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Protective Effect of β-carotene and Turmeric Extract Against Alloxan Generated Oxidative Stress Induced Diabetes

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The present examination was intended to assess the impact of ß-carotene and turmeric extract individually, exclusively and with co-administration on antioxidant status in Allxoan instigated diabetic rats. After the administration of ß-carotene (20 mg/kg) and turmeric extract (40 mg/kg) exclusively and with co administration, the rodent were contemplated for blood glucose level, liver isolate for cell reinforcement compounds like Superoxide dismutase, Catalase, Lipid peroxidation, diminished glutathione and uric corrosive levels and pancreas for histopathology study. All diabetic groups show significantly elevated blood glucose levels compared to the normal control, confirming hyperglycemia. Blood glucose levels increase further (300.56 ± 12.64 mg/dL), indicating worsening hyperglycemia. The combination shows the most substantial reduction in glucose levels (175.25 ± 3.80 mg/dL), nearing normal values, highlighting a synergistic effect. The combination significantly reduces LPO (36.01 ± 0.837), nearing normal levels, suggesting a powerful reduction in oxidative damage. The combination shows the highest improvement among treatments in SOD (58.97 ± 1.26), CAT (0.931 ± 0.025), GSH (32.66 ± 0.554), suggesting a synergistic effect in enhancing enzymatic antioxidant defense. The combination of ß-carotene and turmeric extract (7.612 ± 0.121 g) nearly normalizes liver weight, suggesting a synergistic effect in protecting the liver from diabetes-induced damage. The simultaneous administration of these drugs significantly rejuvenates pancreatic beta cells. The outcomes got from this investigation gives a logical confirmation that ß-carotene and turmeric separate have defensive impact against alloxan creates oxidative pressure prompted diabetes. The combination treatment shows synergistic effect.

Keywords: Alloxan; Anti-diabetic activity; ß-carotene; Free radicals; oxidative stress; Turmeric extract.

Blood glucose levels progress when insulin synthesis and secretion become erratic, resulting in various clinical manifestations such as glycosuria, polydipsia, and polyuria.[1-3] Free radicals, especially reactive oxygen species, are made when glucose auto-oxidation and protein glycosylation happen, which is a result of chronic diabetes. This breaks down tissue.[4,5,6] Free radicals are produced naturally by cells, but there are times when the balance between making reactive oxygen species and cells' defense systems is upset. Cellular damage and dysfunction caused by

this imbalance may lead to tissue harm. Diabetes conditions enhance the levels of reactive oxygen species and free radicals, leading to increased oxidative stress that may affect level of oxidative enzymes like glutathione peroxidase, superoxide dismutase, and catalase. The pancreatic beta cells of the islets exhibited diminished resistance to free radicals, potentially resulting in detrimental effects on tissue.[6,7,8] Changes in the body's natural defenses against free radicals may make it so that reactive oxygen species aren't neutralized enough in people with diabetes mellitus, which could cause tissue damage and oxidative injury. Researchers presently suggest oxidative stress as the underlying mechanism of diabetes and its ramifications[9]. Oxidative stress may play a role in the development of diabetes by creating oxygen-free radicals, nonenzymatic protein glycosylation, and glucose that breaks down on its own. Oxidative stress contributes to the oxidative stress response. Insufficient glutathione metabolism.[10] alterations in antioxidant enzymes [11], and the production of lipid peroxides[12] are all manifestations of this disease. Oxidative stress happens when the body makes too many unstable molecules, like reactive oxygen species (ROS) and reactive nitrogen species (RNS) or doesn't get rid of them properly. By stopping â cell death and the peroxidation chain reaction, antioxidants lower oxidative stress and delay the development of diabetes.[13, 14] Marketed available antioxidants Vit. E and Vit. C reduced oxidative stress temporarily but they might be hard to achieve effectively neutralize free radicals over a long period of time. Marketed antioxidants, Vitamin E and Vitamin C, temporarily decreased oxidative stress; however, they may be ineffective in consistently neutralizing free radicals over an extended duration.

Chronic diabetes often leads to structural and functional impairments that make tissue repair challenging. If oxidative stress has altered gene expression patterns, these changes may become irreversible, hindering the restoration of normal gene function. This underscores the importance of natural antioxidants, as synthetic ones like BHT, BHA, TBHQ, and PG can be harmful to animals [15, 16]. Plants are rich sources of antioxidants, which are present in various parts such as bark, leaves, fruits, roots, flowers, stems, and seeds. Key phytoconstituents include cinnamic acids, phenylpropanoids, lignans, diterpenes, flavonoids, tannins, homoterpenes, and triterpenes. These compounds have shown potential to alleviate oxidative stress and are beneficial in managing conditions like Parkinson's, Alzheimer's, diabetes, and more [17, 18].

————â-Carotene is one of the most beneficial constituents available in many colours, including yellow, green, and orange, present in leaves, fruits, and vegetables. Carotene primarily exists in two primary isomers: á-carotene and â-carotene. It safeguards plant cells against ultraviolet light. Turmeric extract is derived from the rhizomes of Curcuma longa, belonging to the Zingiberaceae family. It is a traditional medication with several applications. Diabetes mellitus ranks among the ten leading causes of mortality worldwide. The literature evaluation indicates that the simultaneous treatment of two antioxidants may help to decreases blood glucose levels [19].

Consequently, the authors considered it essential to assess the therapeutic efficacy of â-Carotene and turmeric extract as a prospective intervention for oxidative stress in diabetes and its associated complications, utilizing animal models while acknowledging the documented assertions and chemical constituents of this plant. Thus, the authors examined the protective effects of â-Carotene and turmeric extract against oxidative stress generated by alloxan in diabetic conditions [20, 21].

