Investigation of anti-diabetic, anti-oxidant activity of leaf extracts of *Eclipta alba and Ziziphus jujube* in animal models

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Abstract

In alloxan-induced diabetic rats, hepatoprotective and blood glucose levels were assessed following oral administration of an ethanol leaf extract of *Eclipta alba* and *Ziziphus jujube*. The data obtained after oral treatment with the extract and glibenclamide for 7 and 21 days showed a comparable hypoglycemic impact. After 21 days, the greatest reduction in blood glucose level (15.18-26.7%) was seen. When compared to diabetic controls, the animals treated with a combined dose of plant extracts showed the greatest increases in GSH and SOD levels and a reduction in MDA levels. Additional research can reveal the plant drug's mechanism of action with regard to its hepatoprotective and anti-diabetic effects.

Keywords: *Eclipta alba*, *Ziziphus jujube*, antioxidant and anti-diabetic

1. Introduction

The prevalence of diabetes and related metabolic disorders is currently pandemic across the globe. It is a metabolic condition characterized by impaired protein, lipid, and carbohydrate metabolism as a result of insulin imbalance or inappropriate insulin action. On the other hand, the imbalance between the production of reactive oxygen species (ROS) and nitrogen species (RNS) and the decreased function of the body's own antioxidant system is known as oxidative stress. Free radical-induced damage during this type of redox activity has been shown to play a role in the pathogenesis and pathophysiology of many chronic health issues, including cancer, emphysema, and neurological diseases (Gulcin, 2020). As a result, understanding antioxidants is important for the treatment of many diseases.

Folklore related to phytomedicine has a wonderful history and expertise in China and India. Natural compounds have always captivated chemists and biologists because of their amazing and varied properties. According to studies, about half of the medications that were approved were made from natural sources (D.J. Newman, G.M. Cragg 2007; M.S. Butler *et al*., 2014; S. Hasani-Ranjbar, *et al*., 2013). Natural substances originating from plants, such as flavonoids, terpenoids, and steroids, have drawn a lot of attention lately because of their wide range of pharmacological activities, which include antioxidant and anticancer activity. Humans are protected against infection and degenerative diseases thanks to antioxidants' crucial role in blocking and scavenging radicals. Antioxidants are substances that aid in inhibiting a variety of oxidation reactions brought on by free radicals such as singlet oxygen, superoxide, peroxyl, hydroxyl and peroxy nitrate, thereby preventing or postponing harm to cells and tissues. UV oxidative stress contributes to a number of diseases, either directly or indirectly.

A different herb, *E. alba*, member of the Asteraceae family, also known as "Bhringraj." Nearly everywhere in the world, including India, China, Nepal, and Brazil, it is easily distributed and propagated. *E. alba* has been extensively researched, with a primary focus on its ability to promote hair development (K. Datta *et al*., 2009). *E. alba* extract has anti-inflammatory (S.S. Kumar *et al*., 2005), antimicrobial (Panghal *et al*. 2011; A. Ray *et al*. 2019), protective effects against CCl4-induced hepatotoxicity (A.K. Saxena *et al*., 1993). The anti-malarial and anti-cancer properties of *E. alba's* hydroalcoholic extract have both been documented (S. Bapna *et al*., 2007) as part of the plant's diverse potential. There have been reports of E. prostrata's bioactivity as an antioxidant (Chan CF *et al*., 2014; Gani AMS *et al*., 2015; Lee SH *et al*., 2018). With the formation of prostaglandin being extensively researched, EP possesses significant antioxidant activity (Lee HY *et al*., 2017). Ethyl acetate, hexane, ethanol, and water extracts of *E. prostrata's* aerial portion all showed antioxidant activity (Jahan R *et al*., 2014; C.F. Chan *et al*., 2014; Kim D. I *et al*., 2008).

Plants that produce substances that can prevent the conversion of carbohydrates to glucose have the potential to be anti-diabetes mellitus treatments. When given to rats with alloxan-induced diabetes, the substance ecclalbasaponin II (an extract of E. prostrata in methanol) significantly reduced blood glucose levels. When compared to diabetic rats not receiving treatment, blood glucose was dramatically lowered by E. prostrata extract (300 mg/kg) and ecclalbasaponin II (10 mg/kg) (Rahman MS *et al*., 2011).

