"Protective effects of *Aloe vera* Gel ethanolic extract against Streptozotocin-induced hepato-pancreatic toxicity in Female Albino Rats"

ABSTRACT

Aims: Organ toxicity results from the accumulation of toxic substances in the organs which ultimately culminates in failure of the organ. Allopathic medications are not very effective in organ protection. Hence, it is imperative that a natural and safer organoprotective agent should be found.
Study Design: To assess the organoprotective effect of *Aloe vera* gel against STZ for renal, hepato and pancreatic toxicity in albino Wistar rats.
Place and Duration of Study: Whole work had been completed at the Microbiology and Molecular biology labs of IMBB, The University of Lahore during 2019- 2020.
Methodology: Organoprotective ability of different doses of ethanolic extracts of *Aloe vera* (*A. vera*) gel was evaluated against intraperitoneal induction of 55mg/kg Streptozotocin (STZ)-induced pancreatic, renal and hepatic toxicity in female albino Wistar rats by keeping metformin (100mg/kg) as a positive protective control.
Results: Results revealed that 200 mg/kg ethanolic extracts of *A. vera* gel showed significant organoprotection as ALT (57.5±7.45I U/L), AST (39.8±3.45I U/L), ALP (438±103I U/L), urea (74.1±8.71mg/dl), creatinine (0.688±0.146 mg/dl), amylase (1247±75 U/L) and lipase (16.6±2.02 U/L) were significantly less than organotoxic control [ALT (103±7.23I U/L), AST (237±12.7I U/L), ALP (2092±195 U/L), Urea (153±18.6mg/dl), Creatinine (1.54±0.262 mg/dl), amylase (675±83 U/L) and lipase (12.2±1.04 U/L)], and these results were near or equal to organoprotective control [ALT (71.6±8.98I U/L), AST (121±28.1 U/L), ALP (916±103 U/L), urea (115±11.4mg/dl), creatinine (1.14±0.226 mg/dl), amylase (667±80 U/L) and lipase (16.6±2.02 U/L)]. The histopathological analysis also highlighted more organoprotection at this concentration as compared to other doses of extract.
Conclusion: *A. vera* gel extract is an able organoprotective agent. This extract can be studied further for its active ingredients as a source of hepatoprotective and nephroprotective agents.

Key words: *Aloe vera gel, Organoprotection, Hepatoprotective, Nephroprotective, Pancreatoprotective, Streptozotocin (STZ)*

1. INTRODUCTION

Human beings come across thousands of chemicals in their everyday life. Approximately, 200 to 1000 new chemicals are synthesized and released in our environment every year by the chemical industry. As a consequence, humans are threatened by numerous chemicals at their residences, workplaces and even in their approximate environment. Chemicals that can cause toxicity in even Minute amounts are also present in the water we drink, air we breathe and food we ingest [1]. Adverse event recording system of FDA states that between 1969 and 2002, around 2.3 million reports of detrimental drug reactions were put forward across 6000 certified compounds [2].
As toxicity is "the accumulation of injury over short or long periods of time, which renders an organism incapable of functioning within the limits of adaptation.''[3]. Based on this definition, toxicity is mainly dependent on two factors, time and dosage. Toxicity occurs either as a consequence of an interplay between biomolecules and molecules of extrinsic chemicals or it can also manifest after life forms are afflicted with physical mauling [4]. Liver serves a pivotal function in metabolism and the excretion of drugs [5]. Drug induced hepatotoxicity or drug-induced liver injury (DLI) comes with substantial risk of morbidity and if not treated, can also lead to mortality. Hepatotoxicity alludes to malfunctioning of liver or it can also indicate hepatic impairment which is connected with an overwhelming number of drugs

or xenobiotics [6]. Examples of such drugs include allyl alcohols, acetaminophen, ethanol and cadmium chloride [7]. The compounds that lead to hepatic impairment are termed as hepatotoxicants or simply hepatotoxins [8]. Instigating factor for hepatic impairment can either come from the primary compound itself, or it can come from a reactive metabolite or it can also come from a bioimmunological response that involves hepatic parenchymal cells, epithelial cells of the biliary channels and/ or blood vessels of liver [9].

Kidney serves as the primary organ for the excretion of drugs and entertains approximately 25% of resting cardiac output. Weighed against other organs, kidneys play a pivotal role in biotransformation of exogenous drugs following the synthesis of reactive oxygen species and toxic biomolecular waste. The aforementioned compounds lead to escalation of oxidative stress-mediated impairment and may lead to renal injury [10]. Numerous medications including chemotherapeutic drugs, radiocontrast media, nonsteroidal anti-inflammatory drugs (NSAIDs), angiotensin-converting enzyme inhibitors (ACE inhibitors), aminoglycoside antibiotics, and angiotensin II receptor blockers possess nephrotoxic ramifications [11].

