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Hypoglycemic, hypolipidemic and hepato-renal protective activities of *Aloe vera* and *Carica papaya* combination in STZ-diabetic rats

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Abstract: *Aloe vera* (*Av*) anti-diabetic and hypolipidemic in addition to tissue protective capabilities investigation in diabetic rats is the main purpose of our 4 weeks study; in a single form or in a combined form with *Carica papaya (Cp)*. Six rat groups were established in this study; control untreated group, *Av*-treated group (300 mg/kg), *Av*+*Cp* treated group (300 mg/kg each), diabetic group (D), D+*Av* group and D+*Av*+*Cp* group. Herein, *Av* administrations either alone or in combination with *Cp* markedly enhanced the hyperglycemic status resulting from diabetes induction; via decreasing both blood HbA1c and glucose values, while increasing serum C-peptide and insulin levels; in comparison with the diabetic group. Regarding lipid metabolism, *Av* treatment individually or with *Cp* reverted back the elevated lipid fractions in diabetic rats to near normal levels indicating its hypolipidemic capacity. Furthermore, single *Av* usage or *Av*+*Cp* mixture were found to ameliorate both liver and kidney status; as indicated through decreasing many sera liver (total bilirubin and ALT, AST and ALP) and kidney (urea, uric acid and creatinine) function markers levels in diabetic rats; compared to thein regard to diabetic untreated rats. Obtained results, also, showed that both extracts remarkably improved the diabetic-induced oxidative stress status; as reflected by pancreatic MDA and ROS decreased levels; while enhanced antioxidant defense system capacity through elevating CAT, SOD and GSH pancreatic contents. Thus, our findings clearly point out *Av*+*Cp* combination health benefits in ameliorating both metabolic and tissue diabetic complications was superior over single *Av* administration; owing to both plants marked antioxidant capability

keywords: *Aloe vera* (*Av*) – Diabetes – Hypoglycemia – Kidney functions – Liver functions – Streptozotocin (STZ).

1.Introduction

Diabetes mellitus (DM) is a metabolic syndrome which characterized by elevated blood glucose levels, marked hypoinsulinemia and hyperlipedemia; that could result in decease of millions of peoples every year. Recent WHO estimates reported 3% of global peoples were suffering from this disease, which expected to reach 6% in 2025 **[1].** Of note, over 415 million adults were estimated to have DM worldwide in 2015, according to the International Diabetes Federation (IDF)**[2];** with India in the lead **[3];** and a further 318 million were estimated to be at high risk of developing DM in the next 25 years **[4].**

Daily insulin injection has served for the past 100 years as a life-saving effective treatment for a previously incurable and usually fatal DM disease **[5].** Many current synthetic oral anti-diabetic drugs are heterogeneous in their mode of action and associated with drawbacks such as resistance and side effects ranging from abdominal discomfort, weight gain, and diarrhea to increased liver toxicity and cardiovascular risk. Thus, it is not surprising that natural products and complementary medicines are imperative and gaining increasing popularity among patients with hyperglycemia; because of their fewer side effects and affordability. In this frontier, nowadays, there is great interest in *Aloe vera* (*Av*) as a potential source of functional food supplements. It is a perennial succulent belong to the Liliaceae family; which was considered the richest natural sources for human health ever coming; has been known as

"the healing plant"; for its considerable medicinal properties, which could probably be attributed to polysaccharides and phenolic compounds contents. Reported *Av* pharmacological activities include antifungal, antiviral, antibacterial, wound healing, anti-inflammatory and antioxidant actions, in addition to marked antidiabetic capacity**[6]and [7]**.

Hence, in this article, we discuss this plant possible beneficial effects alone or in combination with *Carica papaya (Cp): another* plant known for its marked diabetic activity: for DM metabolic and tissue complications treatment.

2.Materials and methods

Chemicals

STZ was purchased from Sigma Aldrich Company. (St. Louis, Mo 6, USA).

Preparation of Aloe vera leaf pulp and Carica papaya fruits ethanolic extracts

Mature and healthy fresh *Av* leaves, over 3 years old, were washed with water, peeled and cut transversely into pieces. The thick epidermis was selectively removed and the leaf pulp (gel together with latex) was scratched with a spoon. The solid gel was then homogenized (Ultra- Turrax T25, IKA Labortechnik, Germany), and centrifuged at 10000 rpm for 30 min at 4 °C to remove the fibers, resulting in mucilaginous, thick, light green-colored homogenate**[8].** On the other hand, unripe fruits of *Cp* were cleaned with distilled water and the outer green thin layers were peeled and discarded. The underlying epicarp was blended with 50 mL of distilled water to a fine texture form using a blender, then centrifuged at 10000 rpm for 30 min at 4 °C to remove the fibers. The mixture was then filtered using a fine muslin cloth followed by rotor evaporation to remove water, resulting in mucilaginous, thick, pale yellow-colored homogenate**[9].**

