

Investigation of the Synergistic Hypoglycemic Effect of Fermented Aloe vera and Amla Extract in STZ-Induced Experimental Diabetes**Megha Agarwal¹, Divya Singh², Ashutosh Sharma³, Aziz Ahmed⁴**¹Research Scholar, Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, Rajasthan, India²Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, Rajasthan, India³Department of Pharmaceutics, Jaipur College of Pharmacy, Jaipur, Rajasthan, India⁴Department of Pharmacognosy, Jaipur College of Pharmacy, Jaipur, Rajasthan, India

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Corresponding author: Megha Agarwal

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Abstract

The present study was undertaken to evaluate the antidiabetic and protective activity of fermented combined extract of Aloe vera and Amla in streptozotocin (STZ)-induced diabetic Wistar albino rats. Aloe vera leaves and Amla fruits were collected, authenticated, processed, combined, and fermented before pharmacological evaluation. Preliminary phytochemical screening revealed the presence of important bioactive constituents including flavonoids, phenolic compounds, tannins, saponins, glycosides and terpenoids. The acute toxicity study indicated that the fermented combined extract was safe at the selected doses. Diabetes was induced by STZ, and the animals were divided into six groups: normal control, diabetic control, standard drug-treated, and fermented combined extract-treated groups receiving 100, 200 and 400 mg/kg body weight, respectively. The treatment was evaluated by measuring fasting blood glucose, body weight, lipid profile, liver function markers, kidney function markers, antioxidant parameters, and pancreatic histopathology. The diabetic control group showed marked hyperglycemia, body weight loss, dyslipidemia, alterations in liver and kidney function markers, decreased SOD, CAT and GSH levels, increased MDA, and considerable pancreatic tissue damage. Treatment with the fermented combined extract at 100, 200 and 400 mg/kg produced progressive improvement in these parameters. The 400 mg/kg dose showed the maximum improvement, with marked reduction in fasting blood glucose, total cholesterol, triglycerides, LDL and VLDL, together with improvement in HDL and body weight. The treatment also improved liver and kidney function and restored antioxidant status by increasing SOD, CAT and GSH while reducing MDA. Histopathological examination showed dose-dependent restoration of pancreatic architecture, with the 400 mg/kg group demonstrating marked preservation of islet structure and reduced degenerative changes. In conclusion, the fermented combined extract of Aloe vera and Amla demonstrated promising antidiabetic, antihyperlipidemic, antioxidant, hepatoprotective, nephroprotective and pancreatic protective activities in STZ-induced diabetic rats. The 400 mg/kg dose produced the most pronounced protective effect among the tested extract doses. Further studies are required to establish its molecular mechanisms, long-term safety, and clinical applicability.

Keywords: Aloe vera, Amla, Fermentation, Hypoglycemic activity, Streptozotocin, Synergistic effect, Diabetes mellitus.

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Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most common non-communicable diseases worldwide and is associated with disturbances in carbohydrate, lipid, and protein metabolism. Prolonged elevation of blood glucose levels leads to serious damage to various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels. Diabetes has become a major public health challenge because of

its increasing prevalence, chronic nature, and associated complications.

Plant Profile

Aloe vera: Aloe vera is a well-known succulent medicinal plant widely cultivated in tropical, subtropical, and arid regions of the world. Although it is extensively cultivated and naturalized in many countries, Kew's Plants of the World Online lists its accepted name as Aloe vera

(L.) Burm.f. and notes its native range as northern Oman in the Hajar Mountains.

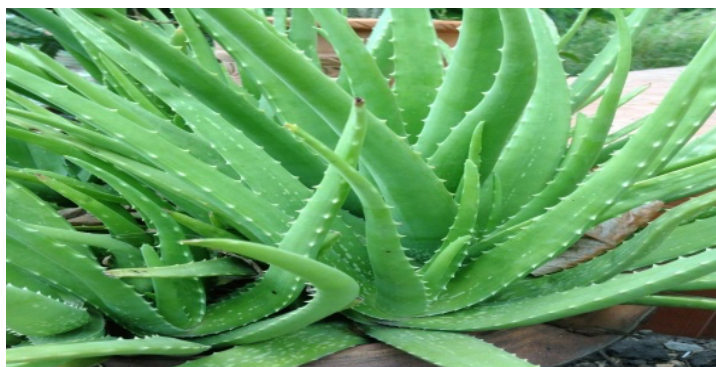


Figure 1: Plant Profile of Aloe Vera

Medicinal Uses of Aloe vera: Aloe vera has been used for centuries in traditional and modern medicine for a variety of therapeutic purposes.

The major medicinal uses include:

Wound healing activity: The gel of Aloe vera is widely used for burns, cuts, abrasions, and wound healing because of its soothing, moisturizing, and tissue-repair promoting effects.

