Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 1813-1817

Received: 20-05-2018
Accepted: 21-06-2018

Manju Lata Mishra
FSN&T, College of Home Science, CSK HPKV, Palampur, Himachal Pradesh, India

Sangita Sood
FSN&T, College of Home Science, CSK HPKV, Palampur, Himachal Pradesh, India

Vikram Patial
CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India

Anil Sood
CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India

Bikram Singh
CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India

UN Shukla
Dept. of Agronomy, College of Agriculture, AU, Jodhpur, Rajasthan, India

Correspondence
Manju Lata Mishra
FSN&T, College of Home Science, CSK HPKV, Palampur, Himachal Pradesh, India

Histo-pathological study of alloxan-induced diabetes effect on rat's organ after intake of different proportion of *Aesculus indica* (*Tatwakhar)* flour

Manju Lata Mishra, Sangita Sood, Vikram Patial, Anil Sood, Bikram Singh and UN Shukla

Abstract
Histo-pathological assessment of *tatwakhar i.e.* processed flour of *Aesculus indica* of different proportion diet was given to the fifty weanling rats weighing 25±5g, which were divided into control, control with diabetic, and three experimental groups, the trial diet consist of 25, 50 and 75 percent *tatwakhar* or processed flour. It was given in powdered form weighing daily 10 g for 40 days. Thereafter the rats were sacrificed and fixed immediately in buffered formalin 10(%), carried to the laboratory, where it was processed to paraffin wax, cut at 3-5 microns, stained by using Hematoxylin and Eosin technique and analyzed their organs viz. heart, liver, kidney, and pancreas under computerized microscope. There was mild congestion and increased interstitial space in heart, increased cytoplasmic granularity in liver, moderate congestion and mild tubular swelling in kidney and mild congestion in pancreas was found in rat having 25 percent *tatwakhar* flour with diabetic group. After giving 50 percent experimental diet, the result showed no congestion, mild increase in interstitial space in heart, mild congestion, increased cytoplasmic granularity, mild tubular swelling and congestion in kidney and reduction in islet of Langerhans in pancreas. When 75 percent *tatwakhar* flour with diabetes was studied, muscle fiber was more eosinophillic, cell inflammation and necrosis in heart, mild cellular swelling and necrosis in liver, mild tubular swelling and congestion in kidney and full developed islet of Langerhans maximum secretion of insulin hormone in pancreas. It might be due to the presence of aescin in the flour. These findings could suggest that the maximum use of *tatwakhar* flour is good effect on pancreas as well as on other organs.

Keywords: Aescin, diabeties, histopathology, organs, *tatwakhar*

1. Introduction
Presently about 3 crore population of our country is enduring diabetes mellitus (DM) and this fastest growing figure will leads to almost more than double i.e. 7 crore by 2030. India has been declared as the "Diabetic capital of world" [4]. Increased in sedentary lifestyle, energy rich diet, obesity, higher life span and others reasons for this higher rate [10]. Altruistic utilization of ethnobotany of medicinal plants like, as they are cheap and effective against the diabetes mellitus in various countries [13]. So, for accomplish it, there is always been discovered different noble food and its active constituents. So here is an Indian Horse Chestnut which is botanically name as *Aesculus indica* and belongs to the family *Hipocastanaceae,* naturally grown at higher altitude or temperate regions of Asia mainly Himachal Pradesh, Jammu and Kashmir in India, Nepal, Pakistan and Afghanistan [12]. In tribal area, people greatly dependent on the various locally available plants group. The fruit has bitter taste which is eaten by wild animals and livestock. The seeds from the tree could be harvested from mid of July to till seed maturation attained during whole winter season [6] and usually goes fritter away because of lack of awareness, inappropriate processing methods for its use as value added products in routine life. Aescin (0.08 g/100 g) is the most active chemical constituent present in *tatwakhar* or processed flour of *Aesculus indica*, which is a mixture of triterpenoid saponin with an excellent anti-inflammatory, anti-oxidant, anti-oedema, antitumor, antiviral, antifungal, antiangiogenic (vascular protection), anti-obesity, antioxidative, and antigenotoxic properties [14]. An unprocessed seed has loads of saponin and tannin and leads to toxic or fatal for human consumption.