MATERIALS AND METHODS

Chemicals were producred from Rajesh Chemicals, India Tris-buffer, Tris-Ethylene Diamine-Tetraacetic Acid (EDTA) pyrogallol, tri-chloroacetic acid (TCA), thio-barbituric acid (TBA), phosphate buffer (0.2 M, pH 6.6), EDTA, and 0.6 mm 5, 5'dithiobis 2-nitrobenzoic acid (DTNB), Ozone International Pvt.Ltd India (Alloxan Monohydrate, â-carotene, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Biolab Diagnostics ltd, India (Glucose GOD-PAP kits). UV Spectrophotometer double beam (Agilent Carry-60), Centrifuges (Remi) and Electronic balance (Wensar) were used.

Preparation of Plant extracts

The dried rhizomes were bought at a nearby store. The dried rhizomes of the turmeric

plant were ground up into a coarse powder and fully extracted using a Soxhlet device and 95% ethanol. The full ethanol extract was vacuum-concentrated until it was thick like syrup [14].

DPPH activity

The DPPH assay was used to test how well the turmeric extract could get rid of free radicals in the lab. Water was used as the solvent to dissolve the extract. Three milliliters of a 0.1 mM DPPH solution made in methanol were mixed with 0.1 milliliter of the test extract. The mixture stayed dark for thirty minutes at room temperature. We used the absorbance at 517 nm to measure how much the DPPH free radicals were reduced. We used the following method to find the percentage of scavenging activities (% inhibition) for different concentrations of extract fractions [22].

$$\% \text{ Radical scavenging activity } = \frac{Ac - As}{Ac} \times 100$$

Ac and As denote the absorbance of the control and the sample, respectively.

Reductive ability

It was mixed with 2.5 ml of 1% potassium ferricyanide and 1 ml of extract fractions at different strengths (25, 50, 100, 200, and 400 g/ml). Then, 2.5 ml of pH 6.6 phosphate buffer was added. The mixture was kept at 50 °C for 20 minutes. What we did was measure the absorption at 700 nm after adding 2.5 cc of 10% TCA, centrifuging the mixture at 3000 rpm for 10 minutes, and then adding 2.5 ml of water and 0.5 ml of FeCl3 (0.1%) to the 2.5 ml of extracted supernatant. A higher absorbance level meant that the reaction mixture could reduce things more effectively. The higher absorption of the reaction mixture showed that the reducing power had been increased [23].

Animal Experiments

The Institutional Animal Ethical Committee of the Anuradha College of Pharmacy, Chikhali District Buldhana, Maharashtra, India, granted us approval with approval number IAEC/2010/2011 (Registration Number 751/03/abc/CPCSEA) for this study. The rats were housed in a controlled environment with a temperature of 25 ± 3ÚC and a relative humidity ranging 35–60%. Wistar rats with a body weight of 150–200 grammes. Rats were given an alloxan solution in pure water (120 mg/kg) to induce diabetes after going without food or liquids the previous evening. After giving alloxan for 72 hours, blood samples were taken through a cut in the back of the orbital sinus. Serum was separated by centrifuging the samples at 3000 rpm. The amounts of glucose in the serum were checked by hand. The standard sample and working reagent were added to three test tubes after the blood was moved there. After mixing the liquids in each test tube, they were kept warm for 15 minutes at 37 °C or 20 minutes at 30 °C. A UV-visible spectrophotometer set to 500 nm was used to measure the optical density of each sample. Equation 1 was used to figure out how much glucose was in the samples.

$$Glucose\left(\frac{mGc}{dl}\right) = \frac{O.D.of\ test}{O.D.of\ Standard}\ x100 \quad ...(1)$$

Experimental design

Animals were divided in groups comprising of six animals each as follows,

Group 1-Normal (control),

Group 2- Diabetic (control), administered with Alloxan (120 mg/kg body weight; ip)

Group 3- â-carotene group, â-carotene given orally (20 mg/kg) for 14 days following alloxan-induced diabetes.

Group 4- *Turmeric extract* group, (40 mg/kg) orally, for 14 days after alloxan induced diabetes.

Group 5- Co-administration, â carotene + *Turmeric extract*, administered with 20 and 40 mg/kg respectively body weight orally for 14 days after alloxan induced diabetes.

The animals were sacrificed using ether-anaesthesia after the 14-day course of drug treatment. A 2 ml blood sample is collected in a sterile test tube and subsequently centrifuged at 3000 rpm for 15 minutes. The serum was stored at a temperature of 0–4 ºC to facilitate the measurement of serum glucose levels. The weights of the livers that were removed from the sacrificed animals were recorded and used to estimate the antioxidant enzymes. The livers were rinsed with ice-cold saline [14].

Estimation of Antioxidant Enzymes

The sample was prepared for assessing its antioxidant activity by following these steps: The liver tissue, which weighed 1 gram, was washed in normal saline after collection. We centrifuged the

tissues at 3000 rpm at 4°C for 30 minutes, and then homogenized them in 10 ml of 0.15 M tris solution (pH 7.4). After that, the supernatant was collected. Gathering 900 mg of liver tissue and washing it in normal saline was the first step in preparing the sample for antioxidant activity testing (CAT). Next, we mixed the tissues with 3.0 ml of M/150 phosphate buffer (pH 7.0), spun them at 3000 rpm in a 4°C centrifuge for 1 hour, and collected the resulting supernatant [24].

Lipid Peroxidation (LPO)

Blend 1 milliliter of homogenized tissue with 1 milliliter of normal saline (0.9% w/v) and 2 milliliters of a 10% TCA solution. To isolate the proteins, we centrifuged a 3000 g mixture for 10 minutes at room temperature. When 2 milliliters of supernatant were heated to 95 degrees Celsius for 60 minutes with 0.5 milliliters of a 1.0% TBA solution, pink MDA was generated. The UV spectrophotometer measured the samples' OD at 532 nm [25]. Equation 2 expressed the lipid peroxides as nM of MDA/mg wet tissue, with an extinction coefficient of 1.56 x 105 M-1 cm[1].

$$\% \ Lipid \ in\square ibition \frac{[A0-A1]}{A0} x100$$

...(2)

Where, A_0 & A_1 are absorbance of the control and sample extract.