Subsequently, one kg of each homogenate was mixed and extracted with 80 % ethanol (300 ml/kg) using cold method of extraction (percolation) till exhaustion. The ethanol extract was filtered, distilled and evaporated under reduced pressure. This procedure repeated 3 times, and the resultant extracts was stored in dry sterilized small containers at 4 °C until further use. Both *Av* leaves and *Cp* fruits were collected and authenticated by Plant Taxonomy staff members of Botany Department, Faculty of Science, Cairo University, Giza, Egypt.

Animals

Male albino rats (*Rattus rattus)* weighing 100-120 g were housed under conventional laboratory conditions (12 h light/12 h darkness photoperiod at 22°C); with free access to both food and water. Animals were randomly divided into six groups each of six animals. Following four days of STZ injection, rats were considered as diabetics when having over 200 mg/dl tail vein's fasting blood glucose level**[10].** Faculty of Science Ethics Committee, Mansoura University, Mansoura, Egypt, had approved this study experimental procedures.

Experimental strategy

1. **Control untreated group:** Rats were given a single dose of citrate buffer(pH4.6)administered intraperitoneally.
2. ***Aloe vera* (*Av*)-treated group:** Rats received orally *Av* ethanolic extract (300 mg/kg), once daily using gastric tube.
3. ***Av* + *Cp* treated group:** Rats received orally *Av*, then *Cp* (after 1 h) ethanolic extracts (300 mg/kg each), once daily using gastric tube.
4. **Diabetic untreated group:** STZ (45 mg/kg) diluted in citrate buffer, pH 4.6, was given to rats as an IP single injection.
5. **Diabetic *Av*-treated group:** Diabetic rats received orally *Av* ethanolic extract (300 mg/kg), once daily using gastric tube.
6. **Diabetic *Av* + *Cp* treated group:** Diabetic rats received orally *Av*, then *Cp* (after 1 h) ethanolic extracts (300 mg/kg each), once daily using gastric tube.

✓ All treatments were started immediately following confirmation of diabetes in diabetic groups and were continued for 4 weeks.

Sample's collection

Before being dissected, diethyl ether was used to anesthetize rats. Directly from the heart, blood samples were withdrawn immediately; where few droplets were collected in

heparinized tubes for glycosylated hemoglobin assessment. However, the remaining of blood samples were collected in non-heparinized tubes, then, centrifuged for 15 min at 3000 rpm, Separated sera were tagged and stored at -20 °C. Pancreas specimens were quickly separated, weighed and homogenized in distilled water forming 10% (w/v) homogenate, and kept at -20 °C.

Biochemical determinations

Serum glucose, HbA1c, lipids fractions [HDL-C, LDL-C, total cholesterol (TC), triglycerides (TG) and total lipids (TL)], total proteins, liver enzymes (ALP, ALT and AST), uric acid, urea, creatinine and total bilirubin concentrations were assessed using kits from Bio diagnostic Company, Egypt. While, both serum insulin and C-peptide were measured by using Boehringer Analyzer ES 300ELISA kits purchased from Boehringer Mannheim, Germany. However, pancreatic levels of MDA, ROS, SOD and CAT were estimated using kits from Bio Diagnostic Company, Egypt.

Statistical analysis

A SPSS 17.5 software was used to evaluate data statistically. All results were expressed as the mean ± SEM for 6 animals in each group. *P* values ≤ 0.05 were considered significant.

3.Results

Diabetic group in **table 1** illustrated a marked serum glucose and HbA1c elevations accompanied by a significant C-peptide and insulin levels decline; in regard to control. However, diabetic rats treated with either *Av* alone or *Av+Cp* mixture exhibited an obvious amelioration in these parameters regarding diabetic rats. While glucose and HbA1c levels were still significantly higher and, insulin and C-peptide levels were still significantly lower in all diabetic rats treated groups, in comparison with control; except for insulin and C-peptide levels in case of *Av+Cp* group which showed non-significant changes compared to control.