That's why it should be mashed and steeped whole night for 10-12 days in wooden basket full of water [11], the saponin rich water may also be used for washing clothes, with continuously changing water till the toxicity removed and dried under sunlight. The dried seeds are grounded to make flour, which is locally called as *tatwakhar* or processed flour [7]. Furthermore, the flour is gluten and fibre free and termed as processed flour or locally known as *tatwakhar*. Hence it becomes advanced choice for gluten receptive patients who have restricted food resource for their existence [8]. The fruit of this species of *Aesculus* are used in the preparation of a nutritious recipe called "sik" which can be stored for 2 to 3 days. This food is given to pre- and post- pregnant ladies. It is also cure excessive bleeding and pain in menses [15]. There was developed and standardized the value-added products prepared as snacks like doughnut, *chatpate* rings, pasta and nibbles made by this flour [9]. Significant stimulation of the cell mediated immunity with no effect on humoral immunity by the use of fresh leaves extract of *Aesculus indica* on rats was found [1]. Use and exploitation of cheap and locally available medicinal food substitute many expensive drugs that are not affordable, accessible to the population [3]. Tribal people used this flour without knowing it's any effect on their internally body. So, with knowing its analyzing effect, it could be a better option to look over it as remedy.

2. Materials and Methods

2.1 Chemicals required

Alloxan, sodium chloride, buffered formalin 10(%), formaldehyde, xylene, 50, 70, 90 and 100% absolute alcohol, hematoxylin, eosin, distilled water, paraffin wax, benzene, embedding cassettes, cassette containers, paraffin wax (melting point 56-57°C), ammonia water or lithium carbonate water.

2.2 Required animals

Germ free 50 weanling male Albino Wistar strains rats weighing 25±5g aged 25 days were obtained from Small Animal House CCSHAU, Hisar. They were maintained on control feed and water ad libitum. The proper consent and approvals for animal studies were obtained from animal ethnical committee, College of Veterinary and Animal Sciences, CSK HPKV, Palampur, HP.

2.3 Diet source and preparation

A control group was fed on control (casein) diet and second group on control diet with diabetes. Whereas Third, fourth and fifth group was depending on 25, 50 and 75 percent *tatwakhar* diet, respectively i.e. the diet consists of 25 percent flour in the prepared diet and remaining 75 percent diet as control having all necessary nutrients for their sustainability. The diets were isoprotenious containing 10% protein and 5% fibre. All the diets contained 10% protein and fat each including the crude protein and crude fat from *tatwakhar* source.

2.4 Diabetics-Induced

Four groups of rats were chosen for inducing diabetes subcutaneously i.e. 140 mg/kg body weight after fasting 48 hour and consecutively left for two days for diabetes-induced. Then the rats were fed on 20 days on control diet to make ensure the diabetes was stable. All the rats were kept in metabolic cages for urine collection (in a beaker). On 21 day, the blood and urine were collected and analyzed via hand glucometer (Model GC Sense) and Benedict's method, respectively. After confirmation of diabetes, the experimental diet was started for the treatment.

2.5 Experimental animal design

The animals were divided into five groups of Ten (10) rats each and treated as follows:

Group I: Control rats were running on control diet and water ad libitum for 21 days for all other groups as well.

Group II: Control with diabetic rats was fed on same diet as group I.

Group III: This group consists of diabetic rats fed on the 25 percent *tatwakhar* or processed flour.

Group IV: This group of diabetic rats was specified as 50 percent *tatwakhar* or processed flour.

Group V: Diabetic rats were depending greatly on the preparation of 75 percent *tatwakhar* or processed flour

2.6 Sample collection for Histopathology and Microscopic study

Histopathology was done in order to determine the efficacy of *Aesculus indica* flour or *tatwakhar* on the organs of rats. After completion of specified 21 days of diabetes induced and fed on experimental diet, the rats were sacrificed using chloroform vapour then dissected to harvest their organs viz. heart, liver, kidney and pancreas and kept in buffered formalin solution immediately. It was analyzed by the protocol given [5]. The microscopic study and histological interpretation was done by computerized microscope at Division of Food and Nutraceutical, CSIR-Institute of Himalayan Bioresource Technology, Palampur, as demonstrated in Plate 1 to 5.