Acute pancreatitis is characterized as an inflammatory disease relating to pancreas and is correlated with substantial morbidity and mortality [12]. In spite of the fact that Drug-induced pancreatitis (DIP) accounts for around 1% to 2% of all the cases of pancreatitis, it has come under the spotlight as a major, albeit indistinct, cause of acute pancreatitis [13]. World Health Organization (WHO) has delineated 525 various drugs which are associated with the incidence of acute pancreatitis [14].

Streptozotocin is an alkylating agent and this feature imparts genotoxicity to the compound thus making it a broad spectrum antibiotic [15]. STZ depicts diabetogenic, antitumor, antibacterial and carcinogenic characteristics [16]. Furthermore, toxicity of beta cells of pancreas was observed with STZ treatment and hence, nowadays, it is used to induce type 2 Diabetes mellitus in laboratory animals [17].

Aloe comes from the family of *Xanthorrhoeaceae*, which encompasses approximately 420 species, and has been utilized in conventional medicines for around 3000 years [18]. The seasonal plant commonly known as *Aloe vera (A. vera)* is actually *Aloe barbadensis* Miller. Nomenclature of *Aloe* is derived from two words; “alloeh” (in Arabic) or “allal” (in Hebrew) or “alsos” (in Greek) which translates to “bitter” and “*vera*” meaning, “true” [19]. *A. vera* is rich with numerous phytochemicals including coumarins, chromones, anthraquinones, flavonoids, enzymes, vitamins [20], anthrones, phenols, alkaloids, carbohydrates and proteins [21].

A wide array of pharmaceutical beneficial effects have been documented for *A. vera* which include anti-inflammatory benefits [22], moisturizing, antiaging effects [23], and antibacterial action [24], among many others. It has been proven safe for human consumption after examination of its acute, subacute and genotoxic studies [25].

The objective of this research is to evaluate the organoprotective role of *A. vera* gel on the functions of the liver, kidney and pancreas in albino Wistar rats *in vitro* following STZ induced organotoxicity.

2. MATERIAL AND METHODS

2.1 Study Location

The following research was held in the animal house of Institute of Molecular Biology and Biotechnology (IMBB) department of The University of Lahore main campus Lahore, Punjab, Pakistan.

2.2 Preparation of *A. Vera* Gel Extracts

A. vera plant was taken from the botanical garden Lahore (GC- BOT 525). The extract was prepared following the methodology of Grieve [26]. Fresh leaves of the plant were plucked and then cut opened from center and the gel was taken out by the help of a spoon. The gel was then collected and homogenized by using a mixer blender. The homogenized mixture was collected in a glass jar and then lyophilized at 4°C by using round bottom flasks. The lyophilized material was then dissolved in 95% ethanol in a 2 liters airtight glass container. The whole solution was shaken once a day so that the lyophilized material does not settles at the bottom of the container. After keeping it for 7 days, the whole solution was filtered with the aid of a filter paper. And the whole filtered solution was placed on a rotatory evaporator at 55 ^{O}C by which ethanol and plant extract were separated. The residue was then kept in petri dishes for two days and then the residue was dissolved in 0.9% normal saline

solution and filtered by using syringe filter. The extract was stored in airtight glass tubes which were then stored at 4°C temperature refrigerator.

2.3 Standard Drug

Metformin had been administered as organ protective control in streptozotocin induced rats by dissolving in citrate buffer (0.1M) as a dosage of 100mg/kg [27].

2.4 *In-Vivo* Studies

Female albino Wistar rats weighed around 180-250 grams were chosen for the study. The rodents were kept in controlled environmental conditions in animal house of IMBB. The University of Lahore, Lahore, and a standard protein and fiber containing food was fed to them. This work has been done with the approval of ethical committee of IMBB, UOL for the commencement of the study.