Anti-inflammatory activity: The plant possesses significant anti-inflammatory activity, which may be useful in inflammatory skin conditions and other disorders.

Antioxidant activity: The presence of phenolics, flavonoids, vitamins, and polysaccharides contributes to the antioxidant property of Aloe vera.

Gastroprotective and digestive uses:

Traditionally, Aloe vera has been used for digestive complaints, although the latex portion must be used cautiously because of its stimulant laxative effects.

Antimicrobial activity: Studies have shown that Aloe vera exhibits antibacterial, antifungal, and antiviral properties.

Cosmetic use: It is extensively used in skin creams, gels, lotions, shampoos, and cosmetic products because of its moisturizing and rejuvenating properties.

Amla: Amla, also known as Indian gooseberry, is an important medicinal and nutritional plant widely used in Ayurveda and other traditional systems of medicine. Kew's Plants of the World Online recognizes *Phyllanthus emblica* L. as the accepted name, while *Emblica officinalis* Gaertn. is a synonym.



Figure 2: Plant Profile of Amla

Medicinal Uses of Amla: Amla has been used for centuries in traditional medicine for the prevention and treatment of various disorders. The major medicinal uses include:

Antioxidant activity: Because of its high tannin and polyphenol content, Amla possesses strong antioxidant properties, which help reduce oxidative

stress and cellular damage.

Hypolipidemic activity: The fruit has been reported to improve serum lipid profile and reduce dyslipidemia, which is often associated with diabetes and cardiovascular disease.

Anti-inflammatory activity: Amla exhibits

significant anti-inflammatory activity, which may be beneficial in chronic metabolic and inflammatory disorders.

Immunomodulatory and rejuvenating activity:

In Ayurveda, Amla is considered a Rasayana drug, meaning it promotes vitality, immunity, and longevity.

Gastroprotective and hepatoprotective activity:

The fruit is also used for digestive support, liver protection, and general health maintenance.

Collection and Authentication of Plant Material:

Fresh, healthy, and mature leaves of Aloe vera and fruits of Amla (*Phyllanthus emblica*) were collected from a local herbal source or market during the study period. Foreign matter, damaged material, and infected samples were removed. The collected materials were washed thoroughly with clean water to remove dust and adhered impurities before further processing. The plant materials were authenticated by a qualified botanist or a recognized department of botany.

Preparation of Hydroalcoholic Extract

Combined extract preparation: The prepared Aloe vera gel pulp and Amla fruit juice/extract was mixed in a pre-determined proportion to obtain a combined herbal extract. The mixture was stirred thoroughly to ensure uniformity. Since both

botanicals have reported antihyperglycemic and antioxidant properties, the combined preparation was selected as the base material for fermentation and pharmacological evaluation.

Fermentation Process: The combined Aloe vera–Amla extract were subjected to microbial fermentation using a lactic acid bacterial culture. Probiotic/lactic acid bacteria such as *Lactococcus lactis* have been successfully used to ferment Aloe vera beverages and improve functional properties. The inoculated extract was incubated under controlled conditions, typically around 30–37°C for 24–48 hours, with fermentation carried out in sterile, closed containers to minimize contamination.

After completion of fermentation, the preparation was filtered through muslin cloth or filter paper to remove coarse particles and microbial residues if required.

Induction of Diabetes: Experimental diabetes was induced by a single dose of streptozotocin (STZ) prepared freshly in cold citrate buffer, usually around pH 4.5, immediately before administration. STZ is unstable in aqueous solution; therefore, fresh preparation and prompt use are important.

Experimental Design: The animals were randomly divided into six groups, with an equal number of rats in each group.

Table 1: Experimental Design (Grouping)

Group	Treatment	Dose & Route
I (Normal Group)	Normal Control	Vehicle only (food & water ad libitum) p.o.
II (Disease Group)	Diabetic Control	Streptozotocin (STZ) (50mg/kg, i.p.) Inducer
III (Standard Group)	Standard Drug	Glibenclamide (5 mg/kg, p.o.)
IV (Treatment Group)	Fermented Aloe vera –Amla extract (Low Dose)	100 mg/kg(p.o.) + Streptozotocin (STZ) (50mg/kg, i.p.)
V (Treatment Group)	Fermented Aloe vera –Amla extract (Medium Dose)	200 mg/kg(p.o.) + Streptozotocin (STZ) (50mg/kg, i.p.)
VI (Treatment Group)	Fermented Aloe vera –Amla extract (High Dose)	400 mg/kg(p.o.) + Streptozotocin (STZ) (50mg/kg, i.p.)

Parameters Studied

Fasting blood glucose: Fasting blood glucose was measured at baseline, after confirmation of diabetes, and at regular intervals during the treatment period using a glucometer or glucose oxidase-based diagnostic kit. Blood glucose reduction is the primary pharmacodynamic endpoint in STZ-induced diabetes studies.

