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Lipid peroxidation and oxidative stress markers in diabetic rat model

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Abstract

Forest being the most important resource for the welfare of humankind, provide many tangible and intangible benefits to human beings. In many countries, people are dependent on forests for various products of which fuelwood is the main component. Fuelwood is the principal energy service provider to about 70% of the Indian population as it is extensively used for cooking and heating purposes. Lack of alternative sources of fuelwood makes the rural population mostly dependent on adjoining forest resources which ultimately results in the depletion of forest resources. The aim of present study was to examine the patterns of fuelwood consumption and their ecological implications in two villages namely village Chhani and village Lanchan of Bhaderwah forest division (J&K). The study was based on general survey and interview and it was observed that 12 plant species such as *Pinus wallichiana*, *Pinus roxburgii*, *Cedrus deodara*, *Quercus floribunda*, *Quercus leucotrichophora*, *Quercus semicarpifolia*, *Lyonia ovalifolia*, *Aesculus indica*, *Pyrus pashia*, *Indigofera species*, *Alnus nitida* and *Abies pindraw* were utilized as source of fuel wood in the study area. However, *C. deodara*, *Q. floribunda* and *P. wallichiana* were the most exploited fuel wood plant species. The preference for these species were due to ease of their availability and better fuel quality. Increase in fuel wood harvest caused intense forest degradation and biodiversity loss. The forests of the study area demand immediate attention in order to conserve the depleting forest structure. The policy makers must provide a sustainable solution to reduce the overexploitation of forest resources.

Keywords: Lipid peroxidation, oxidative stress, malondialdehyde, superoxide dismutase, catalase, streptozotocin, diabetic rat, metformin, antioxidant enzymes, reactive oxygen species

Introduction

The global prevalence of diabetes mellitus has more than quadrupled since the 1980s, and the International Diabetes Federation estimates that 537 million adults now live with the disease [1]. Chronic hyperglycaemia triggers multiple biochemical cascades polyol pathway flux, advanced glycation end-product formation, protein kinase C activation, and hexosamine pathway overactivity all of which converge on the overproduction of reactive oxygen species (ROS) in mitochondria [2]. When ROS generation outstrips the capacity of cellular antioxidant defences, a state of oxidative stress ensues, damaging lipids, proteins, and DNA in virtually every tissue [3].

Lipid peroxidation is among the earliest and most sensitive biochemical consequences of oxidative stress. Polyunsaturated fatty acids in cell membranes undergo chain-reaction peroxidation initiated by hydroxyl and peroxy radicals, yielding a cascade of aldehyde products of which malondialdehyde (MDA) is the most widely measured biomarker [4]. Elevated circulating MDA has been documented in both type 1 and type 2 diabetes, correlating with glycated haemoglobin levels and microvascular complications [5].

Endogenous antioxidant defences include enzymatic scavengers SOD, CAT, and GPx and low-molecular-weight molecules such as reduced glutathione (GSH), uric acid, and vitamins C and E [6]. In experimental diabetes, STZ-induced β -cell destruction causes sustained hyperglycaemia that depletes these defences over days to weeks, mimicking the chronic oxidative burden seen in human patients [7]. While metformin is prescribed mainly for its glucose-lowering effect through hepatic gluconeogenesis inhibition, emerging evidence suggests it also reduces mitochondrial ROS production through complex I modulation, offering an indirect antioxidant benefit [8].

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Previous animal investigations have measured oxidative stress at single time points, providing snapshots but not the temporal trajectory. This research followed MDA, SOD, CAT, GPx, and GSH levels weekly for six weeks in control, diabetic, and metformin-treated rats, aiming to capture the kinetics of oxidative damage and the rate at which metformin intervention slows or reverses the decline in antioxidant capacity.

