RESEARCH PAPER

ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS OF A POLYHERBAL ETHANOLIC FORMULATION IN HFHF-STZ-INDUCED METABOLIC SYNDROME IN RATS

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ABSTRACT

Objective: This study evaluated the effects of ethanolic extracts of *Anthocephalus indicus* fruit and *Terminalia arjuna* stem bark, administered individually and in combination, on oxidative stress and inflammatory biomarkers in high-fat high-fructose (HFHF) diet/low-dose streptozotocin (STZ)-induced metabolic syndrome in rats.

Methods: Male Wistar rats were fed an HFHF diet for 16 weeks followed by STZ (30 mg/kg, i.p.). Animals were divided into seven groups (n=6): normal control, sham treated control, *A. indicus* (500 mg/kg), *T. arjuna* (500 mg/kg), combined formulation (250+250 mg/kg), metformin (100 mg/kg), and atorvastatin (20 mg/kg); treatments were given orally for 21 days. Oxidative stress was assessed using superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and reduced glutathione (GSH); inflammatory status was evaluated using tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β).

Results: HFHF-STZ induction significantly impaired antioxidant status and elevated lipid peroxidation and inflammatory biomarkers. The combined formulation produced significant improvement across all seven biomarkers, restoring SOD, CAT, and GSH and reducing MDA, TNF-α, IL-6, and IL-1β toward normal levels ($p<0.001$ versus sham treated control for all parameters). Its response was comparable to or better than the reference treatments for most parameters.

Conclusion: The combined polyherbal ethanolic formulation demonstrated antioxidant and anti-inflammatory potential in HFHF-STZ-induced metabolic syndrome, supporting further investigation as a candidate approach for metabolic dysfunction.

Keywords: Metabolic syndrome; Polyherbal formulation; Oxidative stress; Inflammation; Antioxidants; Inflammatory biomarkers; *A. indicus*; *T. arjuna*.

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INTRODUCTION

Metabolic syndrome (MetS) is a multifactorial metabolic disorder characterized by the clustering of impaired glucose regulation, insulin resistance, central obesity, dyslipidemia, and hypertension, and it substantially increases the risk of type 2 diabetes mellitus and cardiovascular disease, raising the risk of type 2 diabetes fivefold and cardiovascular events twofold. The prevalence of MetS continues to rise in parallel with the global epidemic of obesity and diabetes, affecting approximately 20–30% of adults worldwide [1]. Oxidative stress is regarded as a central contributor to the initiation and progression of MetS. Metabolic abnormalities lead to increased formation of reactive oxygen species (ROS), resulting in an imbalance between oxidant production and endogenous antioxidant defences, which contributes to lipid peroxidation, membrane damage, and insulin signalling dysfunction. Superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) are the main enzymatic and non-enzymatic antioxidant defences and malondialdehyde (MDA) is a commonly employed marker of lipid peroxidation. MetS is characterised by a chronic oxidative burden that exhausts antioxidant defences by excessive consumption and persistent use of non-enzymatic reserves [2,3]. Oxidative stress is closely interconnected with chronic low-grade inflammation, and the cross-promotion of these two processes creates a feed-forward cycle considered the "common soil" from which obesity, dyslipidemia, and hypertension develop [4]. Elevated free fatty acid flux from adipose tissue further promotes ROS

generation, endothelial dysfunction, and a proinflammatory adipokine profile that impairs insulin signaling [5].

The HFHF diet combined with a low intraperitoneal dose of STZ is an established experimental approach for reproducing the cardinal features of human MetS in rodents. Diet-based approaches are considered the most cost-effective models reflecting the real causes of the syndrome [6], and combined high-carbohydrate, high-fat, fructose-containing diets closely mimic the metabolic, cardiovascular, hepatic, and pancreatic changes observed in human MetS, including visceral fat accumulation, hypertriglyceridemia, reduced insulin sensitivity, and increased lipid peroxidation. Fructose supplementation induces hypertension, fasting hyperglycemia, insulin resistance, and dyslipidemia [7,8], while high-fat feeding produces dyslipidemia, obesity, and peripheral insulin resistance. Dietary models alone, however, rarely produce frank hyperglycemia, because adequate beta-cell secretory capacity can compensate for insulin resistance [9,10]. The addition of low-dose STZ addresses this limitation: prolonged high-fat feeding leads to insulin resistance, and diabetes develops only in insulin-resistant, high-fat-fed rats following low-dose STZ administration [11]. Low-dose STZ induces partial beta-cell dysfunction rather than complete beta-cell ablation, reproducing the combination of peripheral insulin resistance and relative insulin deficiency characteristic of human type 2 diabetes [12]. Low doses in the range of 25–35 mg/kg yield stable, reproducible phenotypes with moderate hyperglycemia, insulin resistance, and dyslipidemia, without the severe toxicity associated with high-dose STZ. Such models also allow metabolic, oxidative, and inflammatory abnormalities to be investigated simultaneously [13,14].