2.5 Animal Groups

The rats were divided into 7 groups with each group consisting of 5 rats to study the organ protective activity

Group 1: Control (Vehicle or Normal or Without STZ)

Group 2: Organo-toxic Control (STZ induced)

Group 3: Organ protective Control (STZ + Metformin)

Group 4: Experimental group 1 (Normal and EEAV at dose of 100mg/ml/kg)

Group 5: Experimental group 2 (STZ and EEAV at dose of 100mg/ml/kg)

Group 6: Experimental group 3(Normal and EEAV at dose of 200mg/ml/kg)

Group 7: Experimental group 4 (STZ and EEAV at dose of 200mg/ml/kg)

2.6 Administration of STZ (Nicotine amide)

Nicotine amide was administered to 12 hours fasting rats 20 mins before the administration of STZ through intraperitoneal route and the dosage was 110mg/ml/kg. STZ was administered by using 0.1M citrate buffer with pH 4.5 through the same intraperitoneal route and the dosage was 55mg/ml/kg [28]. Then 5% dextrose solution was given to the rats which helped them to face the shock of hypoglycemia. Next day, the glucose level of rats was checked by using a glucometer (Accu-Chek). The blood sample was taken by pricking the tail of the rat from the capillaries of the tail by using a lancet. The rats with BSL of above than 200mg/dl were considered diabetic and were kept under observation for 14 days, which included their weight and other physical conditions.

2.7 Administration of Plant Extracts

The prepared plant extracts were administered by using intra-gastric route by the help of a stainless-steel tube attached to a 1ml syringe. This was continued for once in two days for 14 days.

2.8 Biochemical Tests

The sample tubes were centrifuged at 3500 rpm for 5 minutes to separate the serum and RBCs for following biochemical tests.

2.8.1 Alanine Aminotransferase (ALT)

ALT was determined spectrophotometrically by using ALAT (Merck-Inoline) Brand kit on an automated analyzer (Mindray BS-120). Both the sample and ready to use reagent were placed in the machine and the result was shown on the screen of the analyzer.

2.8.2 Aspartate Aminotransferase (AST)

AST was determined by using (AMD Diagnostics) kit on an automated Analyzer (Selectra Pro lab) by using the serum of the blood sample while results were noted from the result screen of the machine.

2.8.3 Alkaline Phosphatase (ALP)

ALP was performed on Selecta Pro Lab (an automated analyzer) by using ready to use (Merck-Inoline) kit by using serum of the sample.

2.8.4 Urea and Creatinine

Urea content was determined by using Merck-Inoline kit on a biochemistry automated analyzer and the serum creatinine was determined by using (AMD) kit on a (microlab-200) manual analyzer by dissolving the 20µl serum sample in 500µl ready to use reagent in a glass tube and then aspirating the whole solution to the machine. The results were shown on the screen.

2.8.5 Amylase

Amylase test was performed on an automated analyzer (Selectra Junior) by using the prepared (Merck-Inoline) kit by using the serum of the sample.

2.8.6 Lipase

Lipase of the serum was determined photometrically by using the (AMD) kit in Microlab-200 manual analyzer at the wavelength of 340nm by adding 50µl serum in 1.0ml ready to use reagent.

2.9 Histopathology

After treating the animals for 14 days with *A. vera* gel extract and Metformin, the 12 hours fasting rats were sacrificed by using cervical dislocation and chloroform was used for anesthetizing. The blood sample was drawn from the heart and then sample was shifted to a clot activator tube for biochemical analysis. The liver and pancreas were dissected out and weighed and were saved in 10% formalin solution for the histopathology analysis [29].

2.9.1 Tissue Staining

Staining jar with xylene was used for deparaffinizing by situating the sections for 10 minutes in it. 100 % isopropyl alcohol was used for rinsing the deparaffinized sections and staining for 8 minutes with Ehrlich's hematoxylin was done. Using the tap water, the section was cleaned and to remove the excess stain, acid alcohol (8.3% HCL in 70% alcohol) was employed. To proceed bluing (slow alkalization), cleansing for 10 minutes was performed under running tap water. Counter staining sections for 1 min in 1% aqueous eosin (1 gm in 100 ml tap water) was proceeded and excess stain was washed in tap water, allowing the sections to dry. Full dehydration of stained sections was ensured by placement of sections in the incubator at 60 °C for 5 minutes. Mounting in DPX was done after the sections were cooled which had glass optical index. The sections were observed low power objective microscope and other aspects such as cell injury was observed under high power dry objective [30].

2.10 Statistical Analysis

The results were statistically analyzed by using GRAPH PAD PRISM 5.0 software. The mean and standard deviation was calculated by using the software mentioned above. The graphs were prepared by using ONE WAY ANOVA.