Table (1): Serum glucose, HbA1c, insulin and C-peptide levels**.**

	Control	***Av***	***Av+Cp***	**D**	**D+*Av***	**D+*Av+Cp***
Glucose (mg/100ml)	90.00± 2.08	95.50± 2.21	89.66± 3.51	407.83± 6.71 [a]	126.00± 3.69[ab]	113.00±2.51[ab]
HbA1c(%)	4.02± 0.20	4.05± 0.29	3.73± 0.14	10.68± 0.44 [a]	6.91± 0.47 [ab]	5.98± 0.25 [ab]
Insulin(µ I U/ml)	16.98± 0.34	16.18±0.66	16.81± 0.24	8.28± 0.57 [a]	15.55± 0.31 [ab]	16.12± 0.14 [b]
C-peptide(ng/ml)	0.77± 0.02	0.76 ±0.02	0.77±0.01	0.36± 0.05 [a]	0.56± 0.04 [ab]	0.70± 0.02 [b]

Values expressed as mean ± SEM (n = 6). [a] and [b] are Significant differences (P ≤ 0.05) comparing to control and diabetic groups respectively.

Lipid fractions' results in t**able 2** reported a marked elevation; except for HDL-C; in diabetic rats regarding control. All tested parameters showed a marked enhancement in diabetic rats treated with either *Av* or *Av+Cp*; when compared to untreated diabetic rats. However, most values were still significantly variable, when compared to control; except values of HDL-C, LDL-C and TG in case of *Av+Cp* treated group which showed non-significant change

Table (2): Serum lipid fractions levels.

	Control	***Av***	***Av+Cp***	**D**	**D+*Av***	**D+*Av+Cp***
TL(mgd)	281.5± 6.79	288± 9.99	294.3±5.54	495.3±23.50	365.5±11.53 [a]	339±15.21 [ab]
TG(mg/dl)	49.00± 2.58	50.17± 3.36	52.00± 2.72	133.17± 22.21 [a]	79.33± 6.96 [ab]	65.17±3.44 [b]
TC(mg/dl)	120.8± 1.87	121.2± 1.14	121.8± 2.40	345.70± 14.40	187.70± 8.27 [ab.]	168.00±10.00 [ab]
LDL-C(mg/dl)	47.66± 0.98	53.17± 1.64	50.17± 1.22	252.17± 27.34 [a]	113.67± 2.33 [ab]	73.33± 1.89 [b]
HDLC(mg/dl)	54.50± 2.77	52.30± 4.43	57.17± 2.40	26.00± 2.14 [a]	42.30± 0.95 [ab]	54.80± 1.67 [b]

Values expressed as mean ± SEM (n = 6). [a] and [b] are Significant differences (P ≤ 0.05) comparing to control and diabetic groups respectively.

T**able 3** illustrated a significant decrease in serum total proteins (TPs) level while marked increases in serum liver function markers in diabetic group; regarding to control. Both diabetic treated groups exhibited a marked

decrease in all liver function maekers while a significant TPs elevation, in regard to diabetic rats. However, values were still significantly variable in *Av* treated diabetic group, comparing to control. On the other hand, results reveal a non-significant change in all mentioned parameters in *Av+Cp* diabetic treated rats indicating that values were reverted back near to normal, compared to control rats; reflecting the hepatic enhancing effect of the mixture extract over the administration of *Av* extract alone.

Table (3): Serum liver function markers levels

	Control	***Av***	***Av+Cp***	**D**	**D+*Av***	**D+*Av+Cp***
TPs(g/dl)	7.07± 0 .10	7.02± 0 .23	7.00± 0 .11	4.06± 0.56 [a]	6.13± 0.14 [ab]	6.70± 0.11 [b]
AST(u/l)	38.50± 1.41	38.17± 1.22	37.67± 1.74	80.67± 8.09 [a]	48.167± 2.4 [ab]	44.50± 1.56 [b]
ALT(u/l)	24.50± 1.56	25.00± 1.79	25.80± 1.50	54.30± 4.02 [a]	39.00± 1.75 [ab]	30.17± 2.30 [b]
ALP(u/l)	237.3± 3.03	235.2± 4.19	236.2± 2.52	425.20± 40.04 [a]	320.33± 20.2 [ab]	287.60±12.37 [b]
Bilirubin(mg/dl)	0.59± 0.24	0.60± 0.20	0.58± 0.20	1.037± 0.11 [a]	0.74± 0.02 [ab]	0.70± 0.05 [b]

Values expressed as mean ± SEM (n = 6). [a] and [b] are Significant differences ($P \leq 0.05$) comparing to control and diabetic groups respectively.

Diabetic rats in **table 4** showed obvious elevation in all kidney function markers in respect to control rats. In contrary, diabetic rats' data treated either with *Av* or *Av+Cp* ethanolic extracts showed a highly significant decrease; in regard to untreated diabetic rats; although values were still significantly higher in all diabetic treated groups; except a non-significant uric acid change in *Av+Cp* diabetic treated group; when compared to normal control group.

