Evaluation of Modulatory Effects of Plant Extracts on Oxidative Stress and Histo pathological Changes in Liver and Pancreas Tissues of Albino Rats.

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ABSTRACT:

In this study we evaluated the oxidative stress and histopathological changes in mango pulp and *Aloe vera* supplementation of streptozotocin (STZ) induced diabetic rats. After experimental period of 21 days the liver and pancreatic tissues were collected from 48 male wistar rats divided into 8 groups and oxidative stress and histopathological changes was evaluated.

Significant reduction of oxidative stress and regeneration of tissues after treatment with Mango pulp and *Aloe vera* supplementation in the liver and Pancreas of diabetic rats due to its phenolic compounds and its metabolites.

Key words: Mangiferin, *Aloe vera*, streptozotocin

INTRODUCTION:

Diabetes mellitus is a disorder of metabolism of carbohydrates, proteins and fats associated with insufficiency of insulin secretion (relative or absolute) and with various degrees of insulin resistance. The cause of diabetes is a mystery, although both genetics and environment appear to play roles. Loss of glucose homeostasis is the primary clinical manifestation of diabetes. Despite treatment (diet, insulin and oral hypoglycaemic agents) most diabetic patients eventually experience one or more of the long-term complications of the disease like cardiovascular diseases, Alzheimer's disease and multiple cancers (liver, pancreatic and endometrial cancers) due to abnormal metabolism-induced immunological disorders **(Bahrani, *et al*., 2012, Lin, 2008, Raffone, *et al*., 2020)**

Experimental and clinical findings suggest that enhanced levels of free radicals found in diabetic patients could be the risk factor explaining the excess morbidity observed in these individuals **(Packer, 1993, Sravani *et al.*, 2023)**. The metabolic alterations which contribute to increased free radicals in diabetes include increased nonenzymatic protein glycosylation **(Brownlee and Cerami 1981; Picard, 1995; Soulis-Liparota *et al.*, 1995)** autooxidation of glucose **(Thornalley *et al.*, 1984)**, activation of aldose-reductase pathway **(Taylor and Agius, 1988)** and localized tissue damage. These mechanisms not only cause direct metabolic alterations but also contribute to increased production of free radicals. Free radicals are very reactive and can react with any molecule present in their vicinity. Protein, carbohydrate, DNA and polyunsaturated fatty acids (PUFA) all are known to be targets of free radicals. Oxidation of proteins and carbohydrates results in fragmentation and cross-linking with subsequent loss of function. Free radical induced DNA cross links could lead to somatic mutations, the production of defective gene products and the generation of malignant changes. When the biological molecule is lipid, the process not only results in a radical chain reaction but membrane lipid oxidation might lead to loss of integrity of the membrane and consequent modification of the activity of membrane bound enzymes **(Halliwell & Gutteridge, 1984)** and lipoprotein oxidation may lead to onset of atherosclerosis **(Goldstein and Brown, 1977; Steinberg *et al*., 1989; Ross, 1993)** and other complications.

The liver is the important insulin-dependent organ, which plays a vital role in glucose and lipid homeostasis and it is severely affected during diabetes (**Giacco & Brownlee., 2010**). Pancreatic β cells are the source of insulin production and are extremely susceptible to oxidative stress **(Wang *et al.*, 2010)**. oxidative stress has also been proposed to play a role in the pathogenesis of hepatic tissues damage (**Ha & Lee, 2001; Amarapurkar & Das *et al.*, 2002; Kashihara *et al.*, 2010**). However, during pathological conditions, the decline free radical production and the protective antioxidant defense system may causes ROS-induced tissue damage including hepatic injury (**Gerstein *et al.*, 2007; Giacco & Brownlee, 2010; Baynes & Thorpe, 1999)**.

Earlier, hormone therapy using insulin is one of the classical approaches to treat diabetes. In recent years, exercise, diet, and medications have been considered as a milestone of the diabetes

therapy. Regular exercise is recommended for the patients with type 2 diabetes mellitus and it reduces the diabetic complications. Medications that achieve the specific glycemic goals can significantly reduce the morbidity and it is the top priority of effective treatment of hyperglycemia.

Ayurveda is a comprehensive, personalized and sustainable health system based on logical principals and involves the treatment with the medicinal plants, change in food habits and life style management. The system has resulted in exploring thousands of medicinal plants for arious physiological abnormalities. Herbal drug development involves a systematic process called reverse pharmacology, which explores and validates the medicinal plants used in Ayurveda **(Bhushan Patwardhan *et al.*, 2004)**. Herbal drug development follows exploratory clinical studies along with the in-vitro and preclinical experimental studies to conclude the mechanistic explanations for the results of clinical studies **(Patwardhan and Hooper, 1992)**.

Nature provided us many plants to treat many diseases among them we selected natural miracle medicinal plant *Aloe vera* and pride fruit of India the mango to treat Diabetes mellitus.

Aloe vera is a wonderful succulent plant widely used in traditional medicine. It contains 75 active compounds like anthraquinones, saponins, carbohydrates, chromones, hormones, minerals, vitamins, enzymes, lignin, salicylic acids, and Amino acids and it has many biological activities **(Sharrif Moghaddasi 2011)**. Many studies suggest that it has anti diabetic properties by controlling the blood glucose level, biochemical parameters and antioxidant stress enzymes in an alloxan or streptozotocin induced diabetic animal models **(Radha Madhavi *et al.*, 2012, Abo-Youssef & Messiha, 2013, Abd El-Kader *et al.*, 2019)**.

Mangifera indica belongs to family Anacardiaceae and usually known as mango. It is used in traditional medicine for the cure of various ailments due to presence of more bioactive constituents like carotenoids, tocopherols, ascorbic acid, dietary fiber, and the phenolic compounds mangiferin, gallic acid and quercetin **(USDA, 2005)**.The stem bark and leaves aqueous extract of mango was reported in lowering of blood glucose in streptozotocin-induced diabetic rats **(Muruganandan *et al.*, 2005)** and glucose-induced hyperglycemia in rats and mice **(Aderibigbe *et al.*, 1999 & 2001)**. High-fat diet fed mice showed positive effect on body composition, blood glucose and lipid profile on supply of freeze-dried mango pulp **(Lucas *et al.*, 2011)**.

Recent studies on *Aloe vera* fresh juices and Mango pulp showed a hypoglycemic effect by decreasing glucose levels and retaining serum enzymes ++. Based on these facts, this research is designed to correlate antidiabetic activity, antioxidant and computational studies to support the in vivo studies.

MATERIALS AND METHODS

Plant Material Preparation

Aloe vera extract

Aloe vera solid translucent gel in the center of the leaf was collected and homogenized resulting mucilaginous, thick and straw-colored homogenate was obtained then filtered with filter paper to avoid fibrous particles in gel. *Aloe vera* gel was extracted freshly every day before dosing (300

mg/kg body weight) throughout the experimental period and administered to rats daily by oral gavage.