Body weight: Body weight of each animal was recorded initially and at regular intervals throughout the study. Diabetes-induced weight loss is a common consequence of insulin deficiency and impaired carbohydrate utilization; improvement in body weight is often taken as an indicator of metabolic recovery.

Food intake and water intake: Daily or periodic food consumption and water intake were recorded for each group. Polyphagia and polydipsia are classical manifestations of experimental diabetes; therefore, changes in these parameters help assess the severity of diabetic state and the response to treatment.

Serum biochemical parameters: At the end of the treatment period, animals were fasted, anesthetized if required under ethical protocol, and blood samples were collected for serum separation. Serum was used for determination of glucose and other biochemical parameters using standard diagnostic kits and validated laboratory methods.

Lipid profile: Serum total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol,

and VLDL-cholesterol were estimated. Dyslipidemia is a common metabolic abnormality in STZ-induced diabetes, and improvement in lipid profile is considered an important supportive effect of antidiabetic therapy.

Liver function markers: Serum AST (SGOT), ALT (SGPT), and alkaline phosphatase, and where relevant total bilirubin, were estimated using standard assay kits. These parameters help evaluate hepatic involvement and any protective effect.

Kidney function markers: Serum urea, creatinine, and uric acid were estimated to evaluate renal function. Kidney function markers are commonly included in diabetic studies because persistent hyperglycemia can impair renal physiology.

Antioxidant parameters: Tissue homogenates or serum was analyzed for antioxidant markers such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) and lipid peroxidation/MDA, depending on available facilities. Oxidative stress is a major component of STZ-induced diabetic pathology, so antioxidant assays are relevant for assessing protective effects of the fermented herbal extract.

Histopathological Study: At the end of the experimental period, all animals from each group

were sacrificed under suitable anesthetic conditions, and the pancreas was carefully removed, washed with normal saline, and fixed in 10% formalin for histopathological examination. The stained sections were examined under a light microscope for evaluation of pancreatic architecture, islets of Langerhans, β -cell integrity, cellular degeneration, and other pathological changes.

Statistical Analysis: All results were expressed as Mean $\pm$ SEM for each group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's test depending on the comparison plan.

Results and Discussion

Preliminary Phytochemical Screening: Preliminary phytochemical screening of the individual extracts of Aloe vera and Amla, as well as the fermented combined extract, was carried out using standard qualitative chemical tests. The fermented combined extract showed a broader phytochemical profile, indicating that fermentation may have enhanced the availability of active constituents.

Table 2: Preliminary Phytochemical Screening of Fermented Combined Extract of Aloe vera and Amla

S. No.	Phytochemical Constituent	Test Name	Result
1	Alkaloids	Mayer's test	+
2	Glycosides	Keller–Killiani test	+
3	Flavonoids	Alkaline reagent test	+
4	Phenolic compounds	Ferric chloride test	+
5	Tannins	Ferric chloride test	+
6	Saponins	Foam test	+
7	Carbohydrates	Molisch's test	+
8	Proteins / Amino acids	Ninhydrin test	+
9	Steroids / Phytosterols	Salkowski test	+
10	Terpenoids	Liebermann–Burchard test	+
11	Anthraquinones	Borntrager's test	+
12	Vitamin C	DCPIP test	+

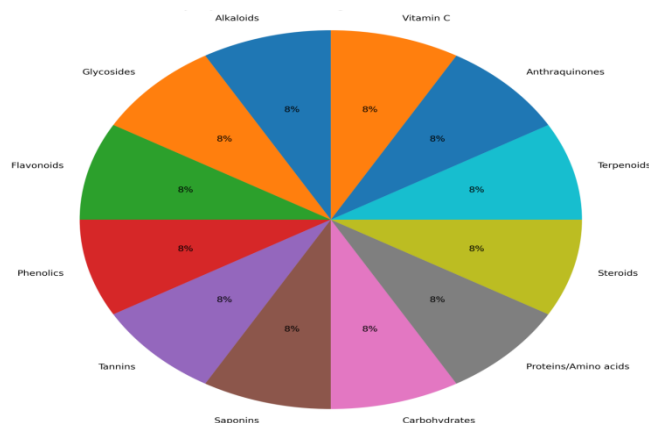


Figure 3: Preliminary Phytochemical Screening

Discussion

Preliminary phytochemical screening of the fermented combined extract of Aloe vera and Amla confirmed the presence of flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, anthraquinones, carbohydrates, proteins, and vitamin C. These phytoconstituents are widely recognized for their antioxidant, anti-inflammatory, and antidiabetic properties.