Table (4): Serum kidney function markers levels.

	Control	***Av***	***Av+Cp***	**D**	**D+*Av***	**D+*Av+Cp***
Creatinine(mg/dl)	9.92± 0.15	10.19± 0.10	9.98± 0.05	43.48± 5.44 [a]	23.52± 1.03 [ab]	20.72± 1.97 [ab]
Uric acid(mg/dl)	1.60± 0.03	1.64± 0.02	1.64± 0.02	2.78± 0.27 [a]	2.35± 0.21 [ab]	1.98± 0.08 [b]
Urea(mg/dl)	20.50± 1.30	21.00± 1.50	20.80± 1.30	77.30± 6.30 [a]	56.00± 5.03 [ab]	56.00± 2.50 [ab]

Values expressed as mean ± SEM (n = 6). [a] and [b] are Significant differences ($P \leq 0.05$) comparing to control and diabetic groups respectively.

Data presented in **table 5** revealed a highly significant elevation in oxidative stress status in contrast to the decreased antioxidants levels in diabetic group. On the other hand, the results revealed that diabetic rats were given either Av or *Av+Cp* ethanolic extracts showed an obvious significant decrease in oxidative stress markers (MDA and ROS) accompanied by a significant elevation in antioxidants contents (SOD and CAT); when compared to diabetic rats; while values in *Av*-treated group were still variable in regard to control. Such date reflects the antioxidant activity superiority of the mixture over Av extract in alleviating the pancreatic oxidative stress-induced status in diabetes.

Table (5): Pancreatic MDA, ROS, CAT and SOD levels

	Control	***Av***	***Av+Cp***	**D**	**D+*Av***	**D+*Av+Cp***
MDA(nmog)	9.92± 0.15	10.19± 0.15	9.98± 0.05	43.48± 5.44	15.52± 2.23 [ab]	11.72± 1.97 [b]
ROS(nmol/g)	1.87± 0.10	1.80± 0.07	1.75± 0.06	3.85± 0.35	2.70± 0.23 [ab]	2.13± 0.09 [b]
SOD(u/gm)	10.58± 0.24	10.43± 0.33	10.55± 0.30	4.55± 0.56	7.72± 0.51 [ab]	9.57± 0.49 [b]
CAT(u/gm)	0.59± 0.01	0.59± 0.01	0.60± 0.02	0.33± 0.04	0.45± 0.03 [ab]	0.55± 0.02 [b]

Values expressed as mean ± SEM (n = 6). [a] and [b] are Significant differences ($P \leq 0.05$) comparing to control and diabetic groups respectively.

Discussion

DM has become over recent decades one of the global health-care crisis that affecting humanity regardless of the geographic location or socioeconomic profile of the population; as it considered the principle cause of great economic loss that can impede nation's development**;** making it the third worldwide human killer (estimated for 6.8% of all deaths) following cancer and cardiovascular disease**[11]and[12]**. *Aloe vera (Av)* may be a promising potential DM treatment candidate.

In concomitant to our results, When compared to control subjects, diabetes participants had greater FBG and HbA1c levels, as well as lower insulin and C-peptide levels**[13];[14]and[15].**Interestingly,**[5]** were found near total loss of insulin secretion following STZ injection in diabetic dogs, since insulin producing cells' number were reduced; which could be attributed to DNA alkylation**[16]** and/or glucose transporter-4(GLUT-4) pancreatic depletion **[17].** On the other hand,**[18]** found that oral administration of *Av* polysaccharides to hamsters with T2DM, markedly lowering FBG levels; by protecting pancreatic β-cells via interfering with ROS generation. Furthermore, *Av* gel could help decrease glucose intolerance by maintaining normal serum HbA1c level to prevent progression of diabetes; clarifying *Aloe* mechanisms in reducing hyperglycemia**[19]**. **[20]** data showed that *Av* might reduce the levels of FBG and HbA1c, in prediabetes and early non-treated diabetic patients. Many explanations were suggested for *aloe* hypoglycemic and antidiabetic effects; such as its potent antioxidant and anti-inflammatory potentials, potent inhibition of pancreatic α-amylase activity, *Av* insulinogenic activity and pancreatic islet's function improvement [21]**and[22]**, in addition to reducing postprandial glucose intestinal absorption to prevent high peaks of blood glucose in diabetic patients **[23].** Various studies hypothesized different *Av* contents being responsible for its favorable effects on HbA1c and FBG; like acemannan polysaccharide and phytosterols**[24]and [25].**