Eclipta alba in mixtures of various herbs Pan-five has been shown to have anti-diabetic and diuretic effects by restoring cell function and pancreatic regeneration. (Feng L *et al*., 2019; Jahan R *et al*., 2014; Mithun N *et al*., 2011). The ethanolic leaf extract of *Eclipta alba* includes sugars, lactones, steroids, terpenoids, glycosides, esters, flavonoids, and tannins that have an anti-diabetic action and are helpful for abnormalities in mice who have been given alloxan to cause diabetes (Nivedita and Vijay 2015). Eclipta alba's effectiveness in relation to blood glucose levels in diabetic patients (Sazia *et al*. 2015).

Ziziphusjujuba originated in Central Asia and has since been cultivated there as well as in Asia, Europe, and North America. The Chinese date palms also occur in a number of other locations as naturalised populations. Additionally, this species can be grown on integrated organic farms and in little-known private gardens.

Recent phytochemical studies of *Z. jujube* confirms the anti-inflammatory, antioxidant, immune stimulating, hepato protective, antiobesity, anticancer, and gastrointestinal protective activities (Gao *et al*., 2013; Abedini *et al*., 2016; Rajopadhye and Upadhye, 2016; Keerthi *et al*., 2016; Zozio S *et al*., 2014; Wang B *et al*., 2016). In one study, it has been proven that the aqueous extract of *Z. jujube* has antioxidant activity (Wang, 2011; Li *et al*., 2014; Zhang *et al*., 2017).

2. Materials and Methods

2.1 Preparation of the extracts

The leaves of *E. alba and Z. jujube* that were purchased from a local market were authenticated at the Botany, Department of Osmania University in Hyderabad, and a voucher specimen of the plant has been submitted (voucher no. 080). In accordance with the steps outlined in the Indian Pharmacopoeia of 1996, the dried leaves were next coarsely pulverized and the purity and quality of the crude medicine were determined. Round-bottomed glass bottles were used to hold the powdered substance. Each received three liters of 95 percent ethanol, which was then refluxed for four hours. The process was carried out twice. The extracts were filtered before being concentrated in a rotavapor under vacuum at 50°C until dry. We obtained alcoholic extracts of *E. alba & Z. jujuba* and yields were found to be 29.37% (w/w) and 31.24% (w/w), respectively.

2.2 Animals & Diabetes induction

48 adult male Wistar rats (200-250 g) were kept in cages at 24 °C with a 12-hour light-dark cycle. Water and commercial palletized diet were available to the animals *ad libitum* throughout the treatment period. The rats weighed between 200 and 250 g. The Ethics Committee gave its approval to the experimental protocols, and the treatment of the animals was in accordance with standards for laboratory animal care.

The alloxan monohydrate (0.1g) in normal saline was considered for stock solution. Alloxan monohydrate (150 mg/kg) was injected intraperitoneally once to induce diabetes (Szkudelski, T. 2001).

2.3 Experimental design

The animals were allowed two weeks for acclimatization and then randomly divided into nine equal groups (n=6) as follows:

1. Non-diabetic control group treated with 0.5 mL distilled water.
2. Alloxan-induced diabetic control group.
3. Diabetic rats receiving Glibenclamide.
4. Diabetic rats receiving Eclipta alba extract 250mg/kg p.o (EAL).
5. Diabetic rats receiving Eclipta alba extract 500mg/kg p.o (EAH).
6. Diabetic rats receiving Ziziphus jujube extract 250mg/kg p.o (ZJL).

7. Diabetic rats receiving Ziziphus jujube extract 500mg/kg p.o (ZJH).
8. Combination of EAL & ZJL (EAZJL).
9. Vit. C (40mg/kg).

Selection of this dose was based on acute oral toxicity studies. Each dose of extract was dissolved in 0.5 mL distilled water. Mean body weight recorded for all treatment groups at 0, 7, 14 and 21 days.

An instrument called a glucometer was used to measure the blood sugar levels of the rats in each group before the experiment started. Prior to oral administration of extracts and glibenclamide, blood glucose levels were also measured following alloxan treatment to confirm that the rats (from groups 2-8) were diabetic. After receiving extract and glibenclamide treatments, blood glucose levels were also checked at 1, 3, 5, 7 and 9h. Animals in all groups were treated for 21 days.