3.RESULTS

3.1 Analysis of Biochemical Parameters

3.1.1 Control groups

All animals of vehicle showed normal results of ALT ranges between (49 IU/l-58 IU/l), AST (39 IU/l-45 IU/l), ALP (311 IU/l-580 IU/l), Urea (75 mg/dl-89 mg/dl), creatinine levels range was between (0.6 mg/dl-0.9 mg/dl) (Figure 3.5), Amylase (1151 U/l-1320 U/l) and Lipase (18.5 U/l-20.5 U/l). Rats in organotoxic control showed significantly high amount of all biochemical like levels of ALT (95 IU/l-115IU/l), AST (224 IU/l-255 IU/l) and ALP (1850 IU/l-2230 IU/l) were abnormally significant which were showing that the STZ had bad effect on the liver and thus released these enzymes in to blood. In this group, the levels of urea (139 mg/dl-180 mg/dl) and creatinine (1.2 mg/dl-1.9 mg/dl) were also very significant which indicates that the damage was done on kidneys resulting in their inability to

function properly. STZ damaged the pancreas too which also showed significance in levels of pancreas enzymes Lipase (10.7 U/l-13.4 U/l) and Amylase (587 U/l-802 U/l). Organoprotective control group showed significantly better results in comparison to cure organs as compared to Organ toxic control group as the levels of ALT (59 IU/l-85 IU/l), AST (88 IU/l-165 IU/l) and ALP (805 IU/l-1065 IU/l) r were significantly lowered down to normal. Urea (98 mg/dl-135 mg/dl) and creatinine levels (0.9 mg/dl-1.5 mg/dl) also showed significant reduction in their amount while pancreatic enzymes raised significantly [Amylase (850 U/l-1020 U/l) and Lipase (12.4 U/l-19.5 U/l)] (Figure 3.1 and Table 1).

3.1.2 Experimental Groups

The rats in the experimental group 1 showed normal levels of ALT (50 IU/l-70 IU/l) (Figure 3.1), AST (35 IU/l-46 IU/l), ALP (305 IU/l-625 IU/l) (Figure 3.3), Urea (60 mg/dl-85 mg/dl) (Figure 3.4), Creatinine (0.5 mg/dl-0.9 mg/dl), Amylase (1161 U/l-1390 U/l) and lipase (16.0 U/l-21.5 U/l). Results of experimental group 1 were non-significant when compared to normal control group. In experimental group 2, the rats showed merely significant results when compared to Organotoxic and organoprotective control groups as level of ALT (88 IU/l-110 IU/l), AST (234 IU/l-265 IU/l), ALP (1650 IU/l-2031 IU/l), Urea (125 mg/dl-170 mg/dl), creatinine (1.0 mg/dl-1.9 mg/dl), amylase (558 U/l-979 U/l) and lipase (10.4 U/l-13.5 U/l) were parallel to that of organoprotective control group animals. In this group, level of ALT (52 IU/l-75 IU/l) (Figure 3.1), AST (30 IU/l-40 IU/l), ALP (234 IU/l-499 IU/l), Urea (53 mg/dl-79 mg/dl), creatinine (0.8 mg/dl-1.0 mg/dl), Amylase (1168 U/l-1310 U/l) and lipase (17.2 U/l-21.0 U/l) were significantly similar to that of organoprotective control group. This group showed highly significant results when compared to organotoxic results of levels of ALT (82 IU/l-105 IU/l), AST (89 IU/l-169 IU/l), ALP (526 IU/l-1366 IU/l), urea (105 mg/dl-125 mg/dl), creatinine (1.0 mg/dl-1.3 mg/dl) (Figure 3.5), amylase (850 U/l-1250 U/l) and lipase (15.7 U/l-17.9 U/l) (Figure 3.1 and Table 1).

Table 1: Statistical analysis of biochemical parameters of all animal groups (Mean± Standard Deviation)

Groups	ALT (IU/l)	AST (IU/l)	ALP (IU/l)	Urea (mg/dl)	Creatinine (mg/dl)	Amylase (U/l)	Lipase (U/l)
Normal Control	55.9±6.58	40.4±3.74	439±137	79.1±7.36	0.713±0.136	1245±645	19.3±0.680
Organotoxic Control	103±7.23	237±12.7	2092±195	153±18.6	1.54±0.262	675±83	12.2±1.04
Organoprotective Control	71.6±8.98	121±28.1	916±103	115±11.4	1.14±0.226	667±80	16.6±2.02
Experimental Group 1	57.5±7.45	39.8±3.45	438±139	74.1±8.71	0.688±0.146	967±60	18.7±2.07
Experimental Group 2	98.3±8.10	242±9.85	1893±139	138±17.8	1.43±0.315	1247±75	11.6±1.06
Experimental Group 3	60.8±7.96	37.1±3.80	340±76.1	69.0±27.9	0.838±0.0916	739±150	19.3±1.20
Experimental Group 4	93.8±7.32	135±32.9	1044±334	113±7.29	1.11±0.136	739±150	16.8±0.651