Glutathione (GSH)

A solution of 0.02 M EDTA and 0.1 ml of samples should be mixed together and then left on ice for 10 minutes. After that, 2 ml of purified water and 0.5 ml of 50% w/v TCA were mixed together. The juice was put in the fridge for ten to fifteen minutes and then centrifuged at 3000 rpm for fifteen minutes. Two milliliters of 0.4M Tris buffer were added after 1 milliliter of supernatant was collected. After that, 0.05 ml of DTNB solution was added using a blender and mixed well. In about two to three minutes, a spectrophotometer was used to measure the optical density at 412 nm with a reagent blank as the standard [14, 26]. At the same time, the right rules were being followed. Equation 3 for GSH,

$$\% \ GSH \ inhibition = \frac{(A0-A1)}{A0} x100$$

...(3)

Where, A_0 & A_1 are absorbance of the control and sample extract.

Catalase (CAT):

In phosphate buffer at pH 7.0, ten microliters of samples were introduced into a tube containing 3.0 milliliters of hydrogen peroxide (H2O2). To find out how long it takes for the optical density to change by 0.05 of a unit, we used a blank that had the enzyme source in a phosphate buffer that did not contain H2O2 (0.16 ml of 30% w/v H2O2 was mixed with 100 ml of phosphate buffer). Absorbance was recorded at 240 nm following the enzyme addition [27-29]; Ät was measured until the optical density reached 0.45. CAT activity can be assessed as

$$Moles \ of \ hydrogen \ peroxide \ consume/min(units/mg) = \frac{2.3}{\Delta t} x ln\left(\frac{E \ initial}{E \ final}\right) x1.63x10^3$$

...(4)

Where, *E* = optical density at 240nm, and Ä *t* = time required for a decrease in the absorbance.

Superoxide Dismutase (SOD)

The cuvette had 2.8 ml of Tris-EDTA and 100 ål of Pyrogallol (2 mM) in it. It was scanned at 420 nm for three minutes. After that, 50 mL of tissue homogenates, 100 mL of pyrogallol, and 2.8 mL of Tris-EDTA were gathered and looked at at the same wavelength for three minutes. One unit of superoxide dismutase (SOD) activity is equal to the amount of the enzyme that cuts in half the time it takes for pyrogallol to turn into a free radical. Units are used to measure this in milligrams of protein per minute [29-31]. To find the enzyme unit, you can use the following formulas:

$$Rate(R) = \frac{final \ OD - initial \ OD}{3 \ min}$$

$$\% \text{ of inhibition } = \{(blank \ OD - R) \ / blank \ OD\} \ x \ 100$$

Liver Weight

On the 15 day, all animals were sacrificed. Livers were removed, rinsed in ice-cold saline, blotted dry, and weighed with a precision digital balance. The liver weights were documented in grams [30, 31].

Histopathological examination

We isolated the pancreases and submerged the samples in 10% formalin for a minimum of

24 hours to fix them. We subsequently embedded the tissue in paraffin wax, sectioned it, and examined the sections under a light microscope for histological alterations [31, 32].

Statistical analysis

The results were shown as Mean ± S.E.M. for the treatment group compared to the normal group and as $P < 0.001$ for the experimental groups compared to the diabetic (control) group. One-way ANOVA and Dunnett's test were used to find significance.

RESULTS AND DISCUSSION

***In-vitro* activity of Drug**

DPPH Radical Scavenging Activity

Ascorbic acid was used as a baseline to measure and compare the DPPH radical scavenging activity. The IC50 values for ascorbic acid were 75 ìg/ml and for turmeric extract they were 110 ìg/ml. The widely available radical DPPH is lowered by the antioxidant, which shows that the body is responding. DPPH is a stable free radical with a nitrogen core that can quickly accept an electron or hydrogen radical, turning into a stable diamagnetic molecule [33, 34]. When the right reducing agents come in contact with DPPH radicals, the electrons pair up and the matching hydrazine is made. Antioxidants cause a decline in the DPPH radical's absorbance at 517 nm, which assesses its reduction capacity.

Table 1 and figure 1 presents the DPPH radical scavenging activities of ascorbic acid (a standard antioxidant) and turmeric extract at varying concentrations ranging from 20 to 140 µg/ml. The percentage inhibition of free radicals is expressed as mean ± standard deviation for each concentration. At the control (0 µg/ml), no radical scavenging activity was observed for both ascorbic acid and turmeric extract. As the concentration increases, both ascorbic acid and turmeric extract show a progressive rise in their % inhibition, demonstrating a dose-dependent antioxidant activity. Ascorbic acid exhibits higher % inhibition compared to turmeric extract at all tested concentrations, indicating its superior radical scavenging potential. At the highest tested concentration (140 µg/ml), ascorbic acid achieves a % inhibition of 65.04 ± 0.55, while turmeric extract reaches 52.89 ± 0.45 [34, 35].

Reductive Ability

In figure 2, the reducing power of turmeric extract is shown compared to the reference standard, ascorbic acid. The reducing power increased with higher amounts of the extract, just like the antioxidant activity did. Table and figure 2 presents the reductive ability of ascorbic acid and turmeric extract, measured as absorbance at various concentrations ranging from 20 to 400 µg/ml. The absorbance values are reported as mean ± standard deviation, with higher absorbance indicating greater reductive potential. At the control (0 µg/ml), both ascorbic acid and turmeric extract exhibit zero absorbance, indicating no reductive activity in the absence of the compounds. As the concentration increases, the absorbance for both ascorbic acid and turmeric extract progressively rises, demonstrating a concentration-dependent increase in reductive ability. Ascorbic acid consistently shows slightly higher absorbance values compared to turmeric extract across all tested concentrations, reflecting its stronger reducing potential. At the highest tested concentration (400 µg/ml), the absorbance for ascorbic acid is 0.672 ± 0.08, while turmeric extract shows an absorbance of 0.637 ± 0.1. These findings highlight that while turmeric extract has notable reductive ability, ascorbic acid remains the more potent reducing agent among the two. This data emphasizes the antioxidant capabilities of both compounds, supporting their potential use in oxidative stress-related applications [37, 38].