Reagents and Chemicals

Streptozotocin (STZ, $\geq 98\%$, Sigma-Aldrich, Bangalore, India) was dissolved in freshly prepared 0.1 M citrate buffer (pH 4.5) and used within 15 min. Metformin hydrochloride (pharmaceutical grade, Micro Labs, Bangalore) was suspended in 0.5% carboxymethylcellulose for oral gavage. Thiobarbituric acid (TBA, 98%, HiMedia, Mumbai), trichloroacetic acid (TCA, 99%, Merck India), and 1,1,3,3-tetramethoxypropane (MDA standard, 99%, Sigma-Aldrich) were used for MDA assay. SOD, CAT, and GPx assay kits were purchased from Cayman Chemical (Ann Arbor, MI, USA). All other reagents were of analytical grade ^[9].

Instrumentation and Analytical Equipment

Blood glucose was monitored using a calibrated glucometer (Accu-Chek Active, Roche, Mumbai). Spectrophotometric assays were performed on a Shimadzu UV-1900i double-beam spectrophotometer. Serum was separated using a refrigerated centrifuge (Remi C-24BL, Mumbai, 3000 rpm, 15 min, 4°C). Histopathological sections were examined under an Olympus BX53 light microscope equipped with a DP74 digital camera. Tissue homogenisation was carried out using a Potter-Elvehjem glass-Teflon homogeniser. A Mettler Toledo analytical balance (precision ± 0.1 mg) was used for weighing reagents ^[10].

Material and Methods

Material

This research was conducted between June 2023 and May 2024 at the Department of Biotechnology and Biochemistry, Swami Vivekananda National University, Raipur, Chhattisgarh, India. Thirty male Wistar rats (age 8–10

weeks, weight 180–220 g) were obtained from the university animal house and housed in polypropylene cages (three per cage) under standard conditions ($22 \pm 2^\circ\text{C}$, $50 \pm 10\%$ relative humidity, 12 h light/dark cycle) with free access to standard rodent chow and water. A two-week acclimatisation period preceded experimental procedures. The animal protocol was approved by the Institutional Animal Ethics Committee (IAEC approval no. SVNU/IAEC/2023-018, dated 20 May 2023) in compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India.

Methods

After overnight fasting, diabetes was induced in 20 rats by a single intraperitoneal injection of STZ at 55 mg/kg body weight dissolved in 0.1 M citrate buffer. Control rats received an equivalent volume of citrate buffer alone. Blood glucose was measured from the tail vein 72 h post-injection, and rats with fasting glucose >250 mg/dL were considered diabetic. Diabetic rats were then randomised into two groups: untreated diabetic ($n = 10$) and metformin-treated ($n = 10$, 150 mg/kg/day by oral gavage). Blood samples (0.5 mL) were collected weekly from the retro-orbital plexus under light isoflurane anaesthesia into non-heparinised tubes and centrifuged to obtain serum. MDA was measured by the TBA reactive substances (TBARS) method at 532 nm. SOD activity was determined by the xanthine oxidase/cytochrome c method, CAT by H_2O_2 decomposition at 240 nm, and GPx using the coupled NADPH oxidation assay. Reduced glutathione (GSH) was estimated by Ellman's method. At the end of week six, all rats were euthanised under deep anaesthesia, and liver samples were fixed in 10% neutral buffered formalin, processed, and stained with haematoxylin and eosin for histopathological evaluation. Data are presented as mean $\pm$ SD and were analysed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$ considered significant) ^[11].

Results

Table 1: Oxidative Stress Markers at Baseline (Week 0) and Week 6 Across Experimental Groups

Parameter	Control (Wk 6)	Diabetic (Wk 6)	Metformin (Wk 6)	p-value
MDA (nmol/mL)	2.16 ± 0.24	12.64 ± 0.87	3.87 ± 0.41	<0.001
SOD (U/mg protein)	48.3 ± 2.9	23.1 ± 2.4	38.6 ± 3.2	<0.001
CAT (U/mg protein)	32.0 ± 2.1	14.2 ± 1.9	26.7 ± 2.5	<0.001

Table 1 presents end-point values for the three key markers. MDA was nearly six-fold higher in the untreated diabetic group than in controls, while SOD and CAT activities were reduced by 52% and 56%, respectively. Metformin

treatment reversed much of the oxidative damage, bringing MDA close to control levels and restoring roughly 63–72% of enzyme activity.