The animals were starved for 12 hours at the conclusion of the 21-day period, anesthetized with chloroform, and then dissected. Whole blood obtained by cardiac puncture into plane tubes was allowed to clot for about 2 h and thereafter centrifuged (3000 rpm/10 min) to remove cells and recover serum, which was used for the biochemical assays.

2.4 Antioxidant activity

2.4.1 Reduced Glutathione (GSH)

Total reduced glutathione content was measured by following the method of Ellman's (1959). When 5, 5^1-dithio-2-nitro benzoic acid (DTNB) reacts with the compounds containing sulphoydryl groups with a maximum absorbance at 412 nm forms yellow color. 0.5 ml of sample was deproteinized with 3.5 ml of 5% TCA and centrifuged. To 0.5 ml of supernatant, 3.0 ml 0.2 M phosphate buffer, pH 8.0 and 0.5 ml of freshly prepared Ellman`s reagent (19.8 mg DTNB in 100 ml of 0.1% sodium citrate) were added and the yellow color developed was read at 412 nm. A series of standards (4-20 µg) were treated in a similar manner along with a blank. Values are expressed as µm GSH/mg protein.

2.4.2 Malonaldehyde (MDA) Assay

MDA is the end product of the lipid peroxidation of polyunsaturated fatty acids. MDA was estimated by utilizing the reactivity of 2-thiobarbituric acid (TBA). One molecule of MDA reacts with 2 moles of TBA to form a pink colored condensation product, a trimethine which was measured spectrophotometrically at 533nm (Shimadzu UV 2600).

2.4.3 Superoxide dismutase

Superoxide dismutase activity was measured based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. A modified procedure described by Murkland and Murkland (1974) was adopted as followed by Soon and Tan (2002). The assay system contained 2.1 ml of 50 mM phosphate buffer, pH 7.8 containing 1mM EDTA buffer, 0.02 ml of enzyme source (35 µg protein) and 0.86 ml of distilled water. The

reaction was initiated by the addition of 0.02 ml of 10 mM pyrogallol in 0.01N HCl and change in absorbance was monitored at 420 nm for 5 min. The percent inhibition was calculated on the basis of comparison with a blank assay system. One unit of SOD was defined as that amount of enzyme required to inhibit the auto-oxidation of pyrogallol by 50% in standard assay system of 3 ml. The specific activity was expressed as units/100mg protein. /min.

2.5 Statistics

The findings of each experiment were expressed as the mean and standard deviation. A one-way ANOVA with SPSS version 16 software was utilized for the statistical analysis and a significant difference was defined as one with a value of (p<0.05).

3. Results

3.1 Antidiabetic activity

Single dose treatment on blood glucose determined at various time intervals for 9 h are 4.99, 8.43, 7.33, 9.87 and 17.38% were observed in groups treated with Vit.C, *EAH*, *ZIH*, EAZIL and Glibenclamide, respectively (table 1). Six days after alloxan-treatment, blood glucose of diabetic rats was significantly raised when compared with the control group (Figure 1 & 2). First dose treatment caused reduction over the intervals monitored but with significant peak reductions after 1 day for EAH treated group (9.83%), EAZJL (11.03%) and Glibenclamide (26.44%) (Table 2). At the end of the chronic treatment period (21 days) decreases in blood glucose level relative to their initial values of 7.35, 17.98, 10.37, 19.41,26.57 and 37.07% were observed in groups treated with Vit. C, *EAH*, *ZIL*, *ZIH*, EAZIL and Glibenclamide, respectively (Table 2 & Figure 3). When administration of plant extract to diabetic rats showed insignificant (p<0.01) values in body weight as compared to the diabetic control group. (Figure 4).