Interestingly, dyslipidemia progression was found to be related with glycemic control**,** as increased lipid levels accompanied by a great pancreatic destruction were detected in diabetic subjects**[26] . [27]** reported elevated TL, TC, LDL-C, VLDL-C and TG levels with HDL-C decline in serum of STZ-diabetic rats. Inflammation and insulin signalling pathways both play important roles in insulin insufficiency and fat accumulation. **[28].** Insulin fails to regulate hepatic glucose production during DM, but instead enhances irregular lipid synthesis, leading to hyperglycemia and hyperlipidemia. **[29]and[30].**However, many reports revealed that *Av* extract usage could alleviate the abnormal lipid profile; as it significantly reduced TG, TC, LDL-C, VLDL-C, TC to HDL-C ratio and LDL-C to HDL-C ratio and increase HDL-C level; in diabetic patients **[20]** as well as in diabetic rats**[31] .** In this line,**[32]** stated that *Av* gel helps to improve metabolic condition in obese prediabetic patients through decreasing body fat mass. **[24]**suggested that *Av* gel may act as a safe antihyperglycemic and antihyperlipidemic agent for DM patients; as it significantly reduced TC, TG and LDL-C levels via fatty streak development prevention that may minimize the risk for atherosclerosis progress.However,**[33]**found that isolated *Av* gel extract phytosterols; namely lophenol and cycloartenol; improves hyperglycemia and reduces visceral fat mass in diabetic fatty rats through inducing fatty acid oxidation upregulation and the tendency for fatty acid synthesis downregulation in the liver; which favors the improvement of hyperlipidemia and intra-abdominal fat reduction; minimizing the cardiovascular diseases risk.

Regarding liver functions in diabetes, the free radicals marked elevation was found to prompt liver diseases progress through induction of hepatocyte inflammation, fibrogenesis and apoptosis **[34]. [27]** and **[35]** reported that STZ injection resulted in an acute liver damage which could lead to a significant decrease in body weight and serum albumin, globulins and total protein levels of diabetic rats; as a consequence, of their decreased synthesis in liver. Such hepatic abnormalities were confirmed through increased serum levels of AST, ALT and γ-GT. Interestingly, [36] reported that *Av* gel prevents ethanol-induced fatty liver via suppressing the hepatic lipogenic

genes mRNA expression. Recently, **[37]**results indicated that *Av* polysaccharides; the main bioactive components in *Av* gel; bear protective efficacy against aflatoxins B1-induced hepatotoxicity in rats, confirmed by the lowered serum AST and ALT levels compared to the aflatoxins group; which could be related to its excellent anti-inflammatory and anti-oxidative effects.

In regard to kidney functions in diabetes, **[38]**and**[27]** studies showed that increased ROS and glucose levels could leads to serious kidney damage; as evidenced through marked in serum creatinine, urea and uric acid elevation, in diabetic rats regarding control. More recently, **[39]** informed that chronic kidney disease (CKD) is characterized by a combination of marked disturbances in homeostasis of both insulin and glucose, in addition to, increases insulin resistance, which leads to β -cell malfunction and insulin secretion problems; leading to a higher glucose intolerance prevalence. Moreover, elevated serum urea; in CKD advanced stages; could suppress insulin release mechanistically and induce insulin resistance **[40] .** On the other hand, literatures had shown medicinal plants with nephroprotective properties to mediate their protection via antioxidant and/or free radical scavenging activities due to the combined effect of active principles (high concentration of flavonoids and alkaloids) they contain **[41]** . Summing these facts, oral *Av* aqueous extract administration (300 mg/kg, for 10 days) to gentamicin-induced nephrotoxicity in rats, significantly protects the renal cells and reduces the severity of tubular damage caused by gentamicin; which could be attributed to its flavonoids and phenolic contents antioxidant capacity; as indicated by reduced serum uric acid, urea and creatinine levels, compared to the gentamicin group **[42]**.

Of note, oxidative stress plays a principle role in diabetic complications development**,** science it is associated with enhanced production of ROS which reacts with DNA, protein and lipids resulting in oxidative stress-induced cellular damage**[43].** In the present study, STZ-diabetes rats obviously induced an oxidative stress status progression, as demonstrated by the elevated pancreatic MDA and ROS contents concomitant to decreased pancreatic antioxidants levels (SOD and CAT). According to **[44]** STZ increase serum glucose levels resulting in LPO and ROS overproduction accompanied with antioxidant enzymes decreased activity; such as GSH-Px, CAT and SOD; participating in oxidative stress injury in diabetic rats; which they were recognized as the principle etiological factor in DM progress; resulting in considerable cellular damage leading to apoptosis