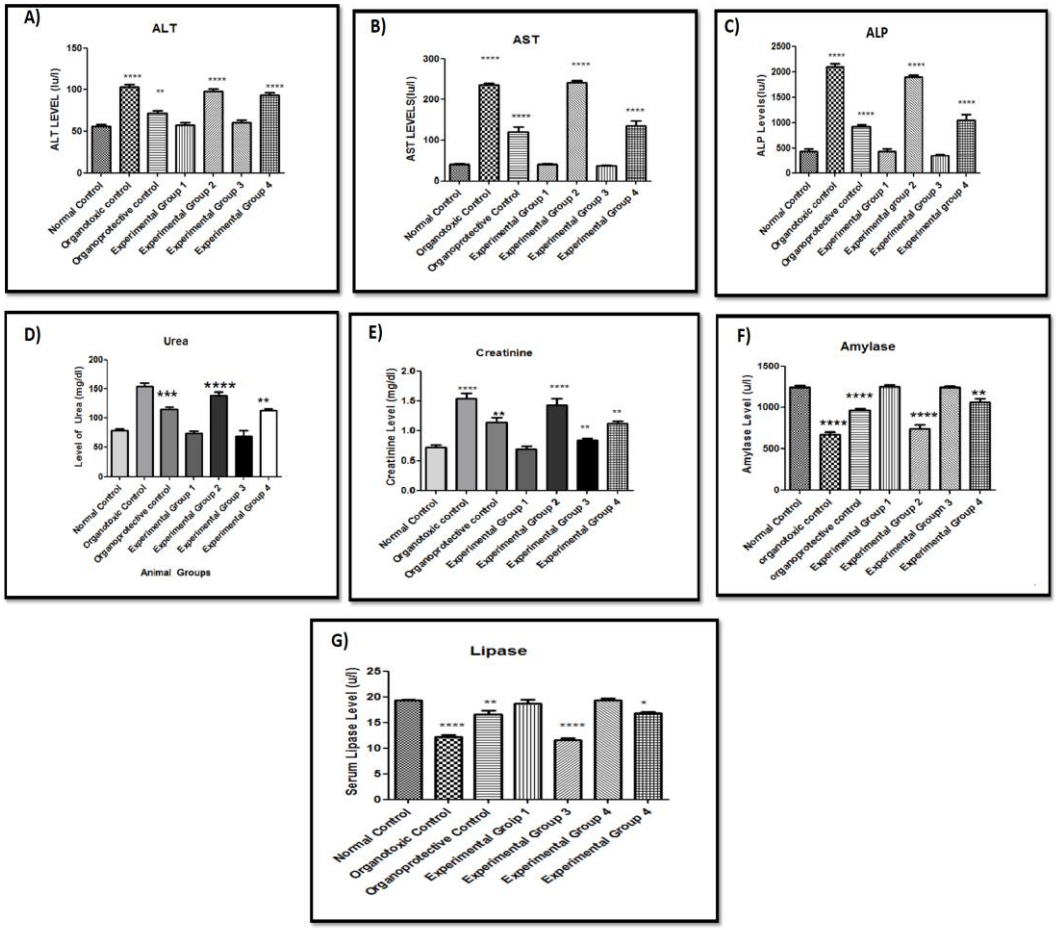


Figure 3.1: A) ALT levels in the rats ***indicates that results are statistically significant (P< 0.05), **** indicate that results are statistically significant (P< 0.01). **B) AST in STZ induced rats ***indicates that results are statistically significant (P< 0.05)**, **** indicate that results are statistically significant (P<0.01). **C) the level of ALP in STZ induced rats ***Indicates that results are statistically significant (P< 0.05) ****** indicate that results are statistically significant (P< 0.01). **D) the different levels of Urea in these groups. ***Indicates that results are statistically significant (P< 0.05) ****** indicate that results are statistically significant (P< 0.01). **E) levels of Creatinine in STZ induced rats. ***Indicates that results are statistically significant (P< 0.05), ****** indicate that results are statistically significant (P< 0.01). **F) the different levels of Amylase in rats. ***Indicates that results are statistically significant (P< 0.05), ****** indicate that results are statistically significant (P< 0.01). G) **the levels of lipase in STZ induced rats. ***Indicates that results are statistically significant (P< 0.05), ****** indicate that results are statistically significant (P< 0.01).