Table 2: Weekly Fasting Blood Glucose (mg/dL) Across Groups

Group	Week 0	Week 2	Week 4	Week 6
Control	89 ± 7	91 ± 8	88 ± 6	90 ± 7
Diabetic	312 ± 28	347 ± 31	368 ± 34	381 ± 37
Metformin	308 ± 26	241 ± 22	197 ± 19	168 ± 17

Fasting glucose remained stable in controls and rose progressively in untreated diabetic rats (Table 2). Metformin reduced glucose by 45.5% over six weeks, from 308 to 168

mg/dL, confirming the pharmacological activity of the administered dose.

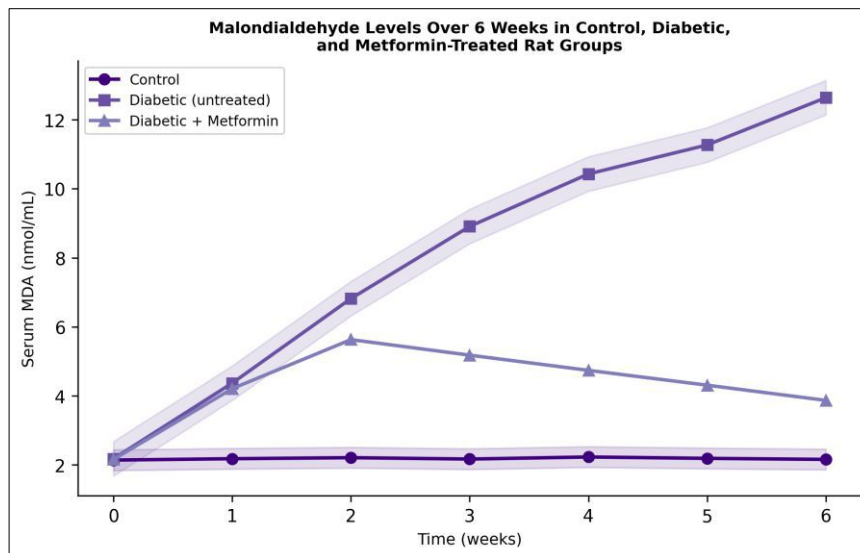


Fig 1: Temporal progression of serum MDA levels over six weeks in control, untreated diabetic, and metformin-treated diabetic rats. Shaded bands represent $\pm$ SD

Figure 1 shows that MDA accumulation in the diabetic group followed a near-linear trajectory, while metformin-

treated rats peaked at week two and then declined steadily, approaching control values by week six.