3. Results and Discussion

The rats were divided into various groups with the number of ten in each group. Control group were treated as standard or reference to the other group. Their diet is balanced as well as complete in all respect. The formulated diets were given to the specific group and the weights of the rats were also recorded on daily basis. At the end of experimental periods, the animals were sacrificed and tissue was collected. The tissues were examined for the histopathology in order to determine the net effect of *tatwakhar* of Indian Horse Chestnut *(Aesculus indica)* and the microscopic results are as following for control group in Plate 1.

3.1 Control group

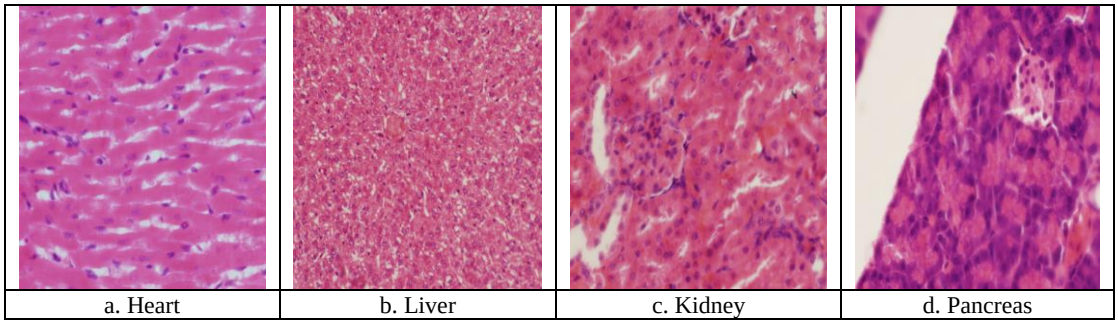
In the performed control group of rats, the following changes were found in the organs viz. heart, liver and kidney of experimental rats.

a) **Heart:** There were normal muscle fibers having elongated nuclei found. The elongation of nuclei might disrupt the basic architect of the cell and results in increased dense area. These nuclei were placed more towards periphery and striations were prominent in the muscle fibers. There was mild to moderate congestion in blood flow in the heart. It might be caused due to the aortic wall shear or stressed condition of the body.
b) **Liver:** The hepatocytes (liver cell) were found to be

normal with mild congestion i.e. only obstruction in blood flow. It might be due to the little pressing or clot in the vessel that might results in portal hypertension if is in excess.

c) Kidneys: The kidneys were also found normal except mild congestion in its tissue. There was also obstruction in blood flow; it might be due the formation of clot in the renal artery.

d) Pancreas: The pancreas of the control group was found normal except mildly congested. Likewise other organs, there was found also an obstruction in blood flow because the blood supply to the pancreas comes from multiple branches and anywhere may be formation of clot or narrowing of arteries.



a. Heart | b. Liver | c. Kidney | d. Pancreas

Plate 1: Histopathological study of control group

Similarly in the same group, most of the cells contain a central rounded nucleus whereas a few binucleated. The blood sinusoids are present between the cords. The sinusoidal endothelium is formed of endothelial lining cells and phagocytic kupffer cells [16].

3.2 Control with Diabetes

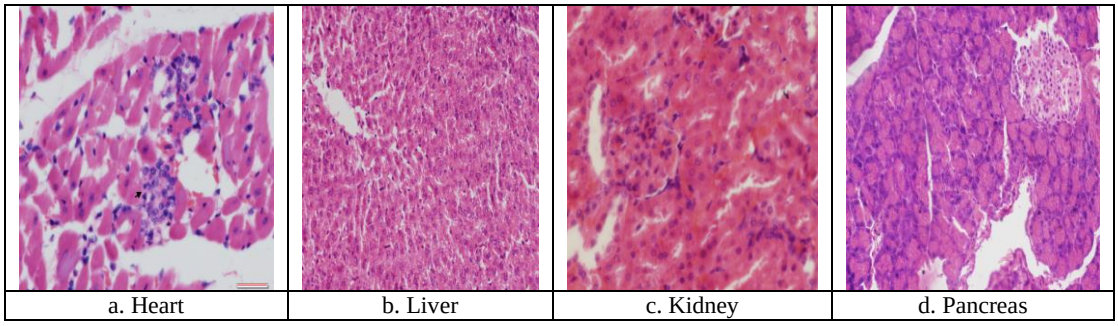
In the following group having control with diabetic experimental rats, Plate 2 reveals the results as summarized below:

a) Heart: In heart, there was no other problem found except mononuclear infiltration in two rats at few places. These mononuclear cells infiltrates showed characteristic of inflammatory lesions, where white blood cells, mainly macrophages and lymphocytes, collected at the site of injury to help clear away the debris. It is the sign of 1 set of graft rejection. And no other prominent change was observed.