Estimation of Blood Glucose Level

In this study, rats were classified as diabetic when their blood glucose levels exceeded 200 mg/dL 48 hours after an alloxan-induced treatment. The effect of different treatments on blood glucose levels was evaluated by comparing the measurements taken on the 1st and 14th days of the experiment. As shown in Table 3, blood glucose levels significantly increased in the diabetic group after 14 days, confirming the establishment of a diabetic condition. In contrast, individual treatments with â-Carotene and Turmeric Extract led to a noticeable reduction in blood glucose levels by the 14th day. Specifically, â-Carotene treatment was associated with a decrease in glucose concentrations, suggesting its potential as a therapeutic agent in managing hyperglycemia. Similarly, Turmeric extract, known for its active

compound curcumin, showed significant effects in lowering blood glucose levels, potentially through its anti-inflammatory and antioxidant properties, which may help reduce insulin resistance.

However, the most pronounced effect was observed in the group receiving the combined treatment of â-Carotene and Turmeric Extract. As illustrated in Figure 3a and Figure 4b, the concomitant administration of both agents resulted in a marked reduction in blood glucose levels, surpassing the effects of the individual treatments. This synergistic action could be attributed to the complementary mechanisms of action of â-Carotene and Turmeric extract.

â-Carotene is a potent antioxidant that has been shown to reduce oxidative stress, a key factor in the pathogenesis of diabetes [39]. It also helps modulate insulin secretion by improving pancreatic â-cell function [40]. Furthermore, â-Carotene has been linked to enhanced glucose metabolism and improved insulin sensitivity [41].

Turmeric Extract, specifically its bioactive compound curcumin, exerts its effects through multiple mechanisms. Curcumin has been shown to improve insulin sensitivity, reduce oxidative stress, and lower inflammatory markers associated with diabetes. Additionally, curcumin has been demonstrated to modulate several

Table 1. DPPH Radical Scavenging Activities

Sr. No.	Concentration µg/ml	% inhibition of Ascorbic acid	% inhibition of *turmeric extract*
1	Control	0.00	0.00
2	20	40.5 ± 1.25	39 ± 1.31
3	40	43.25 ± 1.21	42.06 ± 1.22
4	80	52.02 ± 2.44	46.9 ± 1.52
5	100	56.88 ± 1.78	48.6 ± 1.34
6	120	61.22 ± 0.68	51.18 ± 0.78
7	140	65.04 ± 0.55	52.89 ± 0.45

Table 2. Estimation of reductive ability

Sr. No.	Concentration µg/ml	Absorbance of Ascorbic acid	Absorbance of *turmeric extract*
1	Control	0.00	0.00
2	20	0.509 ±0.06	0.480 ± 0.2
3	50	0.534 ± 0.07	0.522 ±0.09
4	100	0.580 ± 0.06	0.566 ± 0.04
5	200	0.629 ± 0.5	0.598 ± 0.06
6	400	0.672 ± 0.08	0.637 ± 0.1

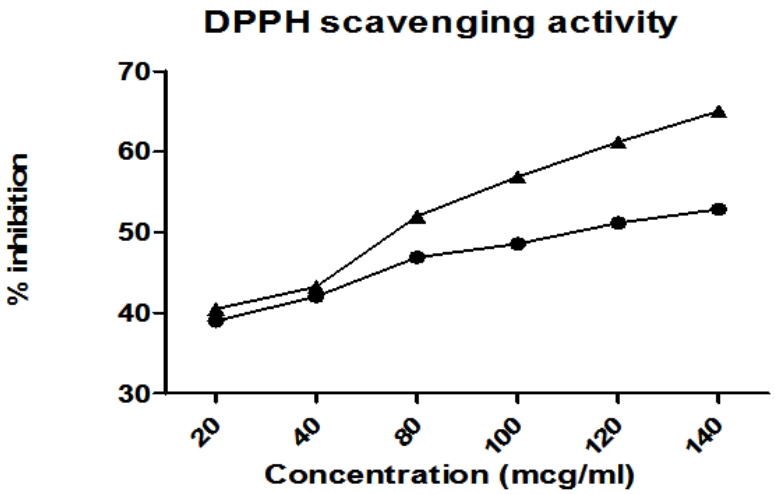


Fig. 1. DPPH Scavenging Activity (Triangle (Δ): % inhibition of DPPH radicals by ascorbic acid, showing higher scavenging activity across various concentrations. (Circle (•): % inhibition by turmeric extract, indicating dose-dependent activity but lower than ascorbic acid.)

signaling pathways, including the AMPK pathway, which plays a crucial role in regulating glucose homeostasis [42].

The combination of these two natural compounds could provide a novel and effective approach to managing diabetes, particularly by reducing oxidative stress and inflammation, both of which contribute to the progression of the disease. The findings presented in Table 3 and Figure 3a and 3b clearly support the potential of â-Carotene and Turmeric extract, both individually and in combination, as therapeutic agents in the management of hyperglycemia. This study emphasizes the importance of exploring natural compounds, such as â-Carotene and Turmeric extract, as complementary or adjunctive therapies to conventional treatments for diabetes. Further investigation is warranted to explore the underlying molecular mechanisms and potential clinical applications of these compounds.