Mango pulp

Mango pulp was purchased from the Srini food park Pvt. Ltd, Chittoor, Bangarupalem. Mango pulp was stored in refrigerator, 5 gms of mango pulp was mixed in 10ml of distilled water daily and administrated orally (300 mg/kg BW**)** to the rats.

Chemicals

Chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and plastics from Nunclon (Roskilde, Denmark).

Animals

Male Wistar albino rats 48 in number weighing around 180 ± 20g of 3 months old obtained from animal house of Bangalore were used for this study. Animals were group housed in clean polypropylene cages contains 6 rats/cage, they were maintained at a room temperature of 22 ± 30C, humidity of 30-70% with 12h light/dark cycle and feed with standard rat pellet supplied by Hindustan Lever Ltd., Bangalore, India and water was supplied ad libitum through plastic bottle provided with nipples. Animals were identified with tail marking using permanent marker.

This study was carried out according to guidelines for the care and use of laboratory animals and approved by the Institutional Animal Ethical Committee at Sri Venkateswara university, Tirupati, India

Ethics committee approval:

(No 10(i)/a/CPCSEA/IAEC//SVU/Zoo/MBR/dt.08.07.2012)

Induction of Diabetes

After fasting for 18 hours, Group IV, V, VI, VII, and VIII rats were injected intraperitonially with a single dose of Streptozotocin (STZ) (40 mg/kg), which is prepared freshly by dissolving in 0.1 M cold sodium citrate buffer, (pH 4.5). After injection, they had a free access to food and water, later given 5% glucose solution to drink overnight to counter hypoglycemic shock. After 96 h of streptozotocin injection, rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment.

Experimental Design

The animals were divided into eight groups. Out of VIII groups, Group I, II, III were selected as control group and group IV, V, VI, VII and VIII as experimental groups. Group I act as control which is fed with standard diet. Group II rats were nourished with standard diet and supplemented with 300 mg/kg BW of mango pulp. Group III rats were nourished with standard diet and supplemented with 300 mg/kg BW of *Aloe vera* gel. Group IV rats were nourished with standard diet and STZ induced diabetic group. Group V rats were STZ induced diabetic group

and treated with 300 mg/kg BW of mango pulp. Group VI rats were STZ induced diabetic group and treated with 300 mg/kg BW of *Aloe vera* gel. Group VII rats were STZ induced diabetic group and treated with *Aloe vera* gel and mango pulp in 1:1 (300+300 mg/kg) ratio. Group VIII rats were STZ induced diabetic group and treated with 600 µg/kg BW of Glibenclamide (Standard drug).

The animals were sacrificed after 24 hrs of the last treatment (21 days) by cervical dislocation and the liver and pancreatic tissues were isolated. The tissues were washed with ice-cold saline, and immediately stored in deep freeze at -80^{0}C for biochemical analysis and enzymatic assays. A part of the tissue was processed for histological studies.

Oxidative enzymes assay

10% (W/V) homogenates of the liver and Pancreas tissues were prepared in 0.25 M ice cold sucrose solution and centrifuged at l000 g for 15 minutes at 4°C. The supernatant fraction was used for MDH, SDH and LDH enzyme assay.

Malate Dehydrogenase (MDH) (E.C: 1.1.1.37)

Malate dehydrogenase (L- Malate NAD+ Oxidoreductase) activity was determined by the method of **Lee and lardy (1965)**. The final volume of 2.0 mL in two separate test tubes were consists of 400 µ moles of sodium malate, 1.0 mL of phosphate buffer (pH 7.4), 100 µ moles of NAD, and 300 µ moles of INT. The reaction was initiated by the addition of 0.2 ml of Liver and Pancreas tissue enzyme sources were added individually to each tube and incubated for 30 minutes at 37°C. The reaction was stopped by the addition of 5 mL of glacial acetic acid. The formazan formed was extracted over night by adding 5 mL of toluene to each tube and kept in refrigerator at 5^{0}C, the final colour was read at 495 nm in a Spectrophotometer. The enzyme activity was expressed in µ moles of formazan formed / mg protein / minute.

Succinate dehydrogenase (SDH) (E.C: 1.3.99.1)

The Spectrophotometerical analysis of SDH (Succinate acceptor oxidoreductase**)** activity was measured at 495 nm according to the method of **Nachlas *et. al.*, (1960)** as modified by **Prameelamma and Swami (1975)** with little changes and expressed in µ moles of formazone formed/mg protein/min. The reaction mixture was prepared separately in two test tubes contained 2 mL possess 400 µ moles of sodium Succinate, 1.0 mL of phosphate buffer (pH 7.0), 100 µ moles of NAD and 300 µ moles of INT. The subsequent steps were followed same as described for MDH analysis.

Lactate dehydrogenase (LDH) (E.C: 1.1.1.27)

According to **Nachlas *et al.*, (1960)** Lactate dehydrogenase activity **(**L-lactate: NAD+ Oxidoreductase) was measured with slight changes followed by **Prameelamma and Swami (1975)**. The reaction mixture was prepared separately in two tubes contained 2 mL of 400 µ moles of sodium lactate, 100 µ moles of NAD and 300 µ moles of INT and 1.0 mL of phosphate

buffer (pH 7.4). The subsequent steps were followed same as described for MDH. The enzyme utilizes NAD and INT was reduces to form formazan then it was extracted overnight into 5 mL of toluene. The activity noted as μ moles of formazan formed / mg protein / minute.

Histopathological Studies

A small portion of liver and Pancreas were fixed in 10% formalin for histopathological studies. Liver and Pancreas sections were taken with 5μm thick, and stained with hemotoxylin and eosin **(Culling 1974)**. Sections were observed under microscope for histopathological changes.

Statistical analysis

The data were statistically analyzed using One-way Analysis of Variance (ANOVA) followed by Dunnet's t-test and 'p' value upto <0.05 was considered significant. The data were presented as mean ± S.D. and analysis was carried out by using SPSS 16.0.1 program.

RESULTS

The results of this investigation is summarized and presented in tables. Present changes all groups comparisons were made and against control group without treatment.

Oxidative Enzymes Activity

Refer table 01 and 02 for Summarized results.

Diabetic animals showed significantly decreased activity of Lactate dehydrogenase, Succinate dehydrogenase and Malate dehydrogenase in both liver and Pancreas tissues. The activity of LDH, SDH and MDH in control group animals treated with *Aloe vera* and mango pulp shows slight increase than group I animals, except SDH activity in liver tissues with shows slight decrease without statistical significance. Diabetic animals treated with *Aloe vera* and mango pulp individually and in combination shows increase in LDH, SDH and MDH activity when compared with diabetic animals without treatment. the activity of LDH, SDH and MDH is slightly higher in animals treated with *Aloe vera* and mango pulp in combination when compared with individually treated diabetic animals and also effect is comparable with the standard drug.