b) Liver: The liver was found comparable to normal with no abnormalities and no effect on the rats with diabetic group.

c) Kidneys: There was found only mild congestion *i.e.* mild obstruction in blood flow in kidney.

d) Pancreas: In pancreas, islet of Langerhans was found well developed. As insulin secretion helps to reduce the blood glucose level. It might be due to the ageing factor and self-defense mechanism of body.



a. Heart | b. Liver | c. Kidney | d. Pancreas

Plate 2: Histopathological study of control with diabetic group

The diabetic untreated rats were having degenerative islet cells of pancreas which might be due to alloxan used in this trial of histopathological study [2].

3.3 Experimental diet consists of 25 percent *Tatwakhar* with diabetes

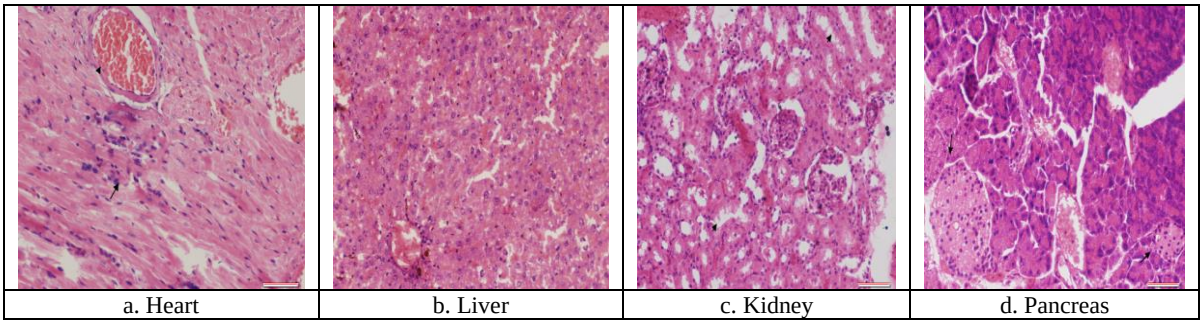
There are following changes depicted in Plate 3 in the heart, liver, kidneys and pancreas as:

a) Heart: Mild congestion and mild increase in the interstitial space were the common changes observed in the tissues whereas mononuclear cell infiltration was also present at few places. There was obstruction in blood flow with increased pressure at the blood arteries. And might be formation of clot and cell injury at specific places could be a reason for these changes.

b) Liver: Moderate congestion with occasional areas of necrosis and mononuclear infiltration was common findings. Increased cytoplasmic granularity was one of the features found in hepatocytes.

c) Kidneys: There was moderate congestion and mild tubular swelling found in kidneys.

d) Pancreas: The pancreas was found normal except mildly congested.



a. Heart	b. Liver	c. Kidney	d. Pancreas

Plate 3: Histopathological study of 25 percent *Tatwakhar* flour with diabetic group

3.4 Experimental diet consists of 50 percent *Tatwakhar* with diabetes

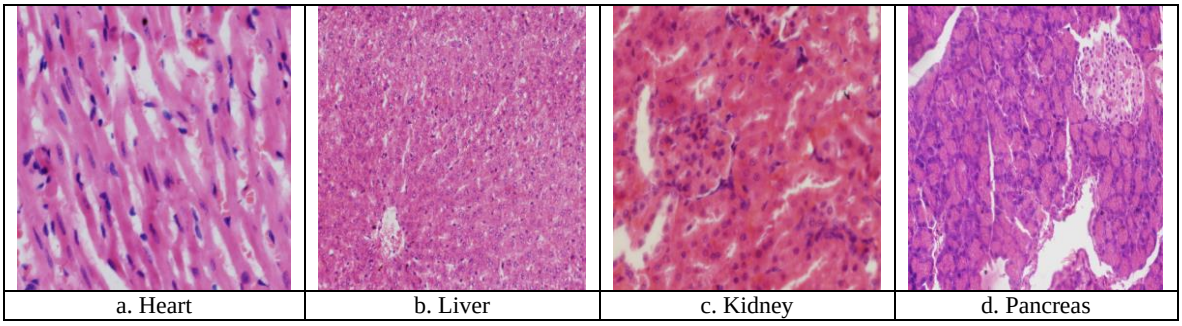
Histopathological changes were observed as in Plate 4 of different vital organs after feeding 50 percent *tatwakhar* experimental diet:

a) Heart: No congestion was established however mild increase in interstitial space and focal area of cellular infiltration was found. Increased interstitial space disrupt the basic architecture of the cell and increased more interstitial fluid with more blood flow that results in venous pressure and ultimately affects heart.

b) Liver: In liver, mild congestion with increased cytoplasmic granularity was found. An increase in cytoplasmic granularity is considered to be one of the signs of cell injury in tissue and indication of toxicity in the screening of chemical agents. These changes might be found because of the inappropriate optimum concentration of aescin content.

c) Kidneys: In kidney, mild tubular swelling and congestion was found. Only mild inflammation and obstructive blood flow was interpreted.

d) Pancreas: In pancreas, there was reduction in the number of islet of Langerhans found. It means that there was decreased secretion of insulin hormones which might be disturbing the blood glucose level.



a. Heart	b. Liver	c. Kidney	d. Pancreas

Plate 4: Histopathological study of 50 percent *Tatwakhar* flour with diabetic group

3.5 Experimental diet consists of 75 percent *Tatwakhar* with diabetes

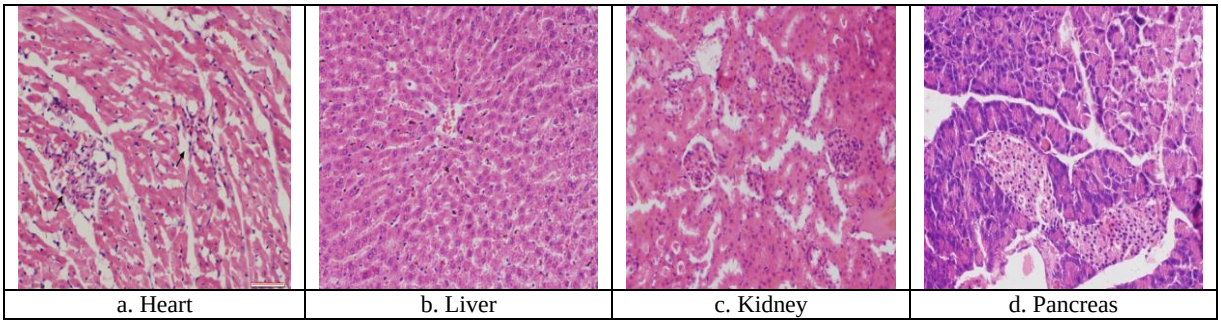
The histopathological changes in 75 percent *tatwakhar* fed diet was observed and reported as in Plate 5 are following:

a) Heart: There was slight increase in interstitial space with branching of muscle fiber i.e. Muscle fiber appeared more eosinophilic. In few focal area mononuclear cell infiltration (cell inflammation) and necrosis (cell death) was present.

b) Liver: There was observed a mild cellular swelling and necrosis at the focal point.

c) Kidneys: Only mild tubular swelling and congestion was present in the kidney.

d) Pancreas: In pancreas, full development of islet of Langerhans was found. It means there was maximum secretion of insulin hormone, so that it can help to maintain/reduce the blood sugar. It might be due to the presence of aescin in the *tatwakhar* or processed flour.



a. Heart	b. Liver	c. Kidney	d. Pancreas

Plate 5: Histopathological study of 75 percent *Tatwakhar* flour with diabetic group
Note: Mild= + (1), Moderate= ++ (2), Severe= +++ (3)

Conclusion

Overall effect of *tatwakhar* on the glycemic group was found health benefitted to the rats. Tissues of the rats from the different proportion which were used to given in the form of complete diet; a 75 percent *tatwakhar* diet was found best in this group because of the optimum level of aescin present in it. Significant responses were showed by other groups comparatively. There was fully development of islet of Langerhans which means that there is production of proper insulin hormones and decreased the blood glucose level. No prominent changes were found in their heart, liver, kidney and pancreas. And other groups were also not shown any major changes. Furthermore, no toxic effects were reported from the toxicity study. The results indicate that the use of more *tatwakhar* or processed flour of *Aesculus indica* was safe and efficient in diabetes mellitus.