3.2 Histopathology Examination of different tissues at 40X

3.2.1 Liver and Pancreas of Control groups

The histological presentation of liver of vehicle showed the exocrine and endocrine sections of the liver. The hepatic cells were showing no inflammation, necrosis, degeneration or any other deformity. Mostly normal looking hepatocytes were seen (Figure 3.2 A). While its pancreas endocrine and exocrine sections seemed normal. No such kind of inflammation or necrosis was seen. β-cells were present in the islet of Langerhans with other pancreatic acinar cells and did not show any abnormality. No such deformity was seen (Figure 3.2 H).

The histopathological analysis of liver of organotoxic control rats showed normal endo and exocrine molecules. Inflammation of hepatocytes was seen and no such deformity or any degeneration was observed (Figure 3.2 B). Histological representation of pancreas showed limited numbers of β-cells.

Degeneration of β-cells was also observed in the islet of Langerhans. There was no observation of inflammation or deformity of cells (Figure 3.2 I).

The histological visualization of liver of organoprotective control rats showed normal features. No such kind of degeneration, deformity or inflammation was seen. The nucleus of hepatocytes was in an irregular shape (Figure 3.2 C). while histological observation of metformin treated pancreas showed a slight lesser number of β-cells. The degeneration of β-cells was also seen and no abnormality in acinar cells was seen. No kind of inflammation, deformity or granuloma was seen (Figure 3.2 J).

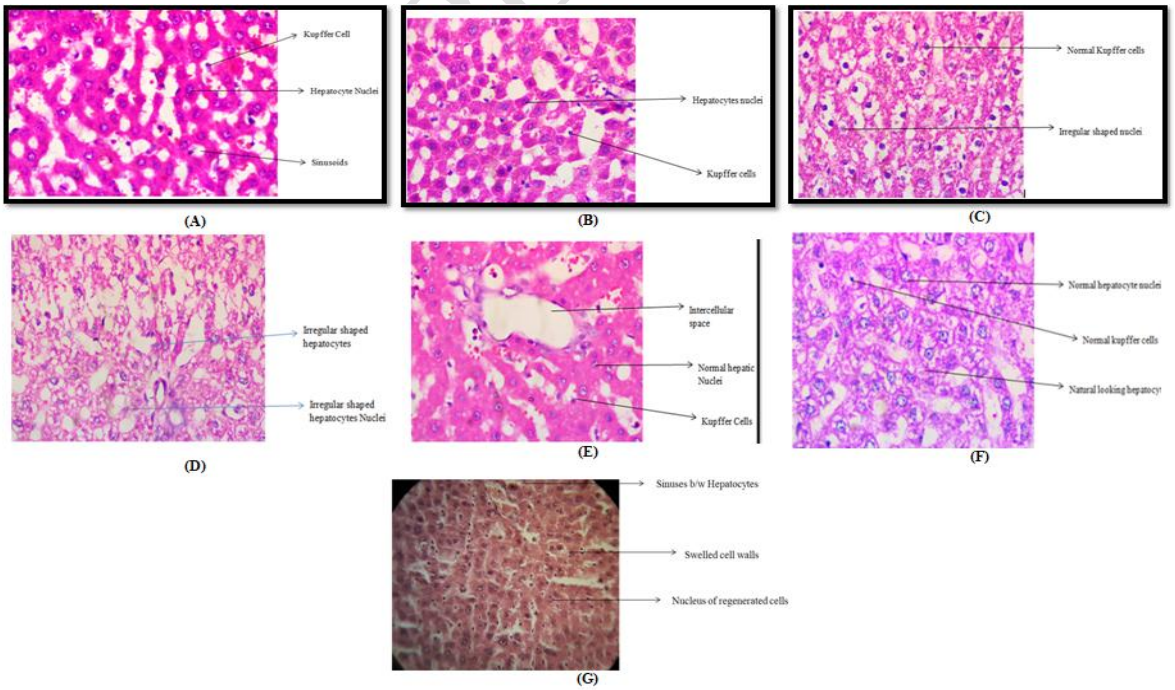
3.2.2 Liver and Pancreas of Experimental Groups

The histological representation of the liver of experimental group I showed normal and healthy hepatocytes. The exocrine molecules were normal looking and Kupffer cells also seemed normal (Figure 3.2 D). While pancreas of this group showed no abnormality in the pancreas. The β-cells were present in the islet of Langerhans and showed no kind of deformity and no degeneration of β-cells was observed. There was no kind of chronic inflammation was seen (Figure 3.2 K).