Recent approaches suggest antioxidants like *Av* and Cp may have a true antidiabetic effect via their antioxidant potential. In this regard, for *Av* and *Cp* treatments, the data in the present study showed decreased pancreatic MDA and ROS levels accompanied by a significant increase in the antioxidant enzymes (SOD and CAT) contents, compared to the diabetic untreated group. However, [45] showed that STZ-diabetic rats administrated *Av* gel extract (300 mg/kg) orally for 60 days exhibited a marked decline in tissues LPO, TBARSs, hydroperoxides and protein oxidation accompanied with marked GSH, SOD, CAT, GPx, GRd, Aldose reductase (AR) and Sorbitol dehydrogenase (SD) levels elevation; pointing out the *Av* gel protective efficacy for lipid peroxidation and oxidative stress; compared to the untreated diabetic subjects. It was evident that *Av* leaf gel phytochemical composition; particularly presence of substantial amounts of antioxidants (vitamins B, C and E, flavonoids, tannins, polyphenols, indoles and alkaloids); enables its acting as reducing agents, XO inhibitor, $NO^{\cdot}$ scavenger, singlet oxygen quencher beside providing some proton-donating abilities; which induce its serving as free radical scavenger beside performing as an antioxidant in a concentration-dependent manner; that may show a great promise in alleviating symptoms associated with/or prevention of DM**[11] .** In concomitant to our results, **[46]** reported that both *Cp* bark and roots ethanolic and methanolic extracts showed promising antioxidant potential. Additionally, **[47] and [48]** showed that4 weeks *Cp* aqueous extract (0.75, 1.5 and 3 g/100 ml drinking water) and *Cp* chloroform extract (31 and 62 mg/kg) administration to STZ-diabetic rats, respectively, prevents oxidative stress; as indicated by an obvious serum $NO^{\cdot}$ level reduction; in comparison with the untreated

diabetic rats. In this regard, **[49]** showed that *Cp* administration under diabetic conditions induced a β-cell function improvement with marked hypoglycemia, suggesting that these effects were associated with the extract's antioxidative properties in preventing oxidative stress and restoring both β-cells structure and mass; by reducing these pancreatic islets' damage in diabetic rats.

In conclusion

Phytochemical based therapies may be developed as novel pharmacological approaches for the treatment of diabetes. This study concluded that the combinational Av and Cp therapy for diabetes treatment was superior to either Av treatment independently; which had better glycemic and metabolic control but did not alleviate diabetic complications as efficiently.. Both plants beneficial effects may involve individual or combinatorial effects of various protective processes, e.g., control of hyperglycemia,hepato-renal ameliorating potency and antioxidant protection capacity. Therefore, we recommend these plants to be used as an adjuvant agent for the prevention and/or management of diabetic complications.

4. References

1- AlFaris, N.A., et al., (2020). Antidiabetic and antihyperlipidemic effect of Duvalia corderoyi in rats with streptozotocin-induced diabetes. *Saudi Journal of Biological Sciences,* **27**(**3**): p. 925-934.

2. Lu, J., Q. Xia, and Q. Zhou, (2017) How to make insulin-producing pancreatic β cells for diabetes treatment. Sci China Life Sci,. **60**(**3**): p. 239-248.

3. Jamil, K., et al., (2017). TNF-alpha -308G/A and -238G/A polymorphisms and its protein network associated with type 2 diabetes mellitus. *Saudi J Biol Sci*, **24**(**6**): p. 1195-1203.

4. Zang, L., et al., (2017). Mesenchymal stem cell therapy in type 2 diabetes mellitus. Diabetology & Metabolic Syndrome, **9**(**1**): p. 36.

5. Jaén, M.L., et al., (2017). Long-Term Efficacy and Safety of Insulin and Glucokinase Gene Therapy for Diabetes: 8-Year Follow-Up in Dogs. Molecular therapy. Methods & clinical development, **6**: p. 1-7.

6. Minjares-Fuentes, R. and A. Femenia, Chapter 3.4 - Aloe vera, (2019) in Nonvitamin and Nonmineral Nutritional Supplements, S.M. Nabavi and A.S. Silva, Editors., Academic Press. p. 145-152.

7. Nicolau-Lapeña, I., et al., (2021) Aloe vera gel: An update on its use as a functional edible coating to preserve fruits and vegetables. Progress in Organic Coatings,. **151**: p. 106007.

8. El Sayed, A.M., et al., (2016). In vivo diabetic wound healing effect and HPLC–DAD–ESI–MS/MS profiling of the methanol extracts of eight Aloe species. Revista Brasileira de Farmacognosia, **26**(3): p. 352-362.