Natural products and medicinal plants have received considerable attention as sources of bioactive compounds with antioxidant and anti-inflammatory properties. Polyherbal formulations (PHFs) are of particular interest because combining extracts from different plants may provide complementary biological activities acting through multiple pathways rather than a single metabolic target, with increased efficacy, diminished toxicity, and reduced adverse effects relative to single-herb preparations [15,16]. *A. indicus* A. Rich. (Rubiaceae), known in the literature under the synonymous generic names Anthocephalus and Neolamarckia and commonly called Kadam, occupies a prominent position in Ayurveda, where its fruits, bark, leaves, and flowers have been used traditionally for diabetes, fever, inflammation, and liver ailments [17]. Phytochemical investigations have revealed indole alkaloids (cadambine, isocadambine, dihydrocadambine), triterpenoids such as quinovic acid, flavonoids, chlorogenic acid, and saponins [18]. Experimental studies have provided support for metabolic importance. Leaf and flower extracts exhibited significant anti-hyperglycemic activity in glucose loaded mice and alloxan-induced diabetic rats, the latter almost equivalent to metformin [19,20]. Ethanolic heartwood extract showed antidiabetic, hypolipidemic and antioxidant activities in STZ/high-fat diet-induced diabetic rats in a dose-dependent manner [21]. In diabetic wound models, the extract reduced lipid peroxidation indicators and increased SOD and catalase activity [22], while the anti-inflammatory potential was established in carrageenan-induced paw oedema [23]. Dyslipidemic diabetic rats treated with root extract of A. indicus (500 mg/kg) showed reduction in plasma glucose, lipids and lipid peroxides and increase in hepatic SOD and catalase activity [24]. *T. arjuna* (Roxb.) Wight & Arnot (Combretaceae), the classical Ayurvedic cardiotonic, contains flavonoids, triterpenoids, cardiac glycosides, tannins, and arjunolic acid, constituents associated with antioxidant, anti-inflammatory, and lipid-lowering actions [25]. Its stem bark has consistently demonstrated hypolipidemic and anti-atherogenic activity, reducing total cholesterol, triglycerides, and LDL while raising HDL [26,27], markedly reduced MDA and improved antioxidant enzyme activities in hyperlipidemic rats [28], and in high-fat diet/STZ-induced diabetic rats lowered fasting glucose and the atherogenic lipid profile while restoring glutathione status and catalase activity [29]. In STZ-induced diabetic rats, *T. arjuna* also improved myocardial function and reduced inflammatory cytokine levels [30,31]. Taken together, the pharmacological profiles of these two plants appear complementary rather than redundant: Kadam demonstrates predominantly antihyperglycemic, antioxidant, and anti-inflammatory actions, whereas Arjuna contributes well-characterized hypolipidemic, antioxidant, and cardioprotective effects. However, comparatively little attention has been directed toward their combined effects on oxidative stress and inflammatory biomarkers in HFHF-STZ-induced MetS.

Therefore, the objective of the present study was to evaluate the effects of a combined ethanolic formulation of *A. indicus* fruits and *T. arjuna* stem bark, relative to each extract alone and to metformin and atorvastatin as reference treatments, on antioxidant status (SOD, CAT, GSH, MDA) and inflammatory biomarkers (TNF-α, IL-6, IL-1β) in HFHF-STZ-induced metabolic syndrome in rats.

MATERIALS AND METHODS

Ethical statement

All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee of Era's Lucknow Medical College and Hospital, Era University, Lucknow, India (IAEC/ELMCH/2/22/-1).

Experimental animals

Healthy adult male Wistar rats weighing 180–200 g were procured from the Animal House Facility of the Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh, India (Registration No. 1652/PO/Re/S/12/CPCSEA). Animals were housed under controlled environmental conditions at 23 ± 2 °C, relative humidity of 40–50%, and a 12-h light/12-h dark cycle, and acclimatized for 7 d before initiation of the experiment. Standard feed and water were provided ad libititum except during specified fasting periods.