Table 01: The levels of Oxidative enzymes in liver tissue of Control and Experimental rats

Parameter		Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
LDH (μ moles of formazon formed/ mg protein /min)	Mean	4.14	4.16	4.19	4.88	4.22	4.29	4.18	4.36
	SD	±0.42	±0.43	±0.41	±0.31	±0.33	±0.32	±0.38	±0.35
	% Change	-	0.48	1.21	17.87	1.93	3.62	0.97	5.31
	-	-	NS	NS	P 0.01	NS	NS	NS	NS

SDH (μ moles of formazon formed/ mg protein / min)	Mean	3.59	3.52	3.55	2.45	3.44	2.99	3.49	3.28
	SD	±0.46	±0.41	±0.42	±0.28	±0.39	±0.26	±0.44	±0.318
	% Change	-	-1.95	-1.11	-31.75	-4.18	-16.71	-2.79	-8.64
	-	-	NS	NS	P<0.001	NS	P <0.05	NS	NS
MDH μ moles of formazan formed / mg protein / minute	Mean	9.12	9.12	9.12	7.83	8.94	8.73	9.04	9.35
	SD	±0.87	±0.90	±0.91	±0.80	±0.83	±0.71	±0.89	±0.86
	% Change	-	0.01	0.04	-14.14	-1.97	-4.28	-0.88	2.52
	-	-	NS	NS	P<0.001	NS	NS	NS	NS

Values are mean, ± S.D. of 6 individual rats

NS means No significant change. P<0.05, P <0.001 statistically significant

Table 02: The levels of Oxidative enzymes in Pancreas tissue of Control and Experimental rats

Parameter		Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
LDH (μ moles of formazan formed/ mg protein /min)	Mean	0.80	0.81	0.83	1.65	0.97	1.04	0.86	0.92
	SD	±0.008	±0.008	±0.008	±0.016	±0.092	±0.102	±0.008	±0.084
	% Change	-	1.25	3.75	106.25	21.25	30.00	7.50	15.13
	--	--	NS	NS	P<0.001	P<0.001	P<0.001	NS	P<0.05
SDH (μ moles of formazan formed/ mg protein / min)	Mean	0.610	0.612	0.613	0.480	0.576	0.567	0.593	0.621
	SD	±0.063	±0.061	±0.065	±0.043	±0.052	±0.053	±0.055	±0.058
	% Change	-	0.33	0.49	-21.31	-5.57	-7.05	-2.79	1.80
	--	--	NS	NS	P<0.001	NS	NS	NS	NS
MDH μ moles of formazan formed /mg protein / minute	Mean	8.025	8.027	8.029	6.2	7.86	7.92	8.024	8.22
	SD	±0.82	±0.83	±0.87	±0.63	±0.72	±0.91	±0.80	±0.074
	% Change	-	0.02	0.05	-22.74	-2.06	-1.31	-0.01	2.43

	--	--	NS	NS	P<0.001	NS	NS	NS	NS

Values are mean, ± S.D. of 6 individual rats

NS means No significant change. P<0.05, P <0.001 statistically significant

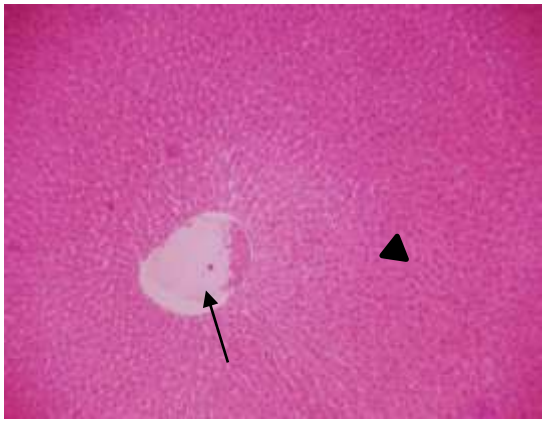
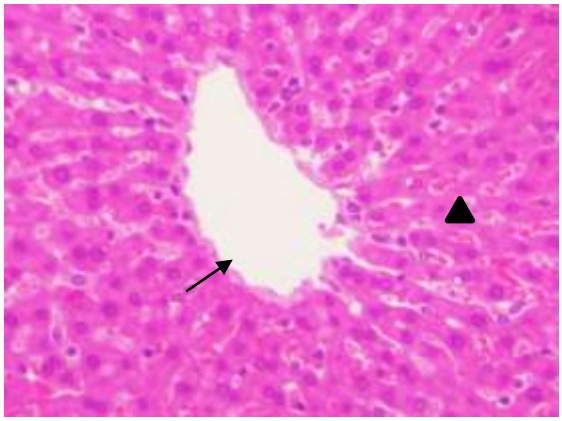
3.2 Histopathology Results

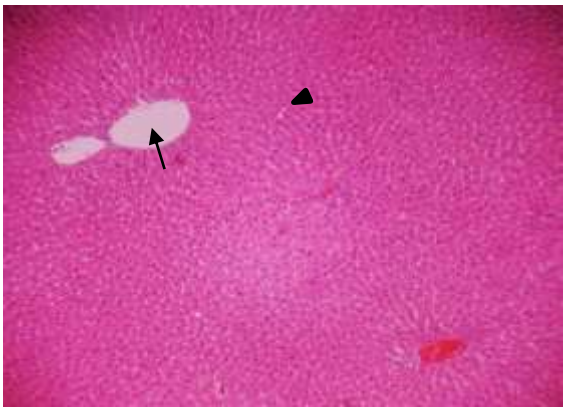
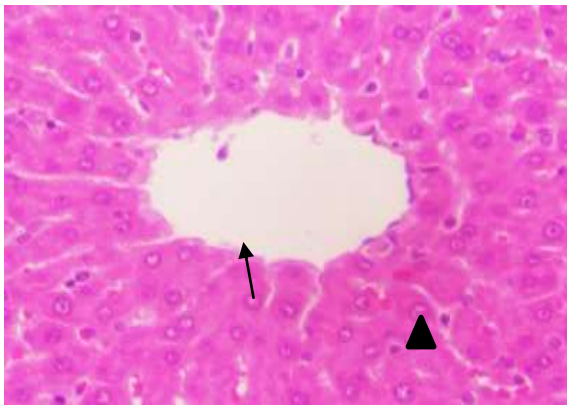
Present study shows histopathology of rat Pancreas was appeared in Fig. 2. The light microscopic appearance of Pancreas sections of control rats revealed the normal appearance of islets of Langerhans and each islet is separated by the surrounding acinar alveoli by a thin layer of reticular tissue. The islets appeared lightly stained than the surrounding acinar cells. The acinar cells are formed of pyramidal cells with basal nuclei and apical acidophilic cytoplasm (Fig. 2A). Similar findings were observed in group-II (Fig. 2B) and group-III (Fig. 2C) rats. However, the group-IV (Fig. 2D) Diabetic induced rats showed pathological changes of both exocrine and endocrine components. The acinar cells were swollen and small vacuoles were observed in most of the acinar cells. Interlobular ducts were lined with flattened epithelium. Islet β-cells are almost entirely lost and atrophied in STZ treated rats (Fig. 2D). On the other hand, group-V (Fig. 2E), VI (Fig. 2F), VII (Fig. 2G) and Group-VIII (Fig. 2H) rats depicted evidence of cellular regeneration among the islets of Langerhans. Comparatively, the experimental groups treated with *Aloe vera* and mango pulp rats showed better improvement in cellular architecture. Atrophic change of the acinar cells was less severe and the border between exocrine and endocrine portions became more distinct showing amelioration of STZ induced diabetic changes in the pancreas.