9. Nayak, S.B., L. Pinto Pereira, and D. Maharaj, (2007). Wound healing activity of Carica papaya L. in experimentally induced diabetic rats. *Indian J Exp Biol*, **45**(**8**): p. 739-43.

10. Koroglu, P., et al., (2015). The effect of exogenous oxytocin on streptozotocin (STZ)-induced diabetic adult rat testes. Peptides, **63**: p. 47-54.

11. Dick, W.R., E.A. Fletcher, and S.A. Shah, (2016). Reduction of Fasting Blood Glucose and Hemoglobin A1c Using Oral Aloe Vera: A Meta-Analysis. *J Altern Complement* Med, **22**(**6**): p. 450-7.

12. Domouky, A.M., et al., (2017). Mesenchymal stem cells and differentiated insulin producing cells are new horizons for pancreatic regeneration in type I diabetes mellitus. *Int J Biochem Cell Biol*, **87**: p. 77-85.

13. Lin, D., et al., (2018). Associations of lipid parameters with insulin resistance and diabetes: A population-based study. Clinical Nutrition, **37**(**4**): p. 1423-1429.

14. Amer, M.G., et al., (2018). Role of adipose tissue derived stem cells differentiated into insulin producing cells in the treatment of type I diabetes mellitus. Gene, **654**: p. 87-94.

15. Gharib, H.M., M.Y. Abajy, and A. Omaren, (2020). Investigating the effect of some fluoroquinolones on C-reactive protein levels and ACh-Induced blood pressure reduction deviations after aging of diabetes in STZ-Induced diabetic wistar rats. Heliyon, **6**(4): p. e03812.

16. Ghosh, S., et al., (2015). Curcumin protects rat liver from streptozotocin-induced diabetic pathophysiology by counteracting reactive oxygen species and inhibiting the activation of p53 and MAPKs mediated stress response pathways. Toxicol Rep, **2**: p. 365-376.
17. Adam, S.H., et al., (2016). Protective effect of aqueous seed extract of Vitis Vinifera against oxidative stress, inflammation and apoptosis in the pancreas of adult male rats with diabetes mellitus. Biomed Pharmacother, **81**: p. 439-452.
18. Kim, K., et al., (2018). ER stress attenuation by Aloe-derived polysaccharides in the protection of pancreatic β-cells from free fatty acid-induced lipotoxicity. Biochem Biophys Res Commun, **500**(**3**): p. 797-803.
19. Cárdenas-Ibarra, L., et al., (2017). Randomized double blind crossover trial of Aloe vera, Cnidoscolus chayamansa and placebo for reducing hyperglycemia in women with early metabolic syndrome. Clinical Nutrition Experimental, **14**: p. 1-12.
20. Zhang, Y., et al., (2016). Efficacy of Aloe Vera Supplementation on Prediabetes and Early Non-Treated Diabetic Patients: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Nutrients, **8**(**7**).
21. Kumar, P., et al., (2013). Enzymatic in vitro Anti-diabetic Activity of Few Traditional Indian Medicinal Plants. *Journal of Biological Sciences*, **13**: p. 540-544.
22. Daburkar, M., et al., (2014). An in vivo and in vitro investigation of the effect of Aloe vera gel ethanolic extract using animal model with diabetic foot ulcer. *J Pharm Bioallied Sci*, **6**(**3**): p. 205-12.
23. Taukoorah, U. and M.F. Mahomoodally, (2016). Crude Aloe vera Gel Shows Antioxidant Propensities and Inhibits Pancreatic Lipase and Glucose Movement In Vitro. Adv Pharmacol Sci, **2016**: p. 3720850.
24. Huseini, H.F., et al., (2012). Anti-hyperglycemic and anti-hypercholesterolemic effects of Aloe vera leaf gel in hyperlipidemic type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. Planta Med, **78**(4): p. 311-6.
25. Devaraj, S., et al., (2013). Effects of Aloe vera supplementation in subjects with prediabetes/metabolic syndrome. Metab Syndr Relat Disord, **11**(**1**): p. 35-40.
26. Levitt Katz, L.E., et al., (2018). Lipid Profiles, Inflammatory Markers, and Insulin Therapy in Youth with Type 2 Diabetes. *J Pediatr*, **196**: p. 208-216.e2.
27. Antony, P.J., et al., (2017). Myoinositol ameliorates high-fat diet and streptozotocin-induced diabetes in rats through promoting insulin receptor signaling. Biomed Pharmacother, **88**: p. 1098-1113.
28. Chen, Z., (2016). Adapter proteins regulate insulin resistance and lipid metabolism in obesity. Science Bulletin, **61**(**19**): p. 1489-1497.
29. Titchenell, P.M., M.A. Lazar, and M.J. Birnbaum, (2017). Unraveling the Regulation of Hepatic Metabolism by Insulin. Trends Endocrinol Metab, **28**(**7**): p. 497-505.
30. Giralt, A., et al., (2018). E2F1 promotes hepatic gluconeogenesis and contributes to hyperglycemia during diabetes. Mol Metab, **11**: p. 104-112.
31. Yimam, M., et al., (2014). Blood glucose lowering activity of aloe based composition, UP780, in alloxan induced insulin dependent mouse diabetes model. Diabetol Metab Syndr, **6**: p. 61.
32. Choi, H.C., et al., (2013). Metabolic effects of aloe vera gel complex in obese prediabetes and early non-treated diabetic patients: randomized controlled trial. Nutrition, **29**(**9**): p. 1110-4.
33. Radha, M.H. and N.P. Laxmipriya, (2015). Evaluation of biological properties and clinical effectiveness of Aloe vera: A systematic review. *J Tradit Complement Med*, **5**(**1**): p. 21-6.
34. Petit, J.M., (2017). Particularités des diabètes associés aux maladies hépatiques. Médecine des Maladies Métaboliques, **11**(**8**): p. 682-686.
35. Jamshidi, M., et al., (2018). The effect of insulin-loaded trimethylchitosan