Preparation of extracts

The fruits of *A. indicus* and stem bark of *T. arjuna* were used for preparation of the respective ethanolic extracts. Approximately 1 kg of each plant material was collected, shade-dried, and pulverized into coarse powder. The powdered materials were separately extracted repeatedly using 95% ethanol. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator at a temperature below 50 °C, subsequently dried under high vacuum to remove residual solvent, and stored under appropriate conditions until further use.

High-fat high-fructose diet

An HFHF diet was prepared in-house for induction of metabolic abnormalities. The diet consisted of fructose/sucrose (220 g/kg), fat (400 g/kg), casein (100 g/kg), maize powder (88 g/kg), gram powder (60 g/kg), whole-wheat powder (6 g/kg), jaggery (80 g/kg), mineral mixture (6 g/kg), vitamin supplement (10 g/kg), cholesterol (20 g/kg), and corn starch (20 g/kg). The fat component consisted of Dalda and vanaspati ghee. The HFHF diet was administered for 16 w.

Induction of metabolic syndrome

Following acclimatization, animals assigned to the metabolic syndrome groups were fed the HFHF diet for 16 w. At the end of the dietary induction period, metabolic abnormalities were further induced by administration of STZ (30 mg/kg body weight, intraperitoneally), a dose within the established low-dose range for this model [13,14]. After 72 h, fasting blood glucose (FBG) was determined and an oral glucose tolerance test (OGTT) was performed. Animals showing FBG >250 mg/dl together with impaired glucose tolerance were considered to have developed the experimental metabolic phenotype and were included in the subsequent treatment protocol.

Experimental groups and treatment

Animals were randomly allocated into seven experimental groups, with six animals per group (n=6): Group I, normal control; Group II, Sham treated control; Group III, *A. indicus* ethanolic extract 500 mg/kg; Group IV, *T. arjuna* ethanolic extract 500 mg/kg; Group V, combined formulation (*A. indicus* 250 mg/kg + *T. arjuna* 250 mg/kg); Group VI, metformin 100 mg/kg; Group VII, atorvastatin 20 mg/kg. The respective treatments were administered orally for 21 d. Metformin and atorvastatin were used as reference pharmacological treatments.

Acute oral toxicity study

The acute oral toxicity of the combined formulation was evaluated according to OECD Guideline 423. Animals were administered the combined formulation orally at doses of 300, 500, 1000, 1500, and 2000 mg/kg and observed for 14 d for mortality and signs of toxicity.

Sample collection and preparation

Samples were obtained at the end of the treatment period according to the approved experimental protocol. Samples for biochemical and inflammatory analyses were processed and stored correctly until analysis. Serum samples for ELISA-based measurements were prepared according to the sample requirements of the respective manufacturers.

Assessment of oxidative stress

Oxidative stress was evaluated by measuring MDA, SOD, CAT, and GSH. SOD activity was determined using the SOD Activity Assay Kit (BioVision, Milpitas, USA; Cat. No. K335-100), a colorimetric enzyme-activity assay based on WST-1 reduction by superoxide; SOD inhibits this reduction, and the resulting percentage inhibition of WST-1 reduction was used to determine SOD activity. CAT activity was determined based on the decomposition of hydrogen peroxide by the method of Aebi, monitored spectrophotometrically at 240 nm, and expressed as U/mg protein. Lipid peroxidation was examined using the thiobarbituric acid reactive substances (TBARS) method of Ohkawa et al., for measurement of MDA, with absorbance of the organic phase read at 532 nm and the results represented as nmol MDA/mg protein. The GSH level was determined using the GSH/GSSG Assay Kit (MedChemExpress, USA; Cat. No. HY-K0311-100T) based on enzymatic reaction between 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and glutathione reductase that was measured kinetically at 412 nm.

Assessment of inflammatory biomarkers

The inflammatory response was evaluated by determining TNF-α, IL-6, and IL-1β concentrations in serum using rat-specific sandwich ELISA kits.

TNF-α was quantified using the Rat TNF-α ELISA Kit (Elabscience, USA; Cat. No. E-EL-R2856), validated for serum with a detection range of 15.63–1000 pg/ml. IL-6 was determined using the Rat IL-6 ELISA Kit (RayBiotech, USA; Cat. No. ELR-IL6), with a minimum detectable dose of 30 pg/ml. IL-1β was determined using the Elabscience Rat IL-1β ELISA Kit (Cat. No. E-EL-R0012), with a detection range of 31.25–2000 pg/ml and a sensitivity of 18.75 pg/ml. Absorbance was measured at 450 ± 2 nm using a microplate reader, concentrations were calculated from four-parameter logistic (4PL) standard curves with correction for the dilution factor, and samples and standards were analyzed in duplicate.