The histological representation of liver of experimental group II showed normal looking exocrine and endocrine substances. The Kupffer cells were also naturally present. A slight chronic inflammation was suggested (Figure 3.2 E) and The pancreas of this group when observed under 40x showed there were slight lesser number of β-cells in islet and degeneration of the β-cells was also observed. No kind of inflammation was seen (Figure 3.2 L).

The histological observation of liver of experimental group III did not show any abnormality. There were natural cells of the organ. The Kupffer cells were also normal and did not show any deformity and showed no inflammation (Figure 3.2F). While histological analysis of its pancreas showed a slight lesser number of β-cells in the islets and a slight degeneration of β-cells and acinar cells. Chronic inflammation was reported. No other deformity or calcification was observed (Figure 3.2M).

The pancreas of experimental group IV showed no kind of degeneration of cells was reported. β **–** cells were naturally present in the islets and acinar cells also showed normal architecture. No kind of inflammation, granuloma or calcification was observed (Figure 3.2 N). Liver of this group received 1.0ml of ethanolic extracts and when observed histopathologically, Kupffer cells were lesser in number but there was no kind of chronic inflammation or deformity and it showed normal exocrine architecture (Figure 3.2 G).



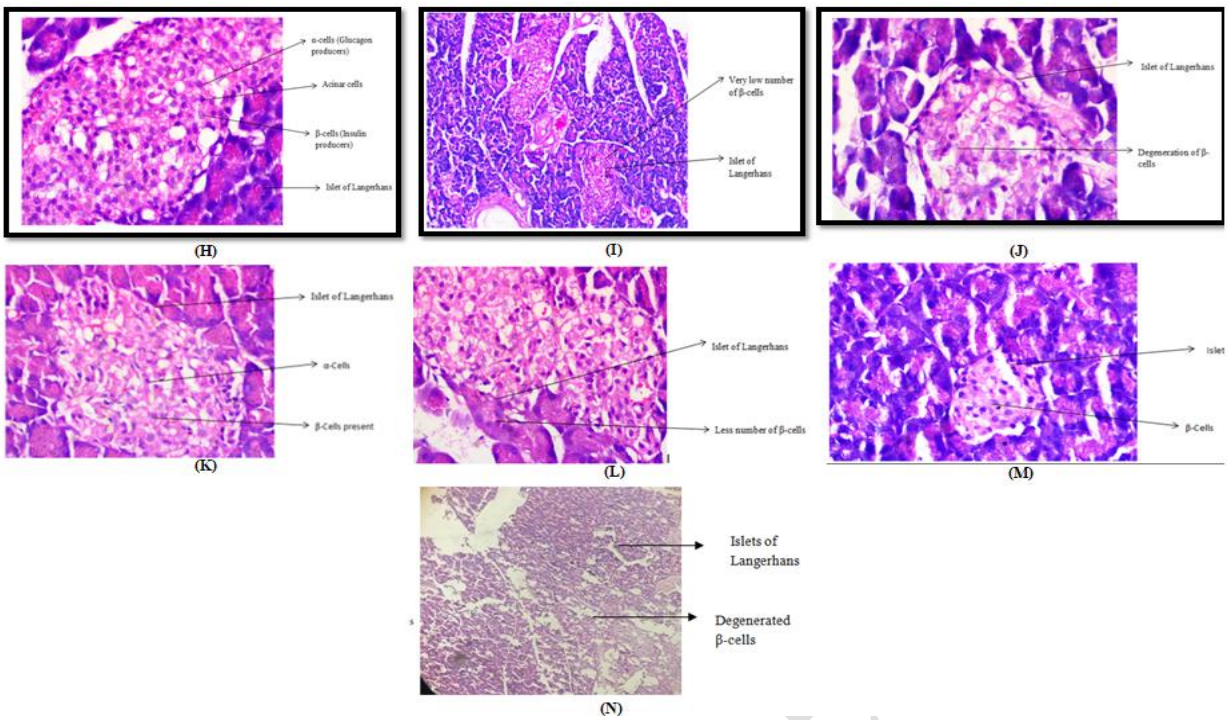


Figure 3.2 Histological representation at 40 X of liver of vehicle(A) Organotoxic control (B) Organoprotective control (C)Experimental group I (D) Experimental group II (E) Experimental group III (F) Experimental group IV (G) and Kidney of vehicle(H) Organotoxic control (I) Organoprotective control (J)Experimental group I (K) Experimental group II (L) Experimental group III (M) Experimental group IV (N)