Acute Toxicity Study: An acute oral toxicity study of the fermented combined extract of Aloe vera and Amla was carried out according to the OECD Test Guideline 423 (Acute Toxic Class Method). According to OECD Guideline 423, substances may be classified based on the observed mortality

pattern across dose levels, and the guideline notes that the use of the 2000–5000 mg/kg range is discouraged for routine testing unless specifically justified.

Effect on Fasting Blood Glucose Levels: Fasting blood glucose levels were estimated at regular intervals in all experimental groups during the treatment period.

The diabetic control group showed a marked elevation in fasting blood glucose when compared with the normal control group, confirming successful induction of diabetes by streptozotocin. This finding suggests that the fermented herbal extract possesses significant hypoglycemic activity in STZ-induced diabetic animals.

Table 3: Effect of Fermented Combined Extract on Fasting Blood Glucose Levels

Group	Treatment	Fasting Blood Glucose Level (mg/dL)
Group I	Normal control	88.42 ± 2.14
Group II	Diabetic control	286.75 ± 6.32
Group III	Standard drug treated	118.36 ± 3.48
Group IV	Fermented extract low dose	198.44 ± 4.26
Group V	Fermented extract medium dose	154.28 ± 3.92
Group VI	Fermented extract high dose	126.53 ± 3.17

Values are expressed as Mean ± SEM (n = 6)

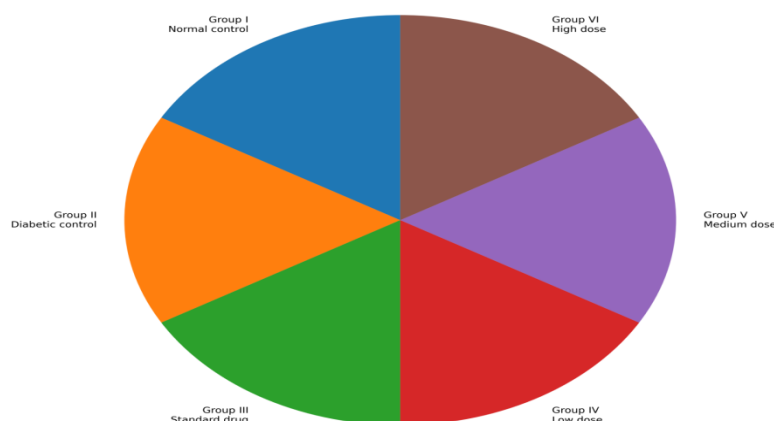


Figure 4: Effect on Fasting Blood Glucose Levels

Discussion

Treatment with the fermented combined extract significantly reduced blood glucose levels in a dose-dependent manner. The high-dose treated group showed glucose levels comparable to those of the standard drug-treated group.

This hypoglycemic effect may be attributed to enhanced insulin secretion, improved glucose utilization, inhibition of intestinal glucose

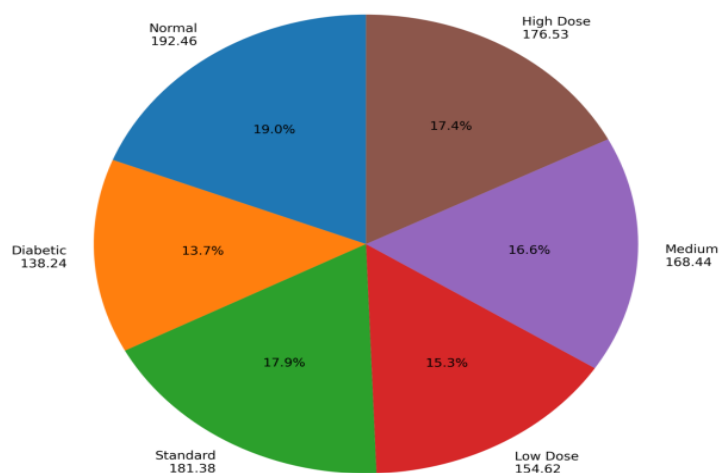
absorption, and the antioxidant activity of the phytoconstituents present in Aloe vera and Amla.

Effect on Body Weight: Body weight of all experimental animals was recorded at regular intervals during the study period. Treatment with the standard drug and the fermented combined extract of Aloe vera and Amla showed improvement in body weight when compared with the diabetic control group.

Table 4: Effect of Fermented Combined Extract on Body Weight

Group	Treatment	Body Weight (g)
Group I	Normal control	192.46 ± 3.25
Group II	Diabetic control	138.24 ± 4.12
Group III	Standard drug treated	181.38 ± 3.74
Group IV	Fermented extract low dose	154.62 ± 3.96
Group V	Fermented extract medium dose	168.44 ± 3.41
Group VI	Fermented extract high dose	176.53 ± 3.28

Values are expressed as Mean ± SEM (n = 6)

**Figure 5: Effect of Fermented Combined Extract on Body Weight**

Discussion

Administration of the fermented combined extract significantly improved body weight compared with the diabetic control group.