Statistical analysis

All quantitative data were expressed as mean ± standard error of the mean (SEM) for n=6 animals per group. Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparisons test, with the Sham treated control group used as the reference group, using GraphPad Prism software. A value of $p<0.05$ was considered statistically significant and is represented as *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

RESULTS

The acute oral toxicity assessment of the combined *A. indicus* and *T. arjuna* ethanolic extract formulation showed no mortality or treatment-related signs of toxicity at doses up to 2000 mg/kg during the 14-day observation period. No marked changes in general behaviour, activity, feeding, or other visible clinical signs were observed. Based on these findings, the oral median lethal dose (LD50) of the combined formulation was considered to be greater than 2000 mg/kg under the conditions of the study.

Effect of treatment on oxidative stress biomarkers

HFHF-STZ induction was associated with a marked alteration in oxidative stress parameters compared with the normal control group. SOD activity was significantly lower in the sham treated control group than in the normal control (57.50 ± 6.61 versus 85.50 ± 2.89 % inhibition of WST-1 reduction, $p < 0.001$). Treatment with *A. indicus* (68.25 ± 4.43, $p < 0.01$), *T. arjuna* (73.75 ± 2.22, $p < 0.001$), the combined formulation (80.75 ± 1.71, $p < 0.001$), metformin (66.00 ± 2.58, $p < 0.05$), and atorvastatin (68.00 ± 3.92, $p < 0.01$) each significantly increased SOD activity compared with the sham treated control, with the combined formulation producing the greatest restoration toward the normal-control level (fig. 1A and Table 1).

Table 1: Dunnett's Multiple Comparison Test for superoxide dismutase (SOD) activity

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sham Control vs Normal	-28.00	10.40	Yes	***	-35.51 to -20.49
Sham Control vs A. indicus	-10.75	3.994	Yes	**	-18.26 to -3.237
Sham Control vs *T. arjuna*	-16.25	6.038	Yes	***	-23.76 to -8.737
Sham Control vs C.F	-23.25	8.638	Yes	***	-30.76 to -15.74
Sham Control vs Metformin	-8.500	3.158	Yes	*	-16.01 to -0.9873
Sham Control vs Atorvastatin	-10.50	3.901	Yes	**	-18.01 to -2.987

Dunnett's post-hoc test following one-way ANOVA; n = 6 per group. Comparisons were made with the HFHF-STZ control group. $P < 0.05$ was considered statistically significant. Data were analyzed using GraphPad Prism.

CAT activity was significantly reduced following HFHF-STZ induction (24.00 ± 2.94 versus 42.25 ± 3.30 U/mg protein, $p < 0.001$). Treatment with *A. indicus* (32.75 ± 2.99, $p < 0.01$), *T. arjuna* (35.25 ± 3.10, $p < 0.001$), the combined formulation (41.25 ± 2.99, $p < 0.001$), metformin (36.25 ± 1.71, $p < 0.001$), and atorvastatin (29.75 ± 2.50, $p < 0.05$) significantly increased CAT activity compared with the sham treated control. The combined formulation showed a response approaching the normal-control level (Fig. 1B and Table 2).

Table: 2 Dunnett's Multiple Comparison Test for catalase activity

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sham Control vs Normal	-18.25	9.111	Yes	***	-23.84 to -12.66
Sham Control vs *A. indicus*	-8.750	4.369	Yes	**	-14.34 to -3.159
Sham Control vs *T. Arjuna*	-11.25	5.617	Yes	***	-16.84 to -5.659
Sham Control vs C.F	-17.25	8.612	Yes	***	-22.84 to -11.66
Sham Control vs Metformin	-12.25	6.111	Yes	***	-17.84 to -

		6			6.659
Sham Control vs Atorvastatin	-5.750	2.871	Yes	*	-11.34 to -0.1591

Dunnett's post-hoc test following one-way ANOVA; n = 6 per group. Comparisons were made with the HFHF-STZ control group. P < 0.05 was considered statistically significant. Data were analyzed using GraphPad Prism.