Table 1: Effect of *E. alba*, *Z. jujube* in rat glucose levels at 0, 1, 3, 5, 7 and 9h

Groups	0 h	1 h	3 h	5 h	7 h	9 h
Control	349.24 ± 17.26	351.97 ± 19.94	353.78 ± 21.27	352.19 ± 14.28	354.87 ± 20.12	350.28 ± 18.09
		(0.78)	(1.30)	(0.84)	(1.61)	(0.30)
ALLOXAN	413.37 ± 15.16	426.21 ± 18.20	460.37 ± 12.84	511.72 ± 20.47	528.25 ± 16.19	532.46 ± 18.12
Glibenclamide	300.92 ± 25.67	291.74 ± 28.47	289.74 ± 21.23	276.53 ± 29.65	267.62 ± 19.28	248.63 ± 23.73
		(-3.05)	(-3.72)	-8.11)	(-11.07)	(-17.38)
EAL	342.92 ± 23.16	342.85 ± 18.55	342.1 ± 25.10	340.29 ± 23.18	338.73 ± 29.82	335.91 ± 26.29
		(-0.77)	(-0.24)	(-0.77)	(-1.22)	(-2.04)
EAH	365.32 ± 18.92	350.62 ± 20.66	342.58 ± 24.62	339.27 ± 20.83	336.79 ± 23.84	334.52 ± 22.97
		(-4.02)	(-6.22)	(-7.13)	(-7.81)	(-8.43)
ZJL	357.28 ± 29.37	354.29 ± 27.43	350.2 ± 28.63	348.67 ± 24.81	345.93 ± 21.01	342.16 ± 25.84
		(-0.84)	(-1.98)	(-2.41)	(-3.18)	(-4.23)
ZJH	348.27 ± 25.62	342.46 ± 24.91	337.61 ± 27.37	331.64 ± 26.35	325.29 ± 21.01	322.73 ± 19.64
		(-1.67)	(-3.06)	(-4.78)	(-6.60)	(-7.33)
EAZJL	335.26 ± 23.82	320.64 ± 27.42	311.37 ± 24.91	310.61 ± 20.49	307.22 ± 18.62	302.18 ± 21.72
		(-4.36)	(-7.13)	(-7.35)	(-8.36)	(-9.87)

Vit. C	361.26 ± 20.69	359.17 ± 22.96	353.14 ± 25.65	350.05 ± 21.94	348.41 ± 21.69	343.22 ± 17.91
		(-0.58)	(-2.25)	(-3.10)	(-3.56)	(-4.99)

Table 2: Effect of *E. alba*, *Z. jujube* in rat glucose levels at 1day 7 day and 21 day

Groups	24 h	7 Day	21 Day
Control	356.21 ± 20.14	361.62 ± 21.49	366.81 ± 19.36
	(2.00)	(3.54)	(5.03)
Alloxan	518.18± 21.38	526.37 ± 17.91	509.36 ± 15.13
Glibenclamide	221.37 ± 26.81	210.84 ± 21.43	189.36 ± 27.60
	(-26.44)	(-29.93)	(-37.07)
EAL	329.16 ± 27.64	324.94 ± 26.81	316.27 ± 25.91
	(-4.01)	(-5.24)	(-7.77)
EAH	329.42 ± 21.52	316.42 ± 24.83	299.64 ± 27.84
	(-9.83)	(-13.39)	(-17.98)
ZJL	337.69 ± 24.69	328.31 ± 20.82	320.23 ± 25.37
	(-5.48)	(-8.11)	(-10.37)
ZJH	320.28 ± 22.73	311.56 ± 21.63	280.66 ± 26.34
	(-8.04)	(-10.54)	(-19.41)
EAZJL	298.28 ± 21.88	284.38 ± (28.27)	246.19 ± 19.28
	(-11.03)	(-15.18)	(-26.57)
Vit. C	341.46 ± 21.01	339.94 ± 24.15	334.72 ± 24.62
	(-5.48)	(-5.90)	(-7.35)

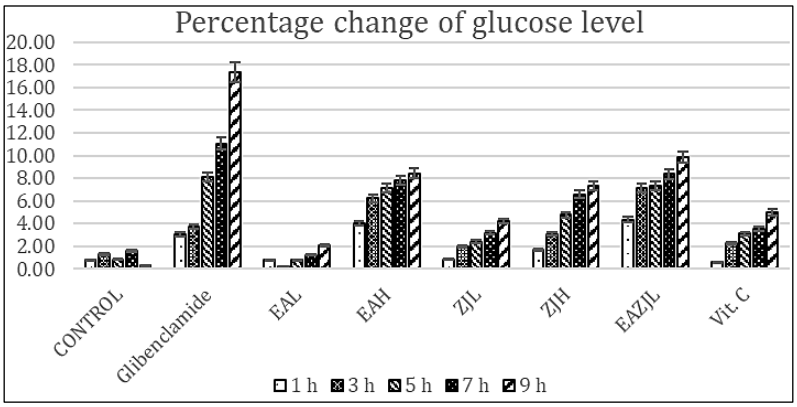

Fig 1: Effect of *E. alba*, *Z. jujube* in rat glucose levels at 0, 1, 3, 5, 7 and 9h