The improvement was dose dependent, indicating restoration of normal metabolism and better utilization of nutrients following treatment. The high-dose group demonstrated the greatest improvement.

Effect on Lipid Profile: The effect of fermented combined extract of Aloe vera and Amla on serum lipid profile in streptozotocin-induced diabetic animals. Treatment with the standard drug and the fermented combined extract significantly improved the altered lipid profile. The fermented extract reduced the elevated TC, TG, LDL, and VLDL levels and increased HDL level in a dose-dependent manner.

Table 5: Effect of Fermented Combined Extract on Lipid Profile

Group	Treatment	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Group I	Normal control	86.24 ± 2.31	78.42 ± 2.14	42.36 ± 1.28	28.20 ± 1.64	15.68 ± 0.43
Group II	Diabetic control	168.53 ± 4.26	156.44 ± 3.95	24.18 ± 1.12	113.06 ± 3.28	31.29 ± 0.79
Group III	Standard drug treated	98.42 ± 2.84	92.36 ± 2.41	38.74 ± 1.35	41.21 ± 1.96	18.47 ± 0.48
Group IV	Fermented extract low dose	142.68 ± 3.92	132.25 ± 3.16	28.64 ± 1.16	87.59 ± 2.74	26.45 ± 0.63
Group V	Fermented extract medium dose	118.36 ± 3.18	108.44 ± 2.73	33.42 ± 1.24	63.25 ± 2.18	21.69 ± 0.55
Group VI	Fermented extract high dose	104.57 ± 2.76	96.28 ± 2.34	36.86 ± 1.31	48.45 ± 2.04	19.26 ± 0.47

Values are expressed as Mean ± SEM (n = 6)

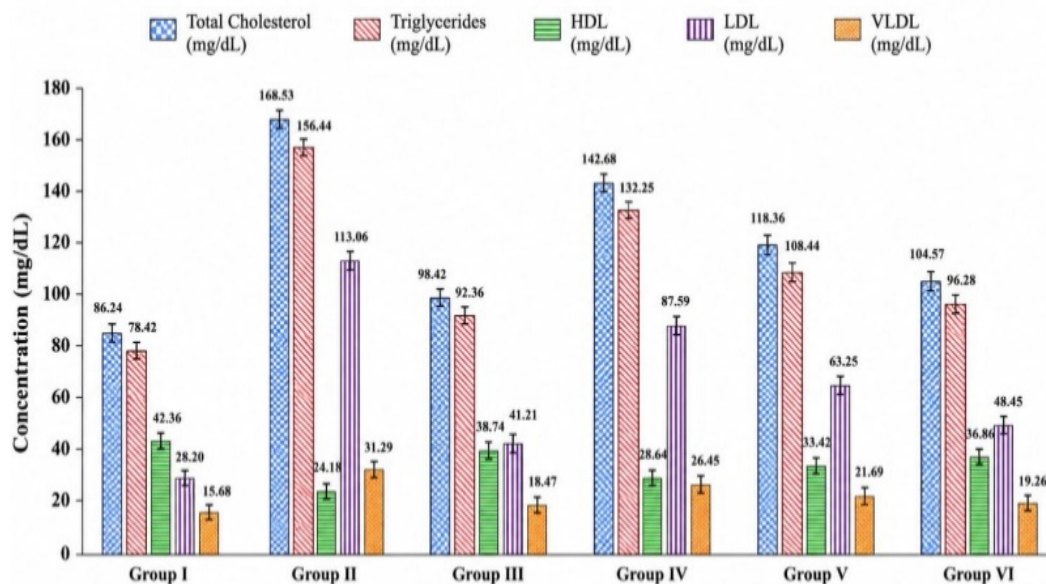


Figure 6: Effect of Fermented Combined Extract on Lipid Profile

Discussion

Diabetic animals exhibited significant dyslipidemia, characterized by elevated total cholesterol, triglycerides, LDL, and VLDL levels with decreased HDL concentration.

Treatment with the fermented combined extract significantly restored these lipid parameters.

Effect on Liver Function Tests: The effect of fermented combined extract of Aloe vera and Amla on liver function markers in streptozotocin-induced diabetic animals. The reduction in SGOT, SGPT, and ALP levels may be attributed to improved glycemic control and the antioxidant activity of phytoconstituents present in Aloe vera and Amla, such as flavonoids, phenolics, and vitamin C.

