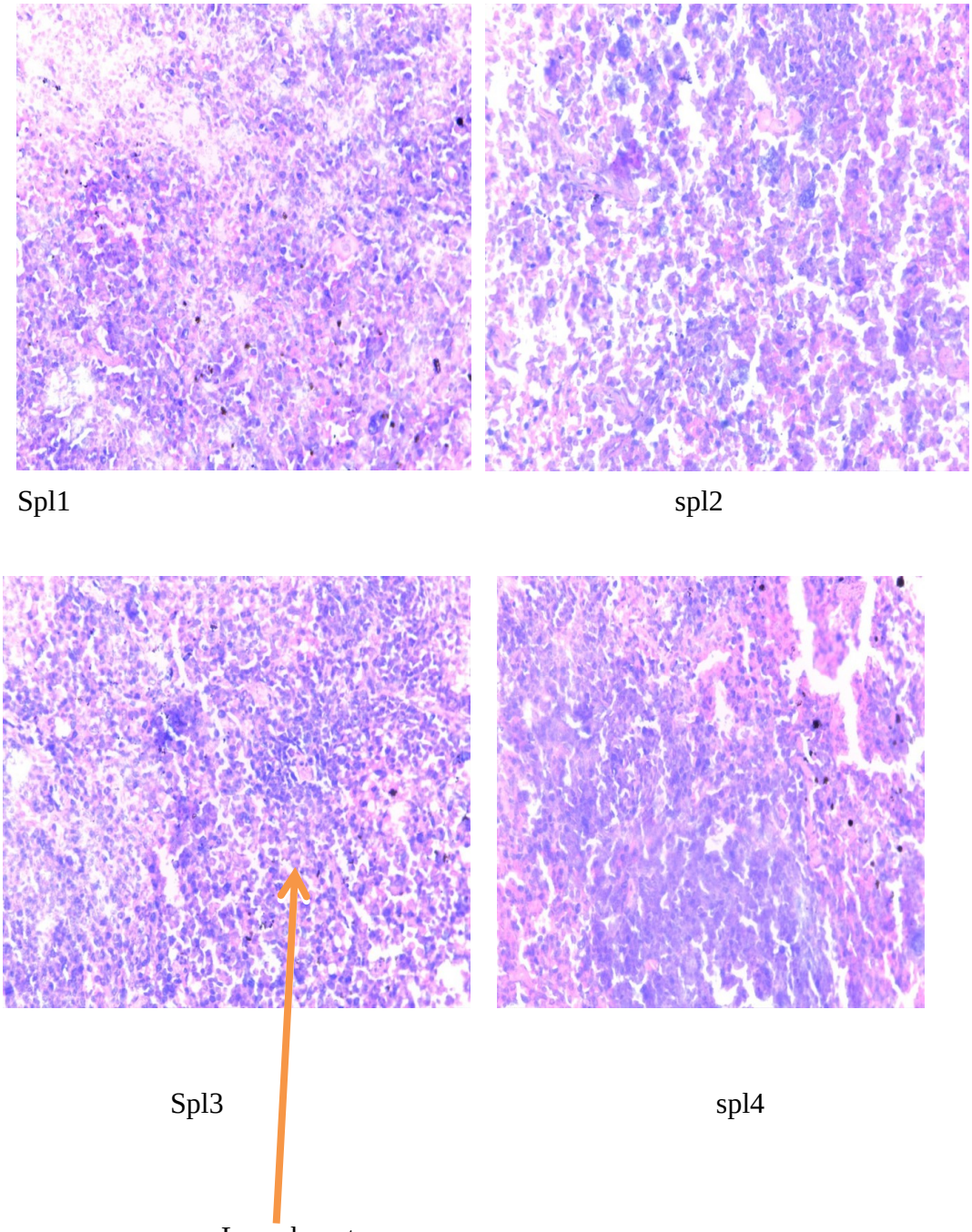

Figure.3 The slides of spline for both control and treated mice

References

Ang-Lee, M.K., Moss, J., Yuan, C.S. 2001. Herbal medicines and peri-operative care. *JAMA*, 286: 208– 216.