5. References

1. Chakraborthy GS. Evaluation of Immunomodulatory activity of *Aesculus indica*. International Journal of Pharm Tech Research. 2009; 1:132-134.
2. Hashemnia M, Ahmad O, Hamidi AR, Mohammadalipour A. Blood glucose levels and pathology of organs in alloxan-induced diabetic rats treated with hydro-ethanol extracts of Allium sativum and *Capparis spinosa*. African Journal of Pharmacy and Pharmacology. 2012; 6:1559-1564.
3. Jose E, Usha PTA. Evaluation of antidiabetic efficacy of *Coccinia indica* in rats. Indian Journal of Animal Research, 2010; 44:168-172.
4. Khajuria P, Raghuwanshi P, Rastogi A, Koul AL, Zargar R, Kour S. Hepatoprotective effect of seabuckthorn leaf extract in streptozotocin induced diabetes mellitus in Wistar rats. Indian Journal of Animal Research, 2017; B-3439:1-6. DOI: 10.18805/ijar.B-3439
5. Luna LG. Manual of histopathology staining methods of the Armed Forces Institute of Pathology, Third edition, Mcgraw hill book company, 1968.
6. Majeed M, Khan MA, Mughal AH, Bashir A. Maturity indices of Indian Horse Chestnut (*Aesculus indica* Colebr) seeds under temperate Kashmir conditions. Silva Lusitana, 2009; 17:211-219.
7. Mishra ML, Sood S, Shukla UN. Phyto-nutritional and mineral composition of Indian Horse Chestnut (*Aesculus indica*) seeds. Journal of Pharmacognosy and Phytochemistry. 2018a; 7:2159-2162.
8. Mishra ML, Sood S, Shukla UN. Standardization, Development and Proximate Composition of Baked Value Added Products by Using Indian Horse Chestnut (*Aesculus indica*) Flour. International Journal of Current Microbiology and Applied Sciences. 2018b; 7:1449-1458.
9. Mishra ML, Sood S, Shukla UN. Standardization, development and sensory evaluation of snacks prepared from *Aesculus indica* flour (*Tatwakhar*). The Pharma Innovation Journal. 2018c; 7:89-93.
10. Oyebadejo S, Bassey E, Oyewunmi A, Archibong V, Usoro E. Histopathological study of the liver of Alloxan induced diabetic rats and macerated *Allium sativum* (Garlic) Ameliorative Effect. Asian Journal of Biomedical and Pharmaceutical Sciences. 2014; 04:72-77.
11. Rajasekaran A, Singh J. Ethnobotany of Indian Horse chestnut (*Aesculus indica*) in Mandi district, Himachal Pradesh. Indian Journal of Traditional Knowledge. 2009; 8:285-286.
12. Syed IR, Sukhcharn S, Saxena DC. Evaluation of Physical and Compositional Properties of Horse-chestnut (*Aesculus indica*) Seed. Journal of Food Processing and Technology. 2016; 7:561. DOI:10.4172/2157-7110.1000561
13. Sirisidthi K, Kosai P, Jiraungkoorskul W. Antihyperglycemic activity of *Ophiocordyceps sinensis*: A Review. Indian Journal of Agricultural Research. 2015; 49:400-406.
14. Sood S, Mishra M, Sood A, Thakur V. Hypoglycaemic and hypocholesterolimic efficacy of horse chestnut (*Aesculus indica*) using rat model. Journal of Clinical Nutrition and Dietetics, 2015; 1:1-8.
15. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. Journal of Ethnobiology and Ethnomedicine, 2006; 2:14.
16. Waer HF, Helmy SA. Cytological and Histochemical Studies in Rat Liver and Pancreas during Progression of Streptozotocin Induced Diabetes and Possible Protection of Certain Natural Antioxidants. Journal of Nutrition & Food Sciences, 2012; 2:9. DOI: 10.4172/2155-9600.1000165.