MDA concentration was markedly elevated in the sham treated control group, indicating increased lipid peroxidation (3.97 ± 0.63 versus 2.18 ± 0.22 nmol/mg protein, p < 0.001). Treatment with *T. arjuna* (3.25 ± 0.24, p < 0.05), the combined formulation (3.05 ± 0.40, p < 0.01), and metformin (3.28 ± 0.17, p < 0.05) significantly reduced MDA levels compared with the sham treated control, whereas *A. indicus* (3.87 ± 0.17) and atorvastatin (4.05 ± 0.31) did not produce a statistically significant change. The combined formulation produced the greatest reduction among all treated groups (Fig. 1C and Table 3).

Table 3: Dunnett's Multiple Comparison Test for malondialdehyde (MDA) level

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sham Control vs Normal	1.800	7.407	Yes	***	1.122 to 2.478
Sham Control vs *A. indicus*	0.1000	0.4115	No	ns	-0.5783 to 0.7783
Sham Control vs *T. Arjuna*	0.7250	2.984	Yes	*	0.04673 to 1.403
Sham Control vs C.F	0.9250	3.807	Yes	**	0.2467 to 1.603
Sham Control vs Metformin	0.7000	2.881	Yes	*	0.02173 to 1.378
Sham Control vs Atorvastatin	-0.07500	0.3086	No	ns	-0.7533 to 0.6033

Dunnett's post-hoc test following one-way ANOVA; n = 6 per group. Comparisons were made with the HFHF-STZ control group. P < 0.05 was considered statistically significant. Data were analyzed using GraphPad Prism.

GSH levels were significantly reduced in the sham treated control group compared with the normal control (48.50 ± 7.59 versus 69.00 ± 18.07 µmol/g protein, p < 0.001). Treatment with *A. indicus* (57.00 ± 3.16, p < 0.001), the combined formulation (66.50 ± 3.70, p < 0.001), metformin (66.00 ± 8.04, p < 0.001), and atorvastatin (55.00 ± 3.27, p < 0.01) significantly restored GSH compared with the sham treated control, whereas *T. arjuna* alone (51.75 ± 5.25) did not reach statistical significance. The combined formulation produced the greatest restoration among the extract-treated groups, approaching the normal-control level (Fig. 1D and Table 4).

Table 4: Dunnett's Multiple Comparison Test for reduced glutathione (GSH) level

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant P < 0.05	Summary	95% CI of diff
Sham Control vs Normal	-32.75	12.76	Yes	***	-39.92 to -25.58
Sham Control vs *A. indicus*	-13.75	5.357	Yes	***	-20.92 to -6.585
Sham Control vs *T. arjuna*	-5.750	2.240	No	ns	-12.92 to 1.415
Sham Control vs C.F	-19.75	7.694	Yes	***	-26.92 to -12.58
Sham Control vs Metformin	-24.50	9.544	Yes	***	-31.67 to -17.33
Sham Control vs Atorvastatin	-10.75	4.188	Yes	**	-17.92 to -3.585

Dunnett's post-hoc test following one-way ANOVA; n = 6 per group. Comparisons were made with the HFHF-STZ control group. P < 0.05 was considered statistically significant. Data were analyzed using GraphPad Prism.

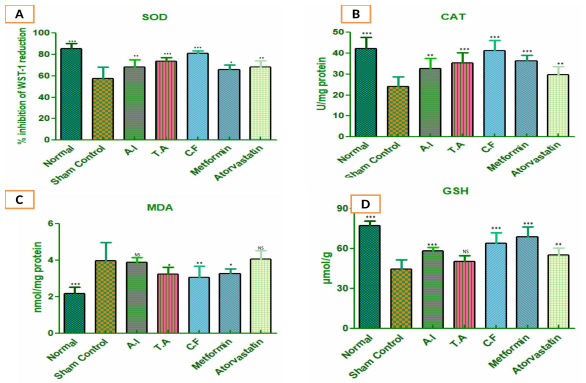


Fig. 1: Effect of treatments on oxidative stress biomarkers in HFHF-STZ-induced metabolic syndrome in rats. (A) SOD activity; (B) CAT activity; (C) MDA level; (D) GSH level. Bars represent mean ± SEM (n = 6); significance markers denote comparisons versus the sham treated control group.