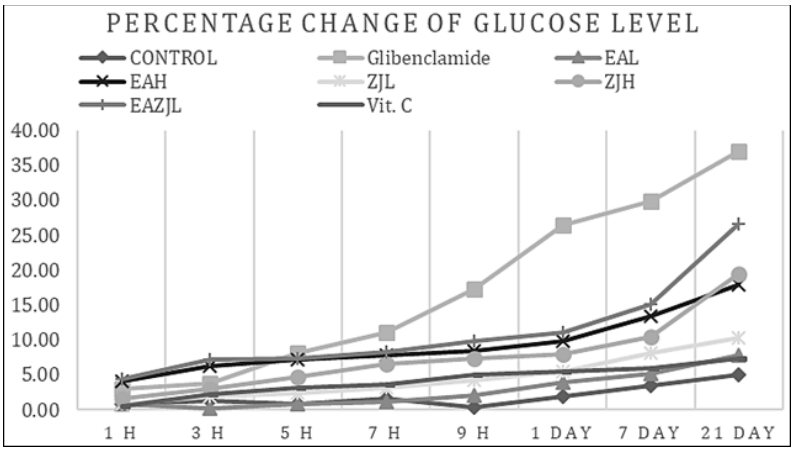

Fig 2: Effect of *E. alba*, *Z. jujube* in rat glucose levels at 0, 1, 3, 5, 7 and 9h

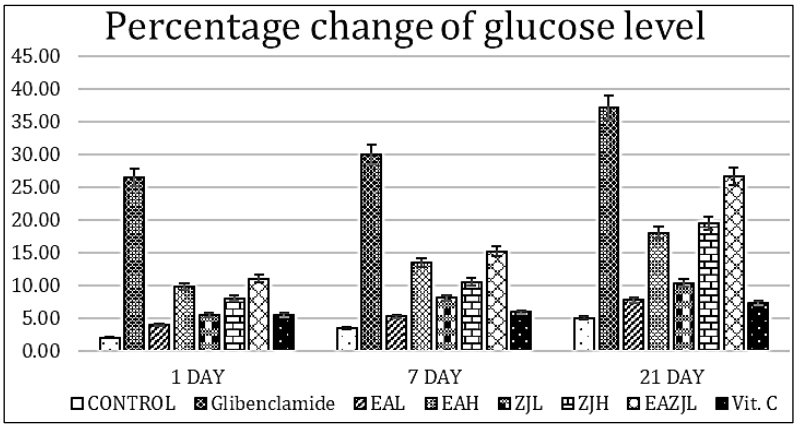

Fig 3: Effect of *E. alba*, *Z. jujube* in rat glucose levels at 1 day, 7 day and 21 day

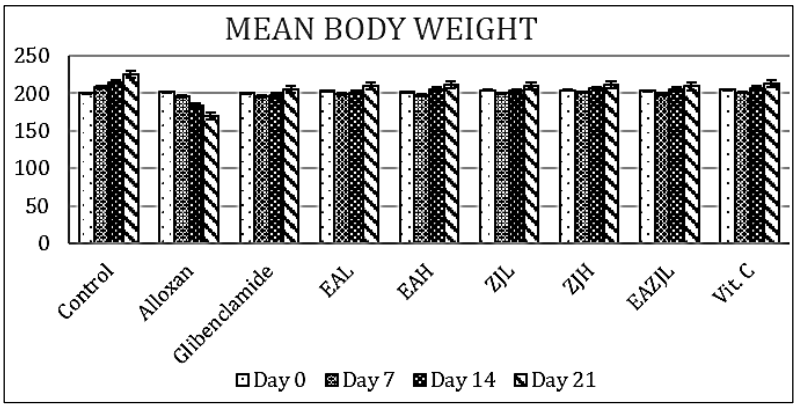

Fig 4: Effect of *E. alba*, *Z. jujube* in body weight of rat

3.2 Anti-oxidant activity

3.2.1 Reduced glutathione

GSH has a multifaceted role in antioxidant defense. It serves as both a cosubstrate for glutathione peroxidases' detoxification of peroxides and a direct scavenger of free radicals. Experimental diabetic animals treated with alcoholic extract of *E. alba* and *Z. jujube* showed significant results of declined SOD level with alloxan treated groups. This effect was more notable when using the ZJH and EAZJL extracts than with the other extracts (Figure 5).