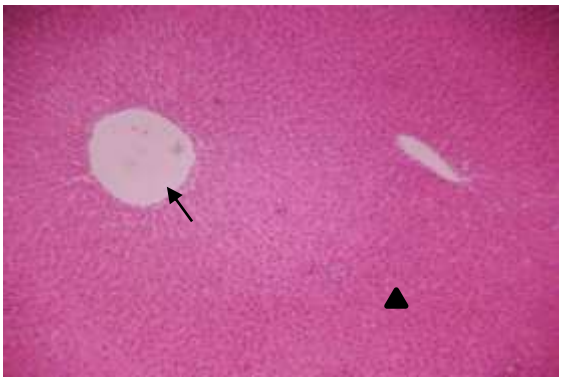
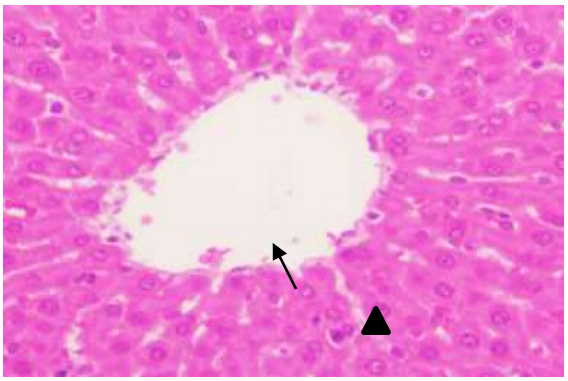
The light microscopic analysis by specific staining (hematoxylin and Eosin) of liver in normal tissues section shows classical hepatic lobules; each was formed of cords of hepatocytes radiating from the central vein to the periphery of the lobule. The cell cords were separated by narrow blood Sinusoids (S). Hepatocytes (H) were in concentric arrangement around the central vein (CV). The cells are large in size with more or less centrally placed prominent nucleus (N). The hepatic cells are hexagonal in nature. (Figs. 1A). In group II (Figs. 1B) and group III (Figs. 1C) the liver tissue exhibited the similar hepatocellular morphology as that of control rats. In group-IV (Figs. 1D), rats with STZ-induced diabetes exhibited hepatic tissue abnormalities, including distorted cell arrangement around the central vein and degenerative changes (DGE) in hepatocytes. Periportal necrosis of hepatocytes near the portal areas was identified, along with dilated and congested portal vessels (C). The capillaries displayed enlargement, and the walls of veins and capillaries appeared thickened. The enlargement of capillaries resulted in the formation of spaces between hepatocytes or widening of sinusoidal spaces (WSS) during the early stages of experimental diabetes. Additionally, inflammation was observed in the diabetic liver, with a notable accumulation of lymphocytes and macrophages near the capillary. In group V (Figs. 1E), VI (Figs. 1F), VII (Figs. 1G), and VIII (Figs. 1H), where the rats were diabetic and treated with Mango pulp, *Aloe vera*, *Aloe vera* + mango pulp, and Glibenclamide respectively, the hepatic cells displayed a concentric arrangement around the central vein, similar to the control rats.

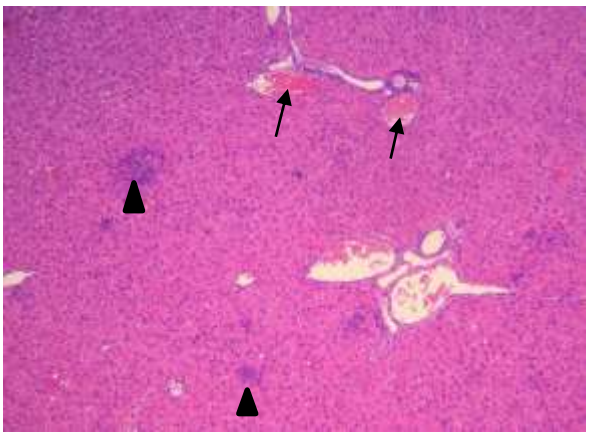
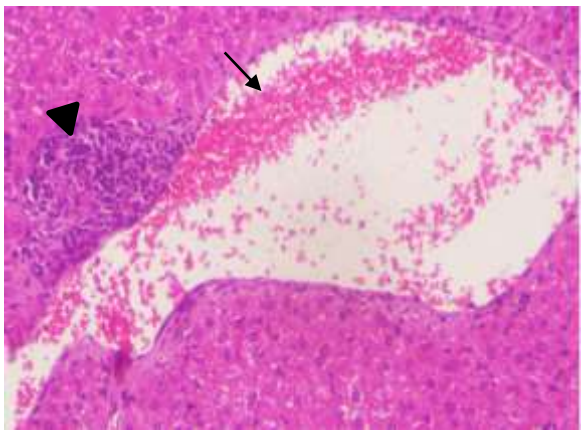
Inflammatory alterations of veins and capillaries were no longer observed, and the recovery of sinusoidal spaces (RSS) was evident. The infiltration of lymphocytes and macrophages near the capillary was minimized in this group. It was found that the reversal of tissue injury was more pronounced in the groups treated with the combined extracts.

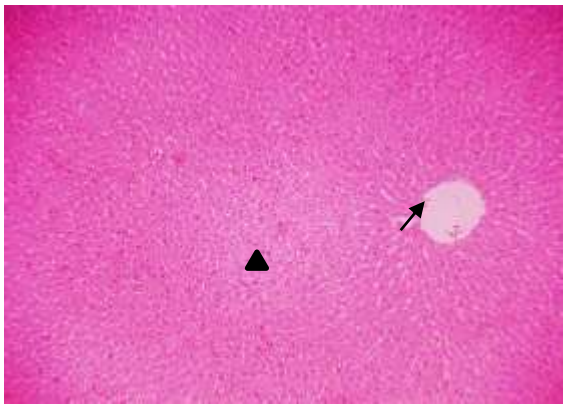
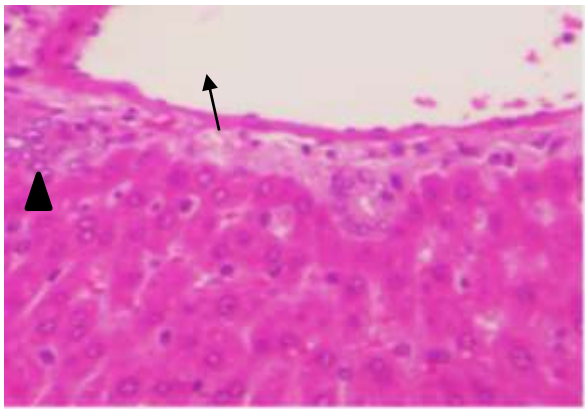
Fig. 1: LIVER:

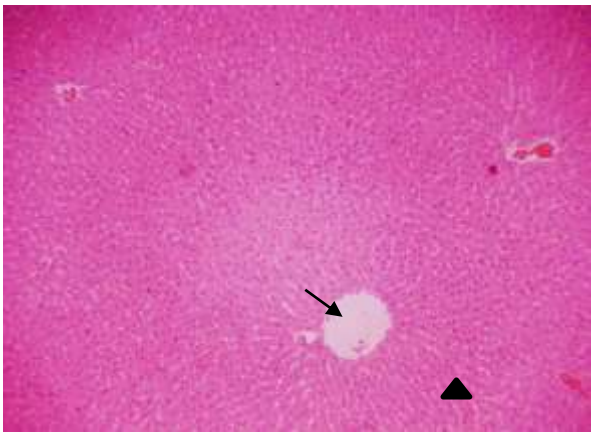
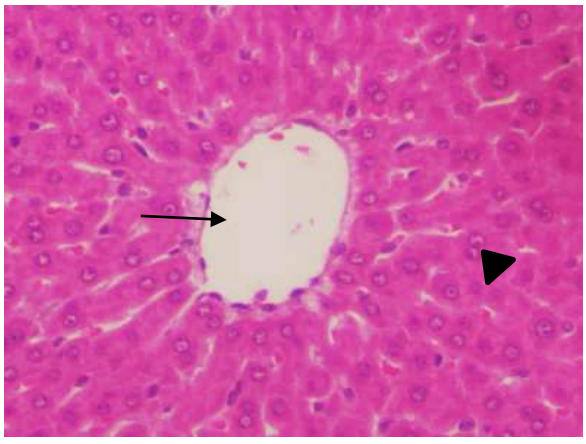
1A. Control (Group I)	
10X	**40X**
	
Control Liver showing normal central vein (arrow) and normal liver cells (arrowhead)	Control Liver showing normal central vein (arrow) and normal liver cells (arrowhead)

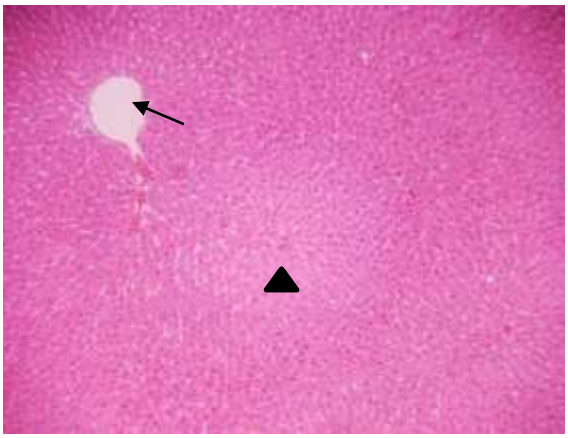
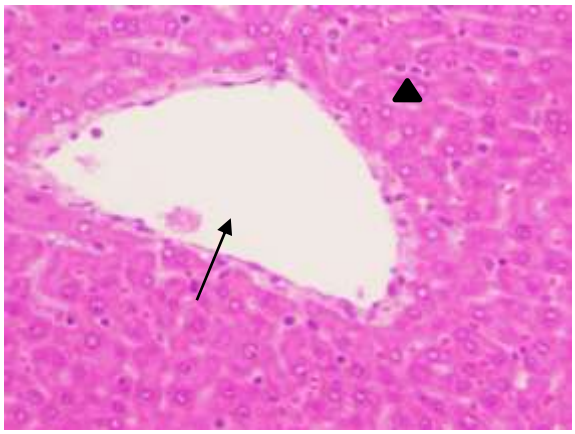
1B. Mango pulp (Group II)	
10X	**40X**
	
Rats treated with Mango pulp- Liver showing normal central vein (arrow) and normal liver cells (arrowhead)	Rats treated with Mango pulp- Liver showing normal central vein (arrow) and normal liver cells (arrowhead)

1C. *Aloe vera* (Group III)	
10X	**40X**
	
Rats treated with *Aloe vera*- liver showing normal central vein (arrow) and normal liver cells (arrowhead)	Rats treated with *Aloe vera*- liver showing normal central vein (arrow) and normal liver cells (arrowhead)

1D. Diabetic Control (Group IV)	
10X	**40X**
	
Diabetic control rats- Liver showing central vein with RBC (arrows), disruption of hepatic cords, and degenerative/necrotic changes (DGE) with mononuclear cell infiltration (arrowheads)	Diabetic control rats- Liver showing central vein with RBC (arrow), disruption of hepatic cords, and degenerative /necrotic changes (DGE) with mononuclear cell infiltration (arrowhead).

1E. Diabetic + Mango Pulp (Group V)	
10X	**40X**
	
Diabetic Rats Treated with *Aloe vera* – Liver showing normal central vein (arrow) and regeneration of liver cells to normalcy (arrowhead)	Diabetic Rats Treated with *Aloe vera* – Liver showing normal central vein (arrow) and regeneration of liver cells to normalcy (arrowhead)

1F. Diabetic + Aloe vera (Group VI)	
10X	**40X**
	
Diabetic Rats Treated with Mango Pulp – Liver showing normal central vein (arrow) and regeneration of liver cells to normalcy (arrowhead)	Diabetic Rats Treated with Mango Pulp – Liver showing normal central vein (arrow) and regeneration of liver cells to normalcy (arrowhead)

1G. Diabetic +*Aloe vera* + Mango pulp (Group VII)	
10X	**40X**
	
Diabetic Rats Treated with *Aloe vera* and Mango Pulp – Liver showing normal central vein (arrow) and regeneration of liver cells (arrowhead)	Diabetic Rats Treated with *Aloe vera* and Mango Pulp – Liver showing normal central vein (arrow) and regeneration of liver cells (arrowhead)