*Values are mean ± SEM (n = 6). Significance from one-way ANOVA followed by Dunnett's multiple-comparisons test: each group compared with the sham treated control. Normal control vs sham treated control was significant (p < 0.001) for all parameters. *p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant. Groups: A. indicus = ethanolic extract 500 mg/kg; T. arjuna = ethanolic extract 500 mg/kg; Combined = A. indicus 250 + T. arjuna 250 mg/kg.*

Effect of treatment on inflammatory biomarkers

HFHF-STZ induction was associated with a significant increase in circulating inflammatory biomarkers. Serum TNF-α concentration was markedly elevated in the sham treated control group compared with the normal control (162.00 ± 2.58 versus 102.00 ± 6.27 pg/ml, p < 0.001). Treatment with *T. arjuna* (141.00 ± 9.93, p < 0.01), the combined formulation (131.25 ± 8.54, p < 0.001), and metformin (135.50 ± 9.98, p < 0.001) significantly reduced serum TNF-α compared with the sham treated control, whereas *A. indicus* (155.25 ± 4.11) and atorvastatin (153.50 ± 4.65) did not reach statistical significance. The combined formulation produced the greatest reduction, approaching the normal-control range (fig. 2A Table 5).

Table 5: Dunnett's Multiple Comparison Test for tumor necrosis factor-alpha (TNF-α) level

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant ? P < 0.05?	Summary	95% CI of diff
Sham Control vs Normal	60.00	11.90	Yes	***	45.93 to 74.07
Sham Control vs *A. indicus*	6.750	1.339	No	ns	-7.322 to 20.82
Sham Control vs *T. arjuna*	21.00	4.165	Yes	**	6.928 to 35.07
Sham Control vs C.F	30.75	6.099	Yes	***	16.68 to 44.82
Sham Control vs Metformin	26.50	5.256	Yes	***	12.43 to 40.57
Sham Control vs Atorvastatin	8.500	1.686	No	ns	-5.572 to 22.57

Dunnett's post-hoc test following one-way ANOVA; n = 6 per group. Comparisons were made with the HFHF-STZ control group. P < 0.05 was considered statistically significant. Data were analyzed using GraphPad Prism.

IL-6 concentration showed a significant increase following HFHF-STZ induction (72.75 ± 2.06 versus 42.75 ± 5.25 pg/ml, p < 0.001). Treatment with *T. arjuna* (63.25 ± 4.03, p < 0.01), the combined formulation (61.75 ± 3.59, p < 0.01), and metformin (60.00 ± 2.58, p < 0.001) significantly reduced IL-6 levels compared with the sham treated control, whereas *A. indicus* (66.50 ± 3.11) and atorvastatin (70.00 ± 2.45) did not reach statistical significance. The combined formulation produced a more pronounced reduction toward the normal-control value (Fig. 2B and Table 6).

Table 6: Dunnett's Multiple Comparison Test for interleukin-6 (IL-6) level

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant ? P < 0.05	Summary	95% CI of diff
Sham Control vs Normal	30.00	12.30	Yes	***	23.19 to 36.81
Sham Control vs *A. indicus*	6.250	2.562	No	ns	-0.5600 to 13.06
Sham Control vs *T. arjuna*	9.500	3.894	Yes	**	2.690 to 16.31
Sham Control vs C.F	11.00	4.509	Yes	**	4.190 to 17.81
Sham Control vs Metformin	12.75	5.226	Yes	***	5.940 to 19.56
Sham Control vs Atorvastatin	2.750	1.127	No	ns	-4.060 to 9.560

Dunnett's post-hoc test following one-way ANOVA; n = 6 per group. Comparisons were made with the HFHF-STZ control group. P < 0.05 was considered statistically significant. Data were analyzed using GraphPad Prism.

IL-1β was significantly elevated in the sham treated control group relative to the normal control (145.50 ± 6.19 versus 77.00 ± 6.32 pg/ml, p < 0.001). Treatment with *A. indicus* (113.00 ± 6.48), *T. arjuna* (103.75 ± 4.86), the combined formulation (84.50 ± 2.65), metformin (90.25 ± 4.11), and atorvastatin (121.25 ± 7.76) all significantly reduced IL-1β concentrations compared with the sham treated control (all p < 0.001). The combined formulation produced the greatest reduction among the extract-treated groups, approaching the normal-control level (Fig. 2C and Table 7).