nanoparticles on rats with diabetes type I. Biomedicine & Pharmacotherapy, **97**: p. 729-735.
36. Saito, M., et al., (2016). Oral administration of Aloe vera gel powder prevents UVB-induced decrease in skin elasticity via suppression of overexpression of MMPs in hairless mice. Biosci Biotechnol Biochem, **80**(**7**): p. 1416-24.
37. Cui, Y., et al., (2017). Evaluating the hepatoprotective efficacy of Aloe vera polysaccharides against subchronic exposure of aflatoxins B1. *Journal of the Taiwan Institute of Chemical Engineers*, **76**: p. 10-17.
38. Hamza, A.H., et al.,(2017). Mesenchymal stem cells: a future experimental exploration for recession of diabetic nephropathy. Ren Fail, **39**(1): p. 67-76.
39. Xie, Y., et al., (2018) Higher blood urea nitrogen is associated with increased risk of incident diabetes mellitus. Kidney Int, **93**(**3**): p. 741-752.
40. Koppe, L., et al., (2016). Urea impairs β cell glycolysis and insulin secretion in chronic kidney disease. *J Clin Invest*, **126**(9): p. 3598-612.
41. Naggayi, M., N. Mukiibi, and E. Iliya, (2015). The protective effects of aqueous extract of Carica papaya seeds in paracetamol induced nephrotoxicity in male wistar rats. Afr Health Sci, **15**(2): p. 598-605.
42. Baradaran, A., et al., (2014). Antioxidant activity and preventive effect of aqueous leaf extract of Aloe Vera on gentamicin-induced nephrotoxicity in male Wistar rats. Clin Ter, **165**(1): p. 7-11.
43. Hossein Nia, B., et al., (2018) The Effects of Natural Clinoptilolite and Nano-Sized Clinoptilolite Supplementation on Glucose Levels and Oxidative Stress in Rats With Type 1 Diabetes. *Can J Diabetes*, **42**(**1**): p. 31-35.
44. Roslan, J., et al., (2017). Quercetin ameliorates oxidative stress, inflammation and apoptosis in the heart of streptozotocin-nicotinamide-induced adult male diabetic rats. Biomed Pharmacother, **86**: p. 570-582.
45. Haritha, K., B. Ramesh, and D. Saralakumari, (2014). Effect of Aloe vera gel on antioxidant enzymes in streptozotocin-induced cataractogenesis in male and female Wistar rats. *Journal of Acute Medicine*, **4**(**1**): p. 38-44.
46. Asghar, N., et al., (2016). Compositional difference in antioxidant and antibacterial activity of all parts of the Carica papaya using different solvents. *Chem Cent J*, **10**: p. 5.
47. Juárez-Rojop, I.E., et al., (2012). Hypoglycemic effect of Carica papaya leaves in streptozotocin-induced diabetic rats. BMC Complement Altern Med, **12**: p. 236.
48. Juárez-Rojop, I.E., et al., (2014.) Phytochemical screening and hypoglycemic activity of Carica papaya leaf in streptozotocin-induced diabetic rats. Revista Brasileira de Farmacognosia, **24**(**3**): p. 341-347.
49. Miranda-Osorio, P.H., et al., (2016). Protective Action of Carica papaya on β-Cells in Streptozotocin-Induced Diabetic Rats. *Int J Environ Res Public Health*, **13**(**5**).