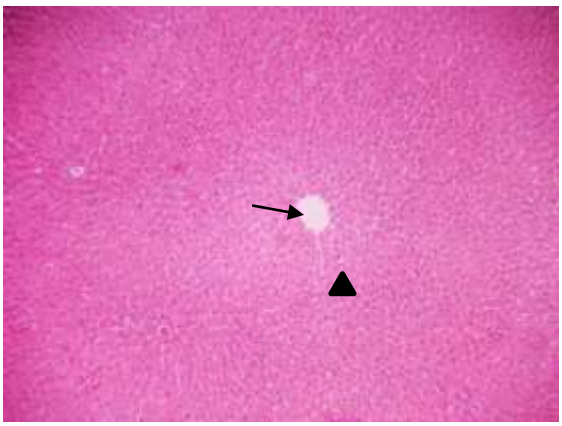
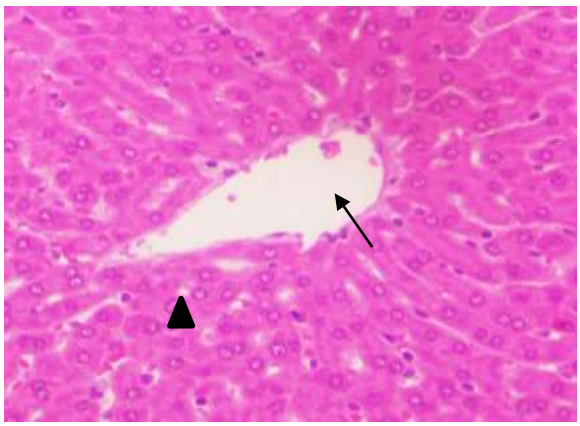
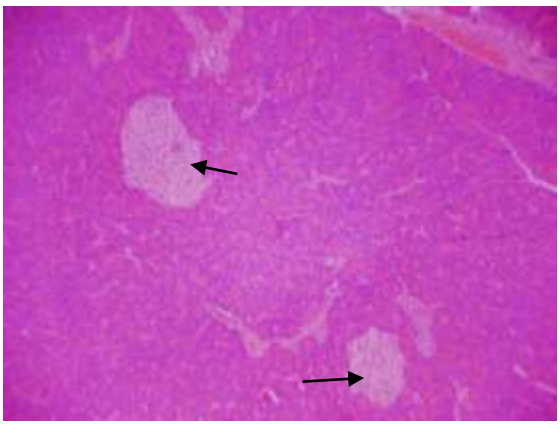
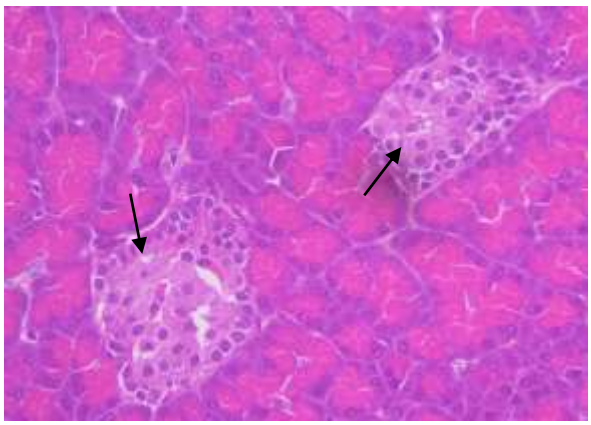
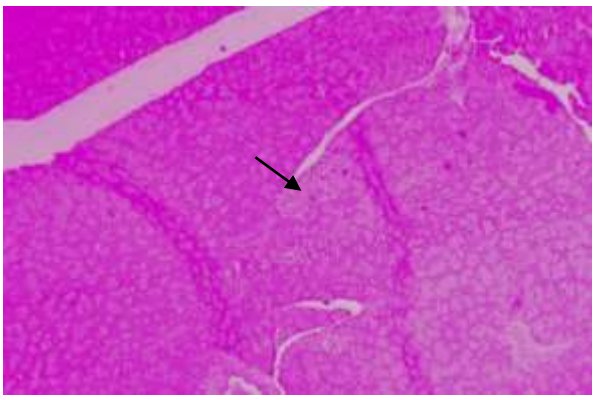
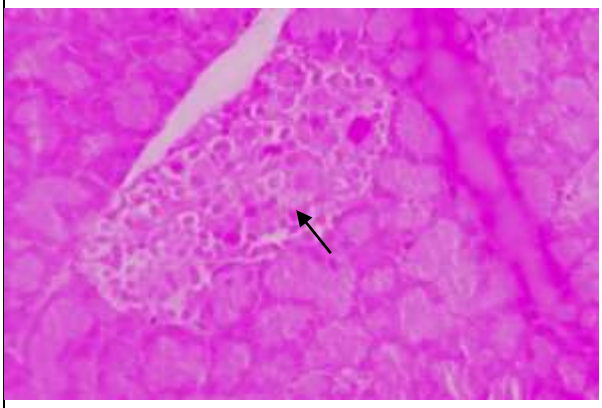
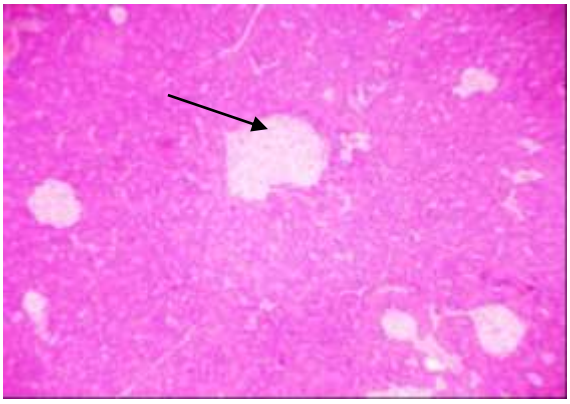
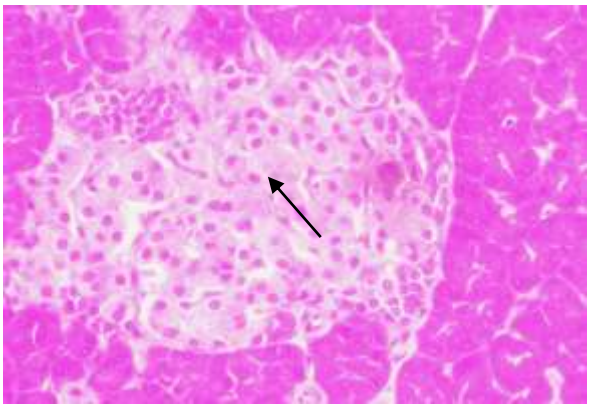
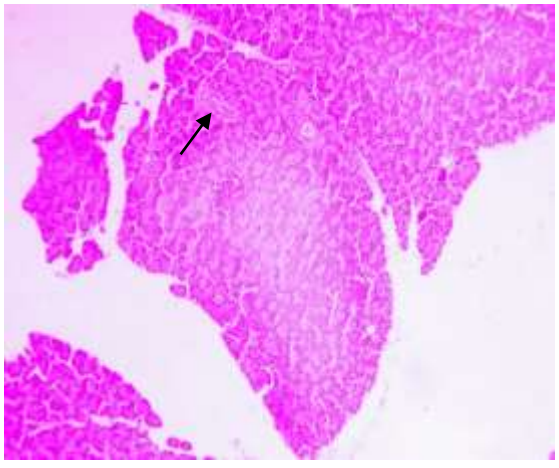
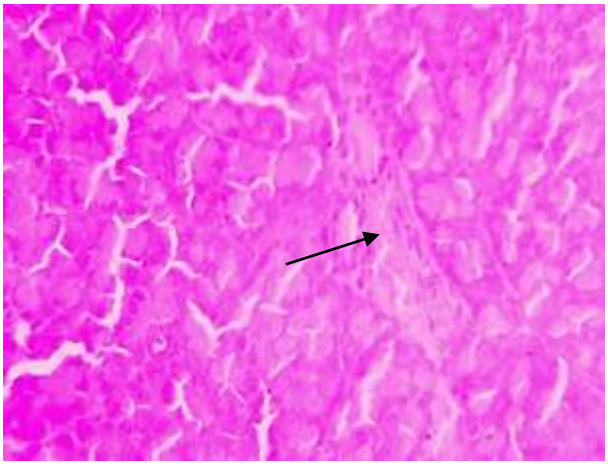
1H. Diabetic + Glibenclamide (Group VIII)	
10X	**40X**
	