Table 7: Dunnett's Multiple Comparison Test for interleukin-1 beta (IL-1β) level

Dunnett's Multiple	Mean	q	Significant	Sum	95% CI

Comparison Test	Diff.		? P < 0.05	mary	of diff
Sham Control vs Normal	68.50	16.97	Yes	***	57.23 to 79.77
Sham Control vs *A. indicus*	32.50	8.052	Yes	***	21.23 to 43.77
Sham Control vs *T. arjuna*	41.75	10.34	Yes	***	30.48 to 53.02
Sham Control vs C.F	61.00	15.11	Yes	***	49.73 to 72.27
Sham Control vs Metformin	55.25	13.69	Yes	***	43.98 to 66.52
Sham Control vs Atorvastatin	24.25	6.008	Yes	***	12.98 to 35.52

Dunnett's post-hoc test following one-way ANOVA; n = 6 per group. Comparisons were made with the HFHF-STZ control group. P < 0.05 was considered statistically significant. Data were analyzed using GraphPad Prism.

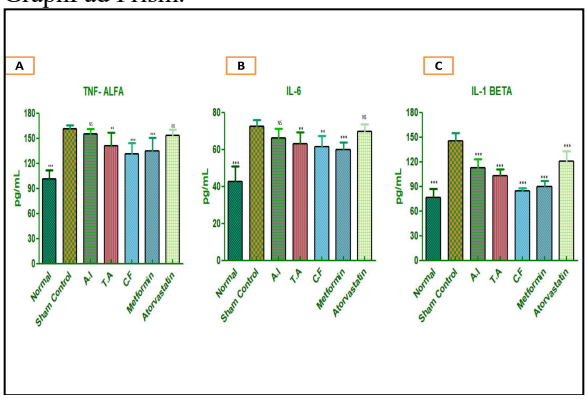


Fig. 2: Effect of treatments on serum inflammatory cytokines in HFHF-STZ-induced metabolic syndrome in rats. (A) TNF-α; (B) IL-6; (C) IL-1β. Bars represent mean ± SEM (n = 6); significance markers denote comparisons versus the sham treated control group.

*Values are mean ± SEM (n = 6). Significance from one-way ANOVA followed by Dunnett's multiple-comparisons test: each group compared with the sham treated control. Normal control vs sham treated control was significant (p < 0.001) for all parameters. *p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant. Groups: A. indicus = ethanolic extract 500 mg/kg; T. arjuna = ethanolic extract 500 mg/kg; Combined = A. indicus 250 + T. arjuna 250 mg/kg.*

Overall, HFHF-STZ induction was associated with increased oxidative stress and inflammatory activity, characterized by reduced antioxidant parameters and increased lipid peroxidation and inflammatory biomarkers. Treatment with the combined *A. indicus* + *T. arjuna* formulation was the only treatment that produced significant improvement across all seven biomarkers, showing a greater tendency toward restoration of antioxidant status and reduction of inflammatory markers compared with the individual extracts.

DISCUSSION

MetS is characterized by the coexistence of impaired glucose regulation, dyslipidemia, insulin resistance, and increased oxidative and inflammatory stress. Persistent metabolic disturbances can promote excessive generation of ROS, leading to lipid peroxidation, depletion of endogenous antioxidants, and activation of inflammatory signalling pathways [2,4]. The present study evaluated the effects of combined ethanolic extracts of *A. indicus* and *T. arjuna* on selected oxidative stress and inflammatory biomarkers in an HFHF-STZ-induced rat model. The sham treated control group showed a clear disturbance in antioxidant status, characterized by lower SOD, CAT, and GSH and increased MDA compared with the normal control, consistent with the development of oxidative stress following dietary metabolic stress combined with STZ-induced pancreatic dysfunction. Increased ROS can overwhelm antioxidant defence systems and initiate membrane lipid peroxidation, leading to higher MDA. Similar changes in SOD, CAT, GSH and MDA have been described in HFD/STZ and STZ-induced diabetic rat models [29]. The decline in SOD reflects a disrupted enzymatic defence against superoxide radicals, the parallel decline in CAT reflects a disruption of the downstream antioxidant defence system, and the depletion of GSH reflects an increased utilisation of the non-enzymatic antioxidant reserve under chronic oxidative stress [3].