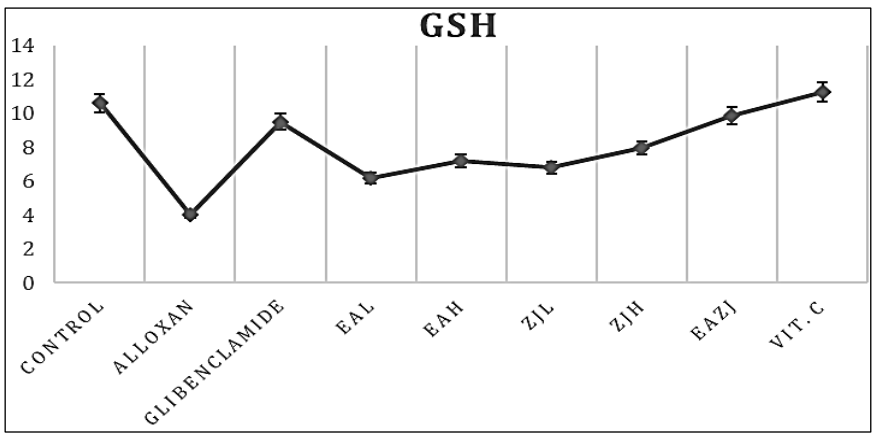

Fig 5: Effect of *E. alba*, *Z. jujube* on GSH

3.2.2 Malondialdehyde (Figures 1 and 2).

Oxidative stress biomarker malondialdehyde (MDA) was measure in liver homogenates of control, untreated diabetic and diabetic animals treated with reference drug and experimental groups treated with alcoholic extract of leaves of *E. alba* and *Z. jujube*. In the liver of alloxan treated diabetic rats, lipid peroxidation levels as evidenced by MDA determination increased significantly as compared to normal control group (p<0.05). In diabetic rats treated with Glibenclamide and Vit. C a significant decrease in MDA was observed (Figure 6). In diabetic rats extract of EAH, ZJH and EAZJL treatment significantly inhibited the increase in MDA. Maximum inhibition in MDA level was observed in the diabetic animals fed with combined plant extract.

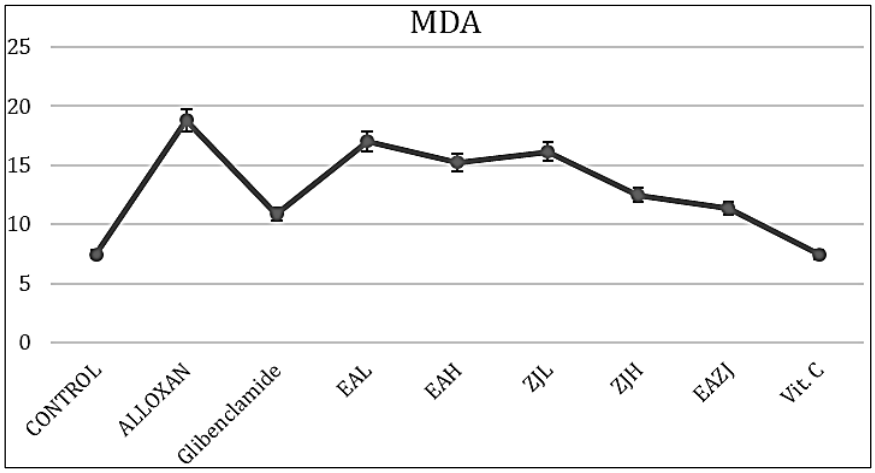

Fig 6: Effect of *E. alba, Z. jujube* on MDA

Superoxide dismutase was assessed in liver of control and experimental diabetic animals treated with alcoholic extract of *E. alba* and *Z. jujube*. The results showed that the SOD level of animals treated with alloxan declined significantly than normal control groups (Fig. 7). Standard Glibenclamide, EAZJL extract for 21 days markedly increased the level of SOD (p<0.05).