When blood glucose levels in rats exceeded 200 mg/dl 48 hours after an alloxan treatment, we classified them as diabetic. Table 3 illustrates the comparison of blood glucose levels between the first and 14th days. It indicates that the diabetic group exhibits a significant increase in blood glucose levels after 14 days. Individual treatments with â-Carotene and Turmeric extract demonstrate a reduction in blood glucose levels following 14 days of treatment, while the concomitant treatment

Table 3. Estimation of Blood glucose level before treatment and after treatment

Animals groups	Blood glucose level when treatment starts (mg/dl)*	Blood glucose level after treatment of 14th days (mg/dl)*
Normal (control)	170.44 ± 3.09	171.39 ± 3.33
Diabetic	249.63 ± 9.56	300.56 ± 12.64
â Carotene	228.52 ± 6.18	184.31 ± 6.52
Turmeric extract	255.30 ± 8.64	178.84 ± 18.04
Co- administration	248.12 ± 11.50	175.25 ± 3.80

*Values are expressed as Mean ± S.E.M.; n=6 for each group. The drug treatment lasted for 14 days. At a significance level of $P < 0.001$, the normal group was compared to the diabetes treated group. Subjects chosen for scientific investigations A one-way analysis of variance (ANOVA) followed by Dunnett's test was used to evaluate significance in comparison to the control group.

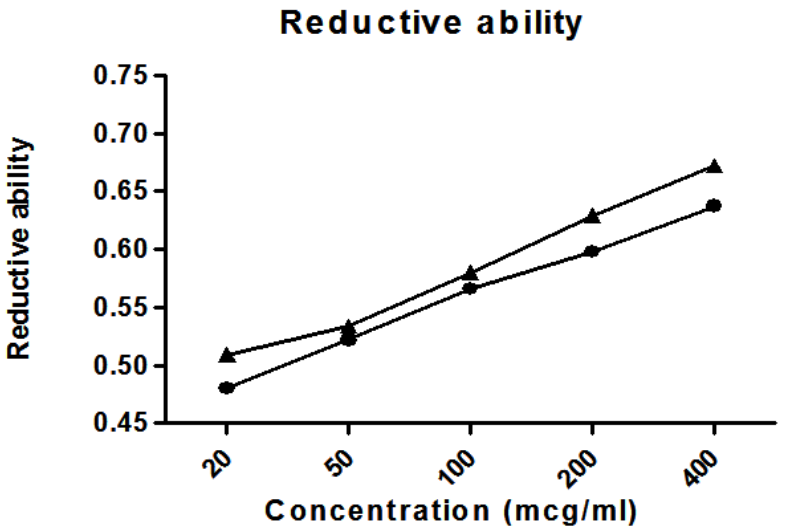


Fig. 2. Reductive ability ((Triangle (Δ): Represents the absorbance values indicating the reductive ability of ascorbic acid across different concentrations (0–400 µg/ml). (Circle (•): Depicts the absorbance values for turmeric extract, showing a concentration-dependent increase in reductive ability.)

of â-Carotene and Turmeric extract results in a significant decrease in sugar levels.

Figures 3a and 3b show an increase in blood glucose levels (300.56 ± 12.64 mg/dL) indicates that hyperglycemia is worsening. With the â Carotene treatment shows significantly reduced blood glucose levels (184.31 ± 6.52 mg/dL) suggest an antihyperglycemic effect. Glycaemic management improves as blood glucose levels decrease more efficiently (178.84 ± 18.04 mg/dL) with Turmeric extract. The combination's considerable decline in glucose levels (175.25 ± 3.80 mg/dL) suggests a synergistic effect, approaching normal values.

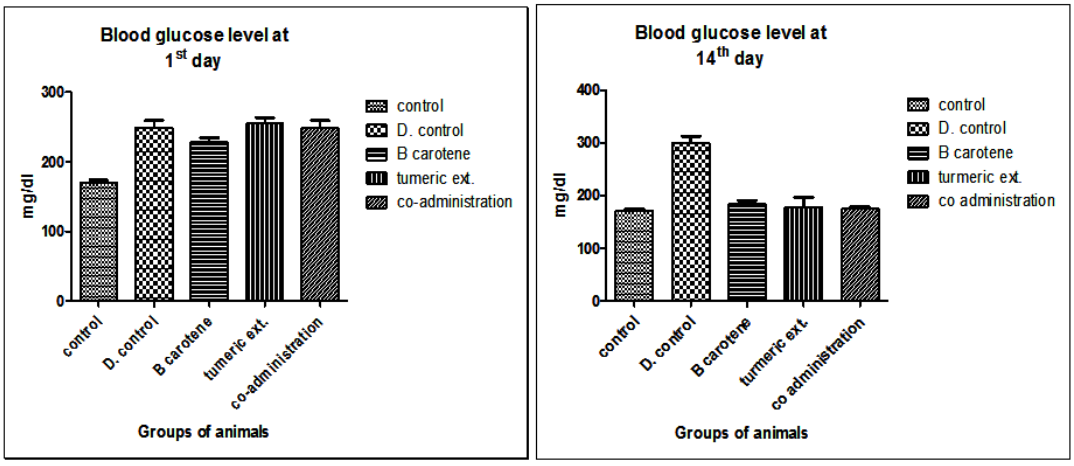
Effect on Tissue with its related Marker Enzyme LPO

An increase in Malondialdehyde MDA levels in the alloxan-induced diabetes group relative to the normal group suggests that LPO is more active in this group. Administration of â-carotene And *Turmeric extract* individually and with co-administration decreases the MDA concentration to normal level. The data are shown in Table 4.

Table 4. Effect of â Carotene and *Turmeric extract* single and combination on SOD, LPO, GSH and CAT levels of Diabetic rats (After 14 days treatment)

Animals groups	SOD (U/mg wet tissue)	LPO (nano mole/mg)	CAT (µM of H_2O_2 decomposed/min/ mg wet tissue)	GSH (ug/mg wet tissue)
Normal (control)	64.79 ± 2.72	20.47±0.730	1.038± 0.007	35.69±0.770
Diabetic	41.30±3.77	60.87±1.42	0.765±0.03	28.59±0.743
β Carotene	49.68± 2.19	51.91±1.35	0.8945±0.018	31.06±0.821
Turmeric extract	43.52±1.20	54.70± 1.47	0.905± 0.037	29.26±0.53
β Carotene + *Turmeric extract*	58.97±1.26	36.01±0.837	0.931±0.025	32.66±0.554

Values are expressed as Mean ± S.E.M.; n = 6 for each group. The drug treatment duration was 14 days. P < 0.001 We compared the diabetic-treated group with the normal group and the experimental groups with the toxicant group, using one-way ANOVA and Dunnett's test to assess significance.