Bailey, C.J. 2003. New approaches to the pharmacotherapy of diabetes. In: Pickup, J.C., William, G. (Eds.), Textbook of Diabetes, Vol. 2, 3rd. Blackwell Science Ltd., UK.

Cristina, C., Rugină, O.D., Socaciu, C. 2012. Plants and Natural Compounds

with Antidiabetic Action. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 40(1): 314.

de Sale, P.M., de Souza, P.M., Simeoni, L.A., de Oliveira Magalhães, P., Silveira, D. 2012. α-Amylase Inhibitors: A Review of Raw Material and Isolated Compounds from Plant Source. *J. Pharm. Pharmaceut. Sci.,* 15(1): 141–183.

Gopinath, S.M., et al. 2013. Invitro Inhibitory effect of polyherbal formulation on alpha-amylase. *IJIRSET,* 2(9): 4556–4566.

Khan, N. 2011. In vitro effects of proteinaceous alpha amylase inhibitors on red flour beetle, *Tribolium castaneum. Sci. Res. Rep.*, 1(2): 10–104.

Kharwar, R.N., Verma,V.C., Strobel, G., Ezra, D. 2008. The endophyic fungal complex of *Catharanthus roseus* (L) G Don. Curr. Sci., 95(2): 228–232.

Malpani, S.L., Manjunath, KP. 2013. In vitro α-amylase inhibition and chromatographic isolation of *Cassia Fistula* linn. Bark. IJAPBC, 2(1): 16–20.

Rekha, M.R., Sasikiran, K., Padmaja, G. 2004. Inhibitor potential of protease and α-Amylase inhibitors of sweet potato and taro on the digestive enzymes of root crop storage pests. *J. Stored Prod. Res.,* 40: 461–470.

Richardson, M. 1991. Methods in Biochemistry, Vol. 5, Academic press, NY, Pp. 259–305.

Saha, P., Mazumder, U.K., Haldar, P.K., Bala, A., Kar, B., Naskar, S. 2011. Evaluation of hepatoprotective activity of *Cucurbita maxima* aerial parts. *J. Herbal Med. Toxicol.,* 5(1): 17–22.

Saha, P., Mazumder, U.K., Haldar. P.K., Naskar, S., Kundu, S., Bala, A., Kar, B. 2011. Anticancer activity of methanol extract of *Cucurbita maxima* against Ehrlich ascites carcinoma. *Int. J. Res. Pharm. Sci.,* 2(1): 52–59.

Shamkhy, A., Ali, A., Nadum, O. 2011. Proper extraction method for amylase inhibitor from different cereals. *J. Cell Plant Sci.,* 2(4): 1.

Shamki, A.W., Ali, A., Abdulaziz, R.S. 2012. Purification and characterization of amylase inhibitor extracted from white kidney bean (*Phaseolus vulgaris*). *J. Cell Plant Sci.,* 3(1): 17–21.

Sharma, A., Sharma, A.K., Chand, T., Khardiya, M., Yadav, K.C. 2013. Antidiabetic and antihyperlipidemic activity of *Cucurbita maxima* Duchense (pumpkin) seeds on streptozotocin induced diabetic rats. J. Pharmacog. Phytochem., 1(6): 108–116.

Strobel, G., Daisy, B. 2003. Bioprosecting for microbial endphytes and their natural products. *Microbial. Mol. Biol. Rev.,* 67: 491–502.

Strobel, G., Daisy, B., Castillo, U. 2005. The biological promise of microbial endophytes and their natural products. *Plant Pathol. J.,* 4(2): 161–176.

Sushruta, K., Satyanarayana, S., Srinivas, N., Raja Sekhar, J. 2006. Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected umbelliferouse fruits used in culinary practices. *Trop . J. pharm. Res.,* 5(2): 613–617.

Tarling, C.A., Woods, K., Zhang, R., Brastianos, H.C., Brayer, G.D., Andersen, R.J., Withers, S.G. 2008. The Search for novel human pancreatic α-amylase inhibitors: high-throughput screening of terrestrial and marine natural product extracts. *Chem. BioChem.* 9: 433–438.

WHO. 2011. Diabetes fact sheet no: 312.