Table 6: Effect of Fermented Combined Extract on Liver Function Markers

Group	Treatment	SGOT / AST (U/L)	SGPT / ALT (U/L)	ALP (U/L)
Group I	Normal control	42.36 ± 1.54	36.24 ± 1.42	96.42 ± 2.84
Group II	Diabetic control	88.44 ± 2.68	79.36 ± 2.31	168.52 ± 4.16
Group III	Standard drug treated	50.28 ± 1.76	43.42 ± 1.58	108.36 ± 3.12
Group IV	Fermented extract low dose	74.62 ± 2.18	68.24 ± 2.04	146.48 ± 3.72
Group V	Fermented extract medium dose	61.36 ± 1.94	54.42 ± 1.76	126.25 ± 3.41
Group VI	Fermented extract high dose	53.18 ± 1.82	46.36 ± 1.63	114.52 ± 3.06

Values are expressed as Mean ± SEM (n = 6)

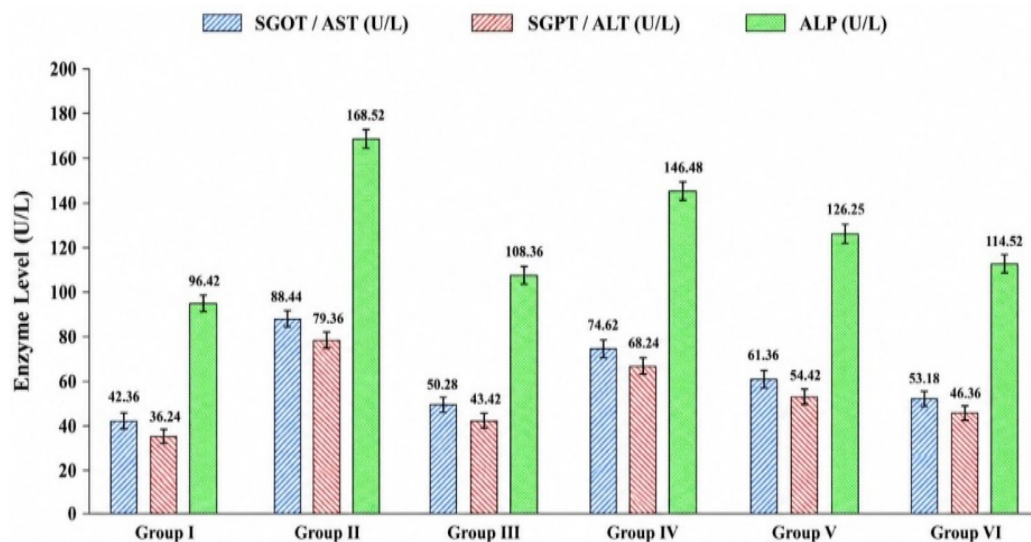


Figure 7: Effect of Fermented Combined Extract on Liver Function Markers

Discussion

Serum AST, ALT, and ALP levels were markedly elevated in diabetic animals, indicating hepatic injury caused by oxidative stress.

Administration of the fermented combined extract significantly reduced these enzyme levels, suggesting hepatoprotective activity.

Effect on Kidney Function Tests: The effect of fermented combined extract on kidney function markers is finding suggests a nephroprotective effect of the fermented formulation. The improvement in renal markers may be due to reduced hyperglycemia and oxidative damage, together with the protective activity of the phytoconstituents present in the fermented extract.

Table 7: Effect of Fermented Combined Extract on Kidney Function Markers

Group	Treatment	Urea (mg/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)
Group I	Normal control	28.42 ± 1.18	0.68 ± 0.04	2.84 ± 0.12
Group II	Diabetic control	62.36 ± 2.04	1.84 ± 0.08	6.42 ± 0.21
Group III	Standard drug treated	34.24 ± 1.36	0.86 ± 0.05	3.24 ± 0.14
Group IV	Fermented extract low dose	52.18 ± 1.82	1.52 ± 0.07	5.48 ± 0.19
Group V	Fermented extract medium dose	42.36 ± 1.54	1.12 ± 0.06	4.24 ± 0.16
Group VI	Fermented extract high dose	36.44 ± 1.42	0.94 ± 0.05	3.52 ± 0.15

Values are expressed as Mean ± SEM (n = 6)