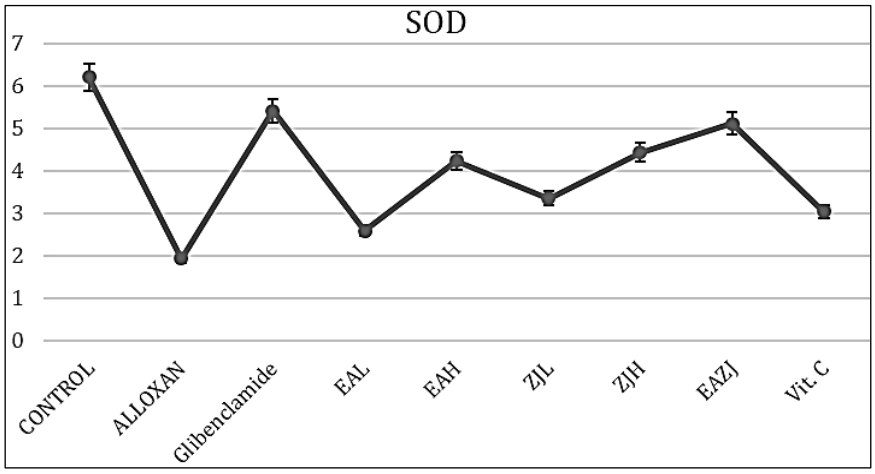

Fig 7: Effect of *E. alba, Z. jujube* on SOD

4. Discussion

The antihyperglycemic effect of ethanolic extracts of *E. alba* and *Z. jujube* combined, *E. alba, Z. jujube* and Glibenclamide have been evaluated in this study. Effect of *E. alba, Z. jujube* on blood glucose was less than that of Glibenclamide, but significantly higher than normal control.

Whereas effect of combined extracts on blood glucose compares fairly well with that of Glibenclamide, which itself compares well with normal control. Within the treatment period, combined extracts effect on blood glucose appears a positive synergy: *E. alba* could not reverse the hyperglycemic state to normoglycemic status, while on the other hand *Z. jujube* tended towards hypoglycemia. In combined form, the extracts tend to complement each other thereby producing the desired normoglycemia.

This study supports the idea that multimodal herbal treatments have advantages over monotherapy (Suvarna, R *et al*. 2021). Although not fully understood, several reports have attempted insights in to the hypoglycemic mechanisms of these plants.

A number of plants have been shown to exert hypoglycemic activity through stimulation of insulin release like Glibenclamide that is reported to enhance the activity of beta cells of pancreas resulting in increased secretion of large amounts of insulin which in turn brings down blood glucose level. There is a report that *E. alba* has prominent anti-adipogenic effects in 3T3-L1 and hMSC derived adipocytes. It was shown to activate Wnt-pathway and alter AKT signaling, additionally their report presupposed increased peripheral glucose uptake by an inhibition (Gupta, A *et al*. 2018; Savova, M. S *et al*. 2021; Singh, A *et al*. 2014).

There are two potential processes for the hypoglycemic effect: one focuses on the islet cells' generation of insulin and the other on the peripheral carbohydrate mechanism. Moreover, Mechanisms involving insulin production are usually more potent. It's not surprising therefore that *Z. jujube*tends to be the most potent hypoglycemic agent.

The increase in GSH, SOD level was maximum among the animals treated with combined dose of plant extracts, as compared to diabetic control. Maximum inhibition in MDA level was observed in the diabetic animals fed with combined plant extract and reference drug. Comparing the experimental groups to the diabetic control group, the plant extracts reduced the oxidative stress caused by diabetes' hyperglycemia and increased the antioxidant capacity of the experimental groups.

It's evident from the results of this work, that whereas *Z. jujube*alone is most potent in blood glucose reduction mechanism and *E. alba* most potent in protecting the liver against damage in diabetic states. Only a combination of the two extracts provided the holistic efficacy desired in management of diabetes. Further studies to confirm this relative advantage of polytherapy is suggested.

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