4. DISCUSSION

Liver disease is one of the most significant and widespread health conditions worldwide. Despite the need for agents to defend the liver against damage, it is difficult to believe that current medicinal products lack an effective liver protection agent [34]. A plethora of attempts have therefore been made to look frantically for the scientifically beneficial and successful treatment of alternative herbal products for the medication of liver diseases [35]. The aloe gel also protects the liver (the primary detoxification organ) from damage in rats by enhancing the activity of a liver enzyme associated with carcinogenic metabolism [36,37].The use of herbal plants proved to be effective in organoprotection because synthetic medications have unintended side effects or contraindications [31]. STZ-induced diabetes can cause organ toxicity and may lead to complications over a long period of time, such as diabetic nephropathy, retinopathy, neuropathy, cardiomyopathy and hyperglycemia. Herbal remedies, from the primitive time to the present, have held a distinct and prominent role. A traditional health care system, which is culturally patterned, exists in every ethnic group. In remote areas, the first and main line of defense appears to be plant-based healthcare. The WHO has already acknowledged their contribution to conventional healthcare. These medications have lesser side effects and it is easy to procure herbs from nature [32]. *A. vera* is considered to be a novel valuable ingredient in treating organ toxicity [33]. Scientific evaluations have demonstrated that both the gel and the rind of *A. vera* leaves contains pharmacologically active ingredients [29]. In addition, compounds such as polyphenols, flavonoids, and many herbal agents have a significant impact in organ protection. This study investigated the organ protective role of *A. vera* on STZ induced organ toxicity.

According to the current evaluation, the ethanolic extract of *A. vera* possessed high phenolic content. The plants with high levels of phenols have been shown to possess antidiabetic capacity in previous studies. Similar results were observed as per the study reported by Manjunath et al. [41].
In order to evaluate the potential effects of *A. vera* on STZ induced toxicity, *A. vera* was administered and levels were measured. Dose-dependent effects have been observed at all dose levels. Slight change was observed when rats were treated with 0.5 ml but 1.0 ml dose of *A. vera*, showed statistically significant results. *A. vera* extract demonstrated improvement over 14 days. Rats gained

in weight showing elevation in body metabolic behavior, suggesting amplification of glucose metabolism by test extract.

STZ induced diabetes is one of the popular models to study liver toxicity. Prospective research indicate that disorders in the levels of liver enzymes are an important risk factor for developing of later-life diabetes [42]. The most frequently measure liver enzymes in our study were ALP, AST and ALT. Increased levels of these enzymes were observed when injured with STZ metformin. These enzymes showed a significant decline in their levels after the rats were treated with 1.0 ml dose but little significance in 0.5 ml doses of *A. vera*. Pretreatment of rats with *A. vera* extract resulted in a significant decrease in enzyme levels, and resulted in a significant reversal of hepatotoxicity. This is in accordance with results previously investigated by Van Fleet and Alberts [43], Korsrud [44] and Dooley [45].

For determining the glomerular filtration rate of the kidneys, creatinine clearance, measured from creatinine concentrations in urine and plasma samples, and urine flow rate, as well as urea clearance, are used. While they are no longer widely performed, they remain useful tests for renal function. Urea and creatinine levels were enhanced when injured with STZ metformin, 0.5 ml dose of *A. vera* showed a reduce level in the concentration of enzymes but 1.0 ml dose showed much significance when compared with injured group. Similar study was conducted by Bolkent et al, [46] and our results are in accordance with it.

Lipase and amylase in normal groups showed an increasing trend and decreased trend in STZ induced injury. However, 0.5 ml showed slight difference compared with normal groups and 1.0 ml doses of A. vera showed significant results compared to injury group. Similar study was performed by Tanvi et al. [47] and our results are in consonance with it.

The biochemical results supporting the hepatoprotective ability of *A. vera* are further confirmed by the histopathology of liver tissue. This study shows that *A. vera* aqueous extract is substantially capable of stabilizing hepatocyte integrity, as shown by improvement in physiological parameters. Similar study was performed by Chandan et al. [48] and our results are in accordance with it. Therefore, based on the findings of the current study and other studies conducted to examine *A. vera*'s **hepatoprotective** function and the indicative mechanisms of *A. vera*'s hepatoprotection, it can be understood that *A. vera* is probably an effective agent for the treatment of liver disease.

ETHICAL APPROVAL

Ethical approval was obtained from the Institutional Review Board of The University of Lahore and all animal experiments were performed in accordance with the Institutional Guidelines for the Care and Use of Animals for Scientific Purposes.

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