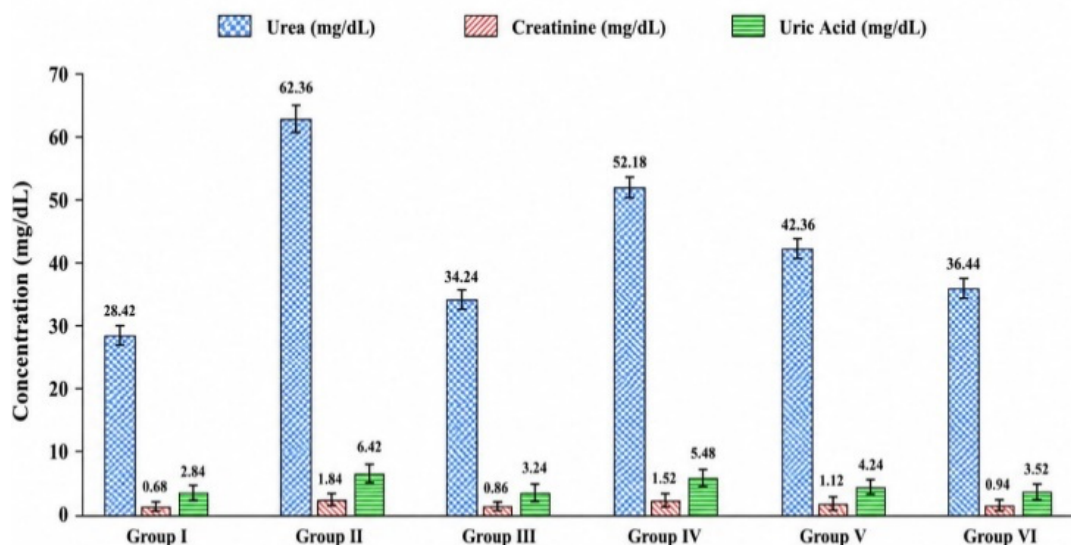


Figure 8: Effect of Fermented Combined Extract on Kidney Function Markers

The diabetic control group showed significantly increased serum creatinine, urea, blood urea nitrogen, and uric acid levels, indicating renal dysfunction.

Treatment with the fermented combined extract significantly reduced these biochemical markers, demonstrating nephroprotective activity.

Effect on Antioxidant Markers The effect of fermented combined extract on antioxidant parameters is shown these findings suggest that the fermented formulation effectively reduced oxidative stress in diabetic animals. The antioxidant effect may be attributed to the presence of phenolics, flavonoids, tannins, and vitamin C in the combined extract.

Table 8: Effect of Fermented Combined Extract on Antioxidant Markers

Group	Treatment	SOD (U/mg protein)	CAT (U/mg protein)	GSH (μ g/mg protein)	MDA (nmol/mg protein)
Group I	Normal control	8.42 $\pm$ 0.26	62.36 $\pm$ 1.84	7.48 $\pm$ 0.22	1.84 $\pm$ 0.08
Group II	Diabetic control	3.24 $\pm$ 0.14	28.42 $\pm$ 1.16	3.12 $\pm$ 0.14	5.84 $\pm$ 0.19
Group III	Standard drug treated	7.36 $\pm$ 0.24	56.28 $\pm$ 1.72	6.82 $\pm$ 0.21	2.24 $\pm$ 0.10
Group IV	Fermented extract low dose	4.68 $\pm$ 0.18	38.24 $\pm$ 1.34	4.46 $\pm$ 0.17	4.62 $\pm$ 0.16
Group V	Fermented extract medium dose	6.14 $\pm$ 0.22	48.36 $\pm$ 1.56	5.84 $\pm$ 0.19	3.28 $\pm$ 0.12
Group VI	Fermented extract high dose	6.98 $\pm$ 0.23	53.42 $\pm$ 1.68	6.46 $\pm$ 0.20	2.56 $\pm$ 0.11

Values are expressed as Mean $\pm$ SEM (n = 6)

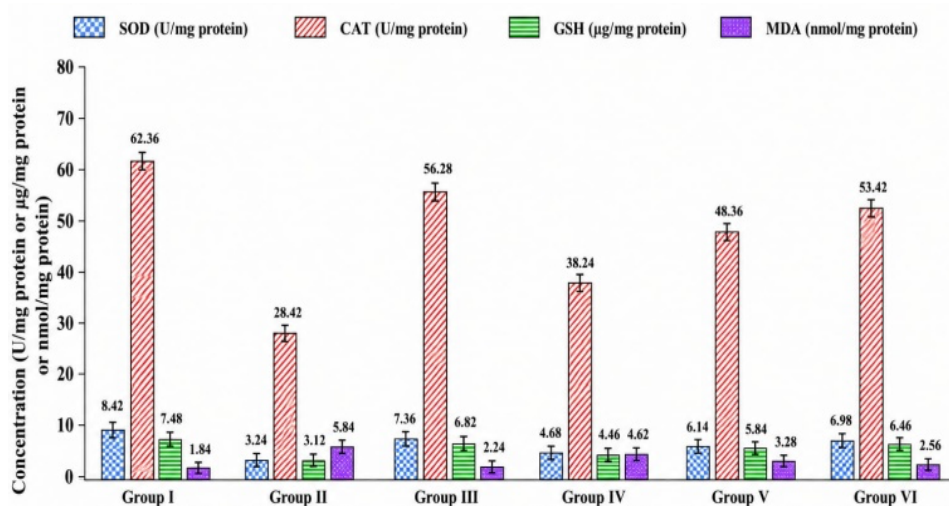


Figure 9: Effect of Fermented Combined Extract on Antioxidant Markers

Discussion

STZ-induced diabetes significantly reduced endogenous antioxidant enzymes such as SOD, CAT, and GSH while increasing malondialdehyde (MDA), indicating enhanced oxidative stress. Treatment with the fermented combined extract restored antioxidant enzyme activity and reduced lipid peroxidation.