Diabetic Rats Treated with Glibenclamide – Liver showing normal central vein (arrow) and normal liver cells (arrowhead)	Diabetic Rats Treated with Glibenclamide – Liver showing normal central vein (arrow) and normal liver cells (arrowhead)

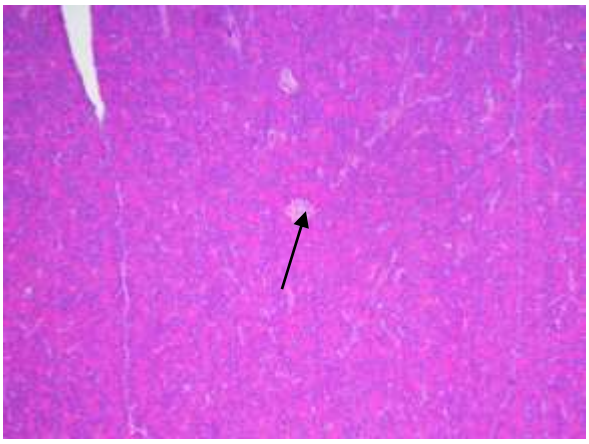
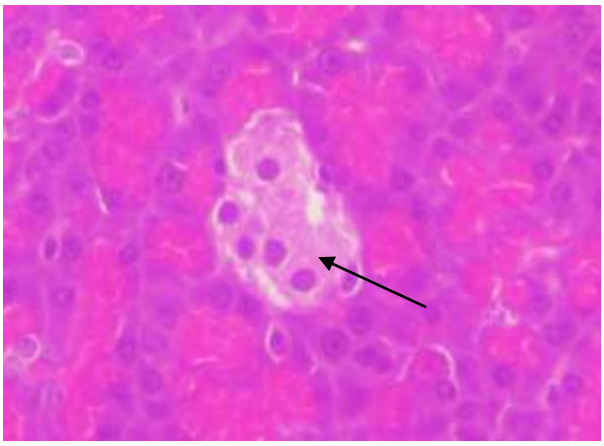
Fig. 2: PANCREAS: (10X and 40X)

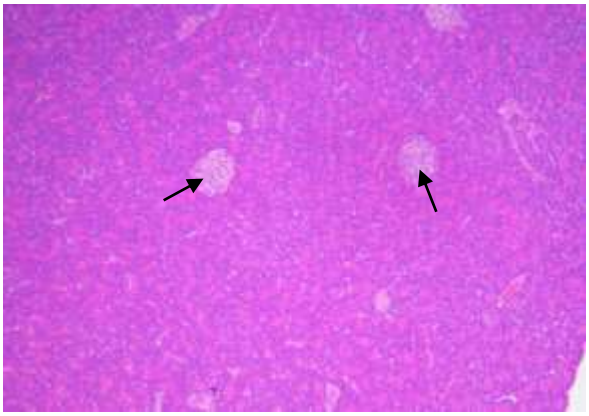
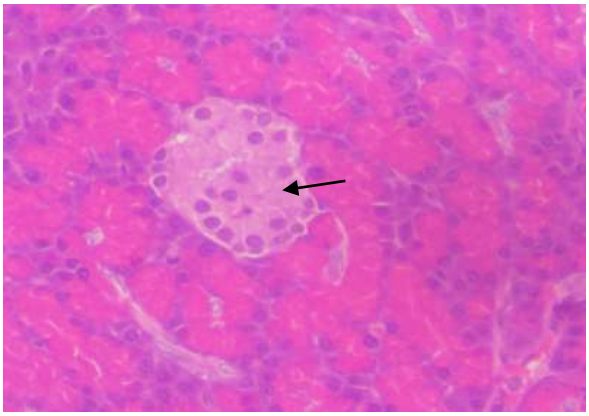
2A. Control (Group I)	
10X	**40X**
	
Control Pancreas showing normal Islets of Langerhans (arrows)	Control Pancreas showing normal Islets of Langerhans (arrows)

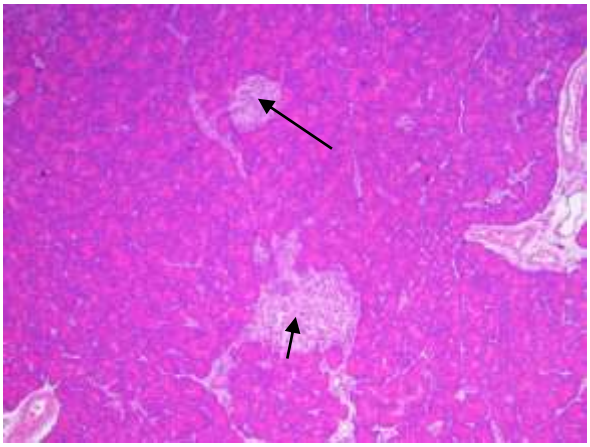
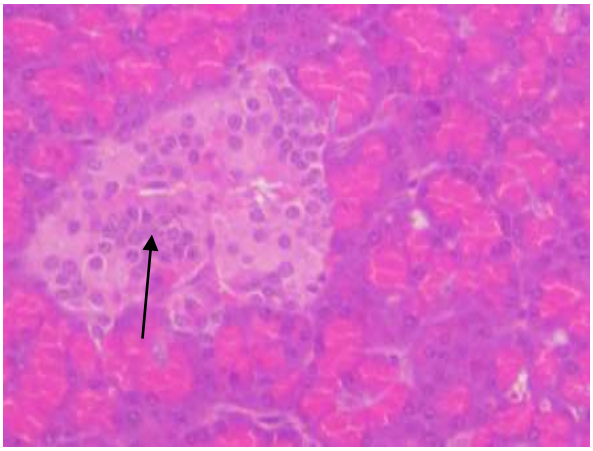
2B. Mango pulp (Group II)	
10X	**40X**
	
Rats treated with mango pulp showing normal Islets of Langerhans (arrow)	Rats treated with mango pulp showing normal Islets of Langerhans (arrow)