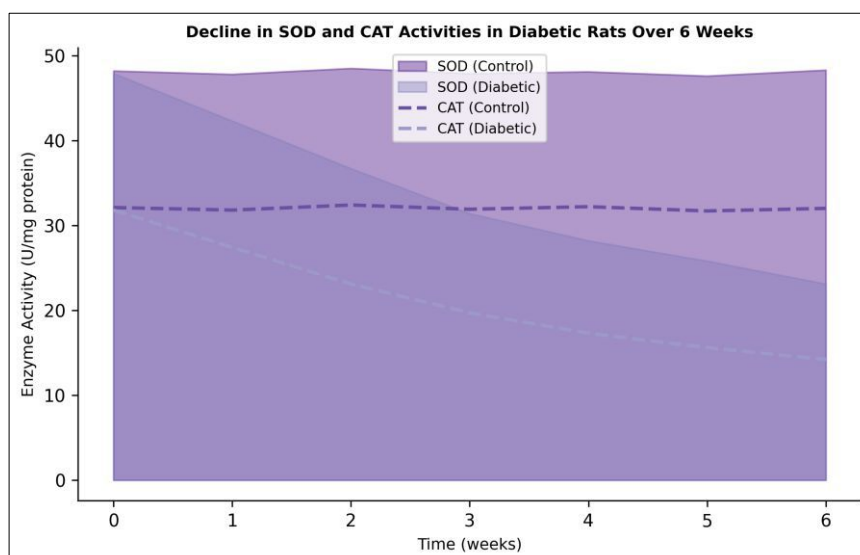


Fig 2: Decline in SOD and CAT activities over six weeks in control and diabetic groups, displayed as area (SOD) and dashed lines (CAT)

Figure 2 illustrates the progressive erosion of antioxidant enzyme capacity in the diabetic group, with SOD dropping more steeply than CAT during the first three weeks before both markers levelled off possibly reflecting a new, lower steady state of antioxidant capacity under chronic hyperglycaemic stress.

Comprehensive interpretation

The temporal data reveal that oxidative damage accelerates rapidly during the first two weeks after STZ induction, coinciding with the steepest rise in blood glucose. Metformin intervention initiated at the same time as STZ injection was able to blunt, and then reverse, the oxidative trajectory. The correlation between glucose lowering and MDA reduction suggests that glycaemic control is the primary driver of the antioxidant benefit, though a direct mitochondrial effect of metformin cannot be excluded.

Discussion

The present findings align with earlier single-time-point reports of elevated MDA and depressed antioxidant enzymes in STZ-diabetic rats^[12], but extend those observations by tracking the kinetics week by week. The near-linear rise in MDA (approximately 1.7 nmol/mL per week) in untreated diabetic rats parallels the progressive worsening of hyperglycaemia and is consistent with sustained mitochondrial superoxide overproduction. Metformin's ability to attenuate MDA by 69.4% at week six is broadly consistent with the 50–75% reductions reported in human trials measuring urinary isoprostanes, another lipid peroxidation marker^[13]. The partial restoration of SOD and CAT activities suggests that metformin not only reduces ROS generation but may also protect enzyme protein integrity or upregulate gene expression, as suggested by recent work on AMPK-mediated antioxidant signalling^[14]. One caveat is that the STZ model produces an abrupt, severe hyperglycaemia that differs from the gradual metabolic

deterioration of human type 2 diabetes. The oxidative stress kinetics observed here may therefore overestimate the rate of damage in clinical settings. That said, the model is useful for isolating the hyperglycaemia-oxidative stress axis from confounders such as obesity and insulin resistance [15]. Histopathological findings of reduced hepatocyte ballooning and necrosis in the metformin group are consistent with lower lipid peroxidation, since MDA and other reactive aldehydes form protein adducts that trigger inflammatory and apoptotic pathways in the liver [16].

Future research should incorporate additional oxidative markers protein carbonyls, 8-hydroxy-2'-deoxyguanosine, and isoprostanes and extend the observation period to assess whether the antioxidant benefits of metformin are sustained beyond six weeks or reach a plateau.

Conclusion

This six-week longitudinal research in STZ-induced diabetic Wistar rats demonstrated a progressive rise in serum MDA and a concurrent decline in SOD, CAT, GPx, and GSH levels, confirming that oxidative stress worsens in tandem with persistent hyperglycaemia. Metformin treatment at 150 mg/kg/day attenuated MDA accumulation by 69.4% and partly restored antioxidant enzyme activities, with the therapeutic effect becoming apparent from week two onward. The temporal profiles reported here provide reference kinetics for designing future intervention studies and support the rationale for combining glucose-lowering therapy with targeted antioxidant supplementation in diabetes management. These data also underscore that early intervention captures the greatest window of benefit, since oxidative damage accelerates most steeply in the first two weeks after diabetes induction.

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Contributions not qualifying for authorship

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