Histopathological Study: Histopathological examination of pancreatic tissue was carried out to evaluate structural changes in the different experimental groups. These findings indicate that the fermented combined extract of Aloe vera and Amla exerted a protective effect on pancreatic tissue and may have preserved or restored β -cell integrity in diabetic animals.

Group I – Normal Control: Animals in this group received only normal vehicle and served as the normal control. Pancreatic tissues of this group were examined to observe the normal histological architecture of the pancreas.

Group II – Diabetic Control: Animals in this group were treated with streptozotocin to induce diabetes and received no treatment. Pancreatic tissues were examined to observe diabetes-induced pathological changes such as shrinkage of islets, β -cell destruction, and degeneration.

Group III – Standard Drug Treated: Animals in this group were treated with streptozotocin followed by the standard antidiabetic drug. Pancreatic tissues were examined to assess the protective and restorative effect of the standard drug on diabetic pancreatic damage.

Group IV – Fermented Extract Low Dose:

Animals in this group were treated with streptozotocin followed by a low dose of fermented Aloe vera and Amla extract.

Histopathological examination was carried out to observe the extent of protection offered by the low dose.

Group V – Fermented Extract Medium Dose:

Animals in this group were treated with streptozotocin followed by a medium dose of

fermented Aloe vera and Amla extract. Pancreatic tissues were studied to evaluate the degree of restoration in tissue architecture.

Group VI – Fermented Extract High Dose:

Animals in this group were treated with streptozotocin followed by a high dose of fermented Aloe vera and Amla extract. Histopathological examination was performed to observe the maximum protective effect of the extract on pancreatic tissue.

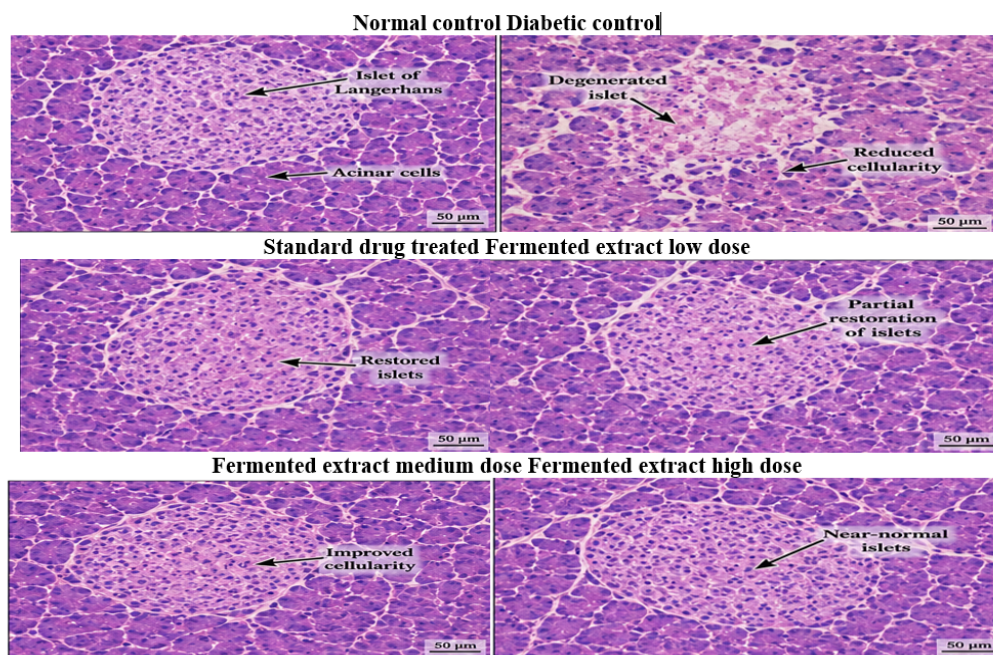


Figure 10: Microscopic Observations of Pancreatic Tissue

Conclusion

The fermented combination extract of Aloe vera and Amla (*Phyllanthus emblica*) showed promising antidiabetic and antioxidant activity in streptozotocin-induced diabetic rats. The 400 mg/kg dose produced the most pronounced improvement in biochemical, lipid, antioxidant, and pancreatic histopathological parameters. Thus, the fermented extract may have potential as a natural antidiabetic agent.

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