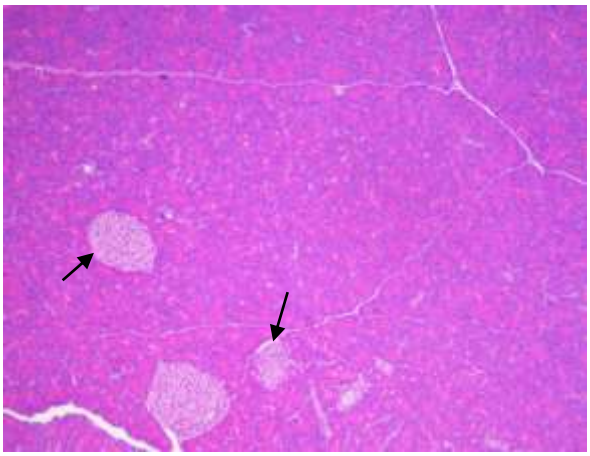
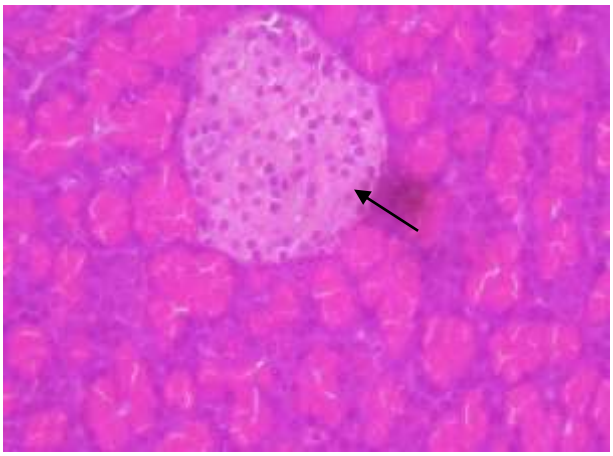
2C. *Aloe vera* (Group III)	
10X	**40X**
	
Rats treated with *Aloe vera* showing normal Islets of Langerhans (arrow)	Rats treated with *Aloe vera* showing normal Islets of Langerhans (arrow)

2D. Diabetic Control (Group IV)	
10X	**40X**
	
Diabetic control rats showing distorted histology and reduced/destroyed islets of Langerhans (arrow)	Diabetic control rats showing distorted histology and reduced/destroyed islets of Langerhans (arrow)

2E. Diabetic + Mango pulp (Group V)	
10X	**40X**
	
Diabetic control rats treated with mango pulp showing regeneration of islets of Langerhans (arrow)	Diabetic control rats treated with mango pulp showing regeneration of islets of Langerhans (arrow)

2F. Diabetic +*Aloe vera* (Group VI)	
10X	**40X**
	
Diabetic control rats treated with *Aloe vera* showing regeneration of islets of Langerhans (arrow)	Diabetic control rats treated with *Aloe vera* showing regeneration of islets of Langerhans (arrow)

2G. Diabetic +*Aloe vera* + Mango pulp (Group VII)	
10X	**40X**
	
Diabetic control rats treated with *Aloe vera* and mango pulp showing regeneration of islets of Langerhans (arrows)	Diabetic control rats treated with *Aloe vera* and mango pulp showing regeneration of islets of Langerhans (arrow)

2H. Diabetic + Glibenclamide (Group VIII)	
10X	**40X**
	
Diabetic control rats treated with glibenclamide showing normal histology of islets of Langerhans (arrows)	Diabetic control rats treated with *Aloe vera* and mango pulp showing normal histology of islets of Langerhans (arrow)

DISCUSSION

The findings of the current study indicate that liver LDH (lactate dehydrogenase) activity was significantly higher in diabetic rats compared to control rats **(Zappacosta *et al.*, 1995)**. In the context of diabetes, alloxan-induced diabetes can lead to tissue damage in the liver, kidney, and heart, mediated by lipid peroxides **(Anuradha and Selvam, 1993)**. These changes can alter cellular properties and functions, potentially resulting in an increased synthesis of certain enzymes, including LDH. Additionally, studies by **Goldberg *et al.*, (1977)** have shown that LDH activity is higher in patients with diabetes compared to normal subjects. The observed increase in LDH activity in diabetes could be attributed to the excessive accumulation of pyruvate. Due to the limited availability of insulin in diabetes, excess pyruvate is converted to lactate, a process requiring LDH enzyme. This may contribute to the elevation in LDH activity **(Chang and Schneider, 1972)**. It is clear from the results that oxidative stress increase LDH activity in diabetic rats. Our results are in agreement with Existing research evidences, where SDH and MDH activity was significantly decreased in both liver and pancreatic tissues of diabetic rats. Multiple lines of research evidence state that SDH activity was inhibited in tissues of diabetic animals in several studies. Diabetic rats on treatment with Mango pulp and *Aloe vera* gel showed elevated levels of SDH activity compared to diabetic rats in both liver and Pancreas. The increase in SDH activity may be due to the varied phytoconstituents present in the Mango pulp and *Aloe vera* gel. There are many reports on the reduction of oxidative stress by Mango pulp and *Aloe vera* gel the active constituents in the extract has the capacity in normalizing the levels of lipid peroxidation.

In our findings histological examination of the pancreas of Diabetic control rats showed a complete destruction of pancreatic islet (Fig. 2D). The acinar cells were swollen and small vacuoles were observed in almost all acinar cells and the histological examination of liver of diabetic rats showed periportal necrosis of hepatocytes near the portal areas with dilated and congested portal vessels as well as areas of inflammatory cell infiltration. (Fig. 1D). The imbalances observed in oxidative and antioxidative enzymes contribute to the decline in β cell function in animal models, as decreased levels of antioxidative enzymes underscore the compromised pancreatic β-cell functionality **(Panigrahy *et al.*, 2017).** The streptozotocin can cause damage to the plasma membrane of beta cells, resulting in morphological and permeability alterations similar to those observed following the administration of STZ. **(Thaete *et al.*, 1985; Sumoski *et al.*, 1989).** In our previous studies we observed alterations of antioxidative and oxidative enzymes in liver and Pancreas of diabetic control (group-IV) rats **(Sravani *et al.*, 2023).** These circumstances known to be receded the islet cells of diabetic animals as β cells and hepatocyte degeneration were considered to be low levels of antioxidant defines and sensitive to oxidative stress **(Muruganandan *et al.*, 2005; Liu *et al.*, 2013).** One particular interesting finding was that in group – V, group-VI experimental rats have mild regeneration of cells were observed (Fig. 1E and 1F). Group-VII and Group-VIII rats had extensively increased the size of

hepatocytes and density of dispersed islet tissue (Fig. 1G and 1H). The observed regeneration of liver and pancreatic cells in this context is consistent with previous findings that underscore the significance of targeting both liver cells and pancreatic β-cells as highly promising strategies for diabetes treatment. **(Enghild *et al.*, 1999).**

CONCLUSION

From the above results we conclude that STZ induced diabetes result in increases LDH and lipid peroxidation and decrease in SDH and MDH activity levels under diseased conditions that can be reverted on treatment with mango pulp and *Aloe vera* gel individually and in combination. The protective effect of the extracts against the changes in LDH, SDH, MDH and development of histological changes in both pancreatic and liver tissues is may be due to antioxidant properties of the phytochemicals present in mango pulp and *Aloe vera* gel. The result concludes that mango pulp and *Aloe vera* gel extract would be the effective against STZ induced diabetes.

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