(3a) (3b)

Fig. (3a): Blood glucose levels (mg/dL) on the 1st day of experiment initiation across all groups: normal control, diabetic control, â-carotene-treated, turmeric extract-treated, and combination-treated rats. **(3b)**: Blood glucose levels (mg/dL) on the 14th day of experiment initiation. The combination treatment shows the most significant reduction in blood glucose levels compared to diabetic control. Data are expressed as ± SEM (n = 6); significant differences ($p < 0.05$) are indicated relative to normal and diabetic controls.

Effect on Non-Enzymatic Antioxidant GSH

The group that was given alloxan and became diabetic had lower amounts of GSH, which is the main non-enzymatic antioxidant, compared to the control group. Normalization of GSH levels happens when â-carotene and turmeric extract are given, either separately or together (Table 3 and 4).

Effect on Antioxidant Enzyme

The major antioxidant such as CAT, SOD were found to be decreased in alloxan induced diabetic group (P<0.001) as compared to the normal group. Administration of the â-carotene and *Turmeric extract* individually and with co-administration after to alloxan induced diabetes restore the activity of CAT, SOD to normal level as compared to the Diabetic group. The data are shown in table 4.

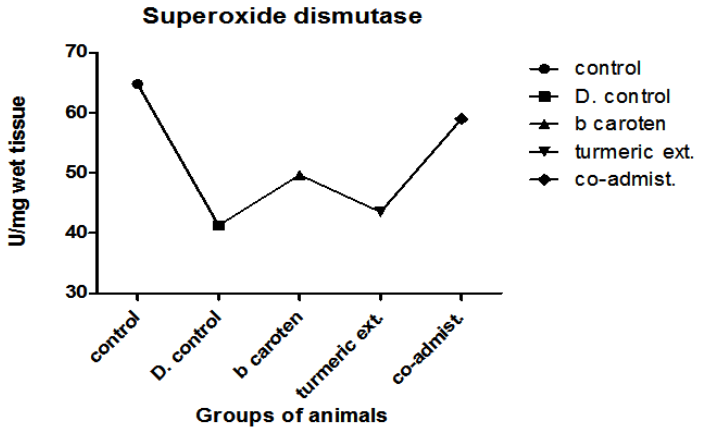


Fig. 5. Superoxide Dismutase level after 14 days treatment. (SOD activity (U/mg protein) in different groups: normal control, diabetic control, â-carotene-treated, turmeric extract-treated and combination-treated rats. The combination group shows the highest recovery of SOD levels among treatments. Data are expressed as ± SEM (n = 6); significant differences ($p < 0.05$) are indicated relative to normal and diabetic controls.)

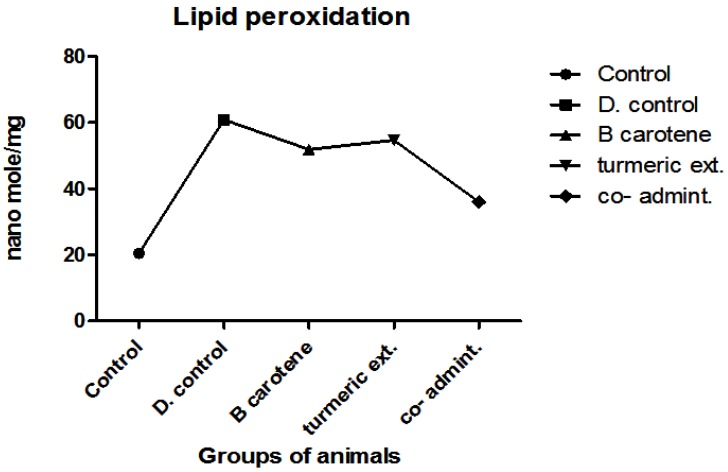


Fig. 6. Lipid Peroxidase level after 14 days treatment. (LPO levels (nmol/mg protein) in different groups: normal control, diabetic control, â-carotene-treated, turmeric extract-treated, and combination-treated rats. Diabetic control rats show the highest LPO levels, while the combination treatment significantly reduces LPO, nearing normal levels. Data are expressed as ± SEM (n = 6); significant differences ($p < 0.05$) are indicated relative to normal and diabetic controls.)

The SOD levels are the highest in the normal group (64.79 ± 2.72), indicating robust antioxidant defense under normal conditions. Diabetic rats show significantly reduced SOD activity (41.30 ± 3.77), reflecting oxidative stress and impaired enzymatic antioxidant defense. Treatment with â-carotene partially restores SOD activity (49.68 ± 2.19), suggesting its role in mitigating oxidative stress. Turmeric extract leads to a slight increase in SOD activity (43.52 ± 1.20), though less effectively than â-carotene. The combination shows the highest improvement among treatments (58.97 ± 1.26), suggesting a synergistic effect in enhancing enzymatic antioxidant defense.

The lowest LPO levels (20.47 ± 0.730) are observed in normal rats, indicating minimal lipid membrane damage. Diabetic rats have the

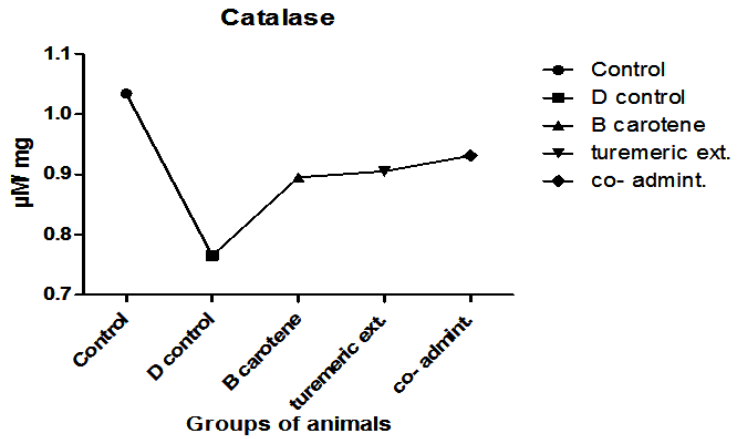


Fig. 7. Catalase level after 14 days treatment (Catalase activity (µM of H, O, decomposed/min/mg protein) in different groups: normal control, diabetic control, â-carotene-treated, turmeric extract-treated, and combination-treated rats. Diabetic control rats exhibit the lowest CAT activity, while the combination treatment shows the highest recovery, nearing normal levels. Data are expressed as ± SEM (n = 6); significant differences ($p < 0.05$) are indicated relative to normal and diabetic controls.)

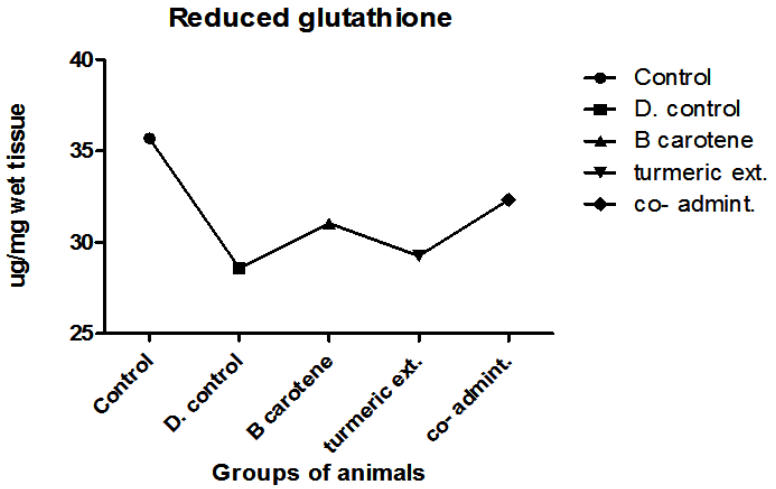


Fig. 8. Reduced Glutathione level after 14 days treatment (GSH levels (µg/mg protein) in different groups: normal control, diabetic control, â-carotene-treated, turmeric extract-treated, and combination-treated rats. Diabetic control rats show the lowest GSH levels, while the combination treatment significantly restores GSH levels, approaching normal values. Data are expressed as ± SEM (n = 6); significant differences ($p < 0.05$) are indicated relative to normal and diabetic controls.)

highest LPO levels (60.87 ± 1.42), showing increased lipid peroxidation due to oxidative stress. Treatment with â-carotene reduces LPO levels (51.91 ± 1.35), indicating its antioxidant properties. Turmeric extract also lowers LPO levels (54.70 ± 1.47), though less effectively than â-carotene. The combination significantly reduces LPO (36.01 ± 0.837), nearing normal levels, suggesting a powerful reduction in oxidative damage.

Normal rats exhibit the highest CAT activity (1.038 ± 0.007), indicating efficient hydrogen peroxide breakdown. CAT activity is reduced in diabetic rats (0.765 ± 0.03), demonstrating compromised enzymatic defense against hydrogen peroxide. â-Carotene treatment increases CAT activity (0.8945 ± 0.018), showing its role in improving antioxidant defenses. Turmeric extract similarly increases CAT activity (0.905 ± 0.037), slightly outperforming â-carotene. The combination results in the highest CAT activity among treatments (0.931 ± 0.025), reflecting synergistic protection against oxidative stress.

Normal rats have the highest GSH levels (35.69 ± 0.770), indicative of a robust non-enzymatic antioxidant defense. Diabetic rats show reduced GSH levels (28.59 ± 0.743), reflecting depleted antioxidant reserves under oxidative stress. â-Carotene treatment partially restores GSH levels (31.06 ± 0.821), enhancing the non-enzymatic antioxidant system. Turmeric extract shows moderate improvement in GSH levels (29.26 ± 0.53). The combination yields the highest GSH levels among treated groups (32.66 ± 0.554), nearing normal values and suggesting synergistic enhancement of antioxidant reserves.

Liver Weight

Diabetic control rats show significantly reduced liver weight (5.516 ± 0.186 g) compared to the normal control (7.712 ± 0.050 g), indicating liver damage or atrophy due to diabetes-induced oxidative stress. Treatment with â-carotene (7.216 ± 0.197 g) and turmeric extract (7.146 ± 0.244 g) partially restores liver weight, demonstrating their hepatoprotective effects. The combination of â-carotene and turmeric extract (7.612 ± 0.121 g) nearly normalizes liver weight, suggesting

Table 5. Liver weight of animals after 14th day treatment

Animals groups	Weight of liver after treatment in gm
Normal(control)	7.712 ± 0.050
Diabetic(control)	5.516 ± 0.186
β Carotene	7.216 ± 0.197
Turmeric extract	7.146 ± 0.244
β Carotene + *Turmeric extract*	7.612 ± 0.121

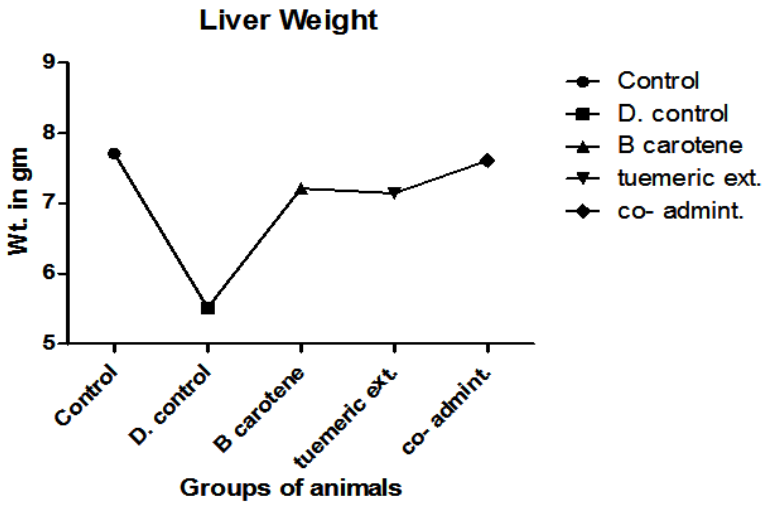


Fig. 9. Liver weight of animals after 14th day treatment (Liver weights (g), Diabetic control rats exhibit significantly reduced liver weight, while the combination treatment nearly restores liver weight to normal levels. Data are expressed as mean ± SD (n = 6); significant differences ($p < 0.05$) are indicated relative to normal and diabetic controls.)

a synergistic effect in protecting the liver from diabetes-induced damage.

Histology

The pancreas segment (A-E) exhibits a range of outcomes in experimental animals. The control group exhibits no substantial alterations in cellular structure (A) in comparison. The diabetic group (B) had a greater extent of pathological alterations, including islet necrosis, reduced islet size, and cellular infiltration. The â-carotene group (C) exhibited a marginal recovery phase, and cell necrosis of the islets was noted in comparison to the diabetes condition. The turmeric group (D) exhibits a notable improvement in cellular structure compared to the diabetic group. Group (E) the simultaneous administration of these drugs significantly rejuvenates pancreatic beta cells. Here, the islet size is maximal as compared to the other groups.

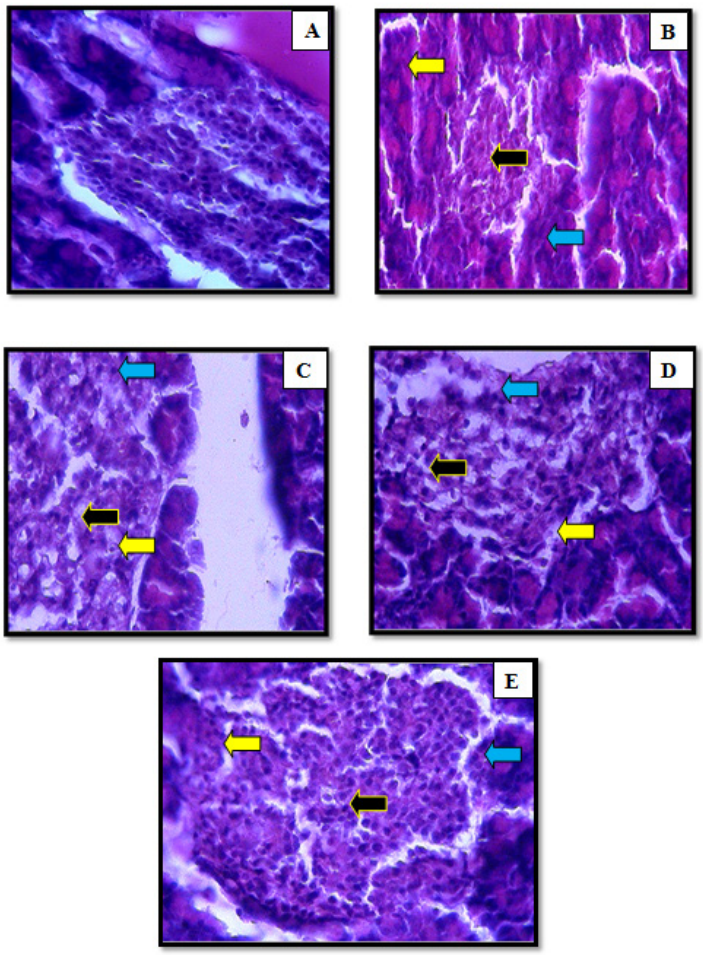


Fig. 10. Photomicrographs of pancreatic sections A, B,C, D, E. A) Photomicrographs of pancreatic sections from a control group rats. (B) Diabetic group rats without any treatment, not only showing ruptured islets but also shrinkage and degenerated cells including increased intracellular space. C) β-Carotene group rat's section compare with the diabetic rats shows slight recovery in pancreatic histoarchitecture. D) *Turmeric extract* group compare with the diabetic rats, shows better recovery compare with diabetic rats. E) β-Carotene + *Turmeric extract* group shows best recovery of islet cell which will be comparatively similar with control group (H and E stain; original magnification × 400) (Black arrow: - Necrosis of islets. Blue arrow: - Cellular infiltration. Yellow arrow: -Degeneration of acinar cells)

CONCLUSIONS

This study investigates the effects of â-carotene and turmeric extract on antioxidant status in alloxan-induced diabetic rats. After administering alloxan, blood glucose levels were assessed, and the study found that both â-carotene and turmeric extract showed antioxidant activity in a concentration-dependent manner. The study also found elevated MDA levels in diabetic subjects, indicating increased production of free radicals. Treatment with â-carotene and turmeric resulted in a reduction of MDA levels, with a modest decrease observed with each prescription individually but a significant reduction noted when both medications were administered together. The study also found that diabetic rats had lower levels of GSH, possibly due to their increased use of GSH to remove free radicals.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

The Institutional Animal Ethical Committee (IAEC) must be consulted before commencing any form of experiment. We received approval with approval number IAEC/2010/2011 from the Anuradha College of Pharmacy's Institutional Animal Ethical Committee in Chikhali District Buldhana (Registration Number 751/03/abc/CPCSEA).

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required

Clinical Trial Registration

This research does not involve any clinical trials

Author contributions

Rahul Sable and Rajendra Mogal wrote and revised the first draft; After English editing by Eknath Ahire; Proofreading performed by Rakesh shelake, all authors are agreed the final version and submitted the article.

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