Treatment with *A. indicus* improved the antioxidant profile compared with the sham treated control, consistent with previous experimental evidence: administration of *A. indicus* root extract at 500 mg/kg in dyslipidemic diabetic rats has been reported to reduce lipid peroxidation and stimulate hepatic SOD and catalase activities [24]. The response observed with *T. arjuna* was likewise consistent with previous studies, in which *T. arjuna* administered at 500 mg/kg in an HFD/STZ diabetic model significantly improved oxidative stress-related parameters including MDA, GSH, and CAT [29], and reduced oxidative stress and inflammatory cytokine levels in STZ-induced diabetic rats [30]. Interestingly, the combined formulation produced a better improvement in antioxidant parameters than any individual extract with SOD, CAT and GSH close to

the values of the normal group and with MDA significantly decreased. These results may suggest that the antioxidant activity of the two plant extracts is complimentary when combined. However, the present findings should be interpreted as an enhanced response to the combined formulation rather than definitive evidence of pharmacological synergy unless a formal interaction or combination-index analysis is performed. The improvement in GSH following treatment is particularly relevant because GSH constitutes an important intracellular non-enzymatic antioxidant involved in maintaining cellular redox balance, and its restoration may reduce the susceptibility of cellular membranes to oxidative damage.

In addition to oxidative stress, the HFHF-STZ model was associated with an increase in the inflammatory biomarkers TNF-α, IL-6, and IL-1β. Metabolic stress and oxidative imbalance can activate inflammatory signalling pathways, including NF-κB-associated pathways, resulting in increased production of proinflammatory cytokines [4,5]. The reduction of these cytokines following treatment suggests that improvement in oxidative status may be accompanied by attenuation of inflammatory responses. The reduction in TNF-α following the combined formulation is particularly relevant because TNF-α is an important mediator of metabolic inflammation associated with insulin resistance and inflammatory tissue responses [5]. Because oxidative stress and inflammation are closely interconnected, restoration of antioxidant defenses may contribute to reduced cytokine production. However, the present study cannot establish a direct causal relationship between antioxidant restoration and cytokine suppression because signalling pathways such as NF-κB, Nrf2, or related molecular targets were not directly measured.

Metformin was included as a reference treatment because of its established use in metabolic disease. Its improvement of antioxidant and inflammatory parameters is consistent with reports that metformin at 100 mg/kg reduces oxidative stress markers and improves antioxidant status in experimental diabetes; however, in the present study metformin did not reach the improvement observed with the combined formulation for several parameters. Atorvastatin requires a more cautious interpretation. Statins can exert effects beyond lipid lowering, including modulation of oxidative and inflammatory processes, but their effects may depend on the experimental model, tissue, and duration of treatment. One study in HFD/STZ diabetic rats reported that atorvastatin at 20 mg/kg increased hepatic ROS and inflammatory mediators while reducing SOD and CAT activity, whereas a more recent study in high-fat diet/STZ diabetic rats reported partial anti-inflammatory effects of atorvastatin 20 mg/kg. Thus, the comparatively weak and frequently non-significant response of the atorvastatin group in the present study, with MDA even slightly above the sham treated control level, should be interpreted in the context of the specific HFHF-STZ model and experimental conditions rather than assuming a uniform antioxidant or anti-inflammatory effect.

Taken together, the findings suggest that HFHF-STZ-induced metabolic stress is accompanied by simultaneous oxidative and inflammatory disturbances. The combined *A. indicus* + *T. arjuna* formulation produced a more pronounced overall restoration of antioxidant and inflammatory parameters than the individual extracts, biologically consistent with the antioxidant and anti-inflammatory properties previously reported for both plants [24,26]. Nevertheless, further mechanistic studies examining insulin signalling, Nrf2/HO-1, NF-κB, and related molecular pathways would be required to establish the mechanisms underlying the observed effects.

CONCLUSION

The present study indicates that HFHF-STZ-induced metabolic stress is associated with marked disturbances in oxidative balance and inflammatory status, characterized by impaired antioxidant defenses, increased lipid peroxidation, and elevation of pro-inflammatory cytokines. Treatment with ethanolic extracts of *A. indicus* and *T. arjuna* improved these alterations, and the combined formulation at 250 + 250 mg/kg was the only treatment that achieved statistically significant improvement across all seven biomarkers, showing a more pronounced overall effect than the individual extracts and comparable to or better than metformin and atorvastatin. These findings support the potential of this combined ethanolic extract formulation as a candidate for further investigation in metabolic syndrome, pending additional studies involving molecular targets, insulin-signalling pathways, and long-term safety.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Nil.

AUTHOR CONTRIBUTIONS

Arvind Kumar Srivastava, Vivek Ranjan Patel, and Aparna Misra contributed to the conception and design of the study. Vivek Ranjan Patel conducted the data acquisition, analysis, and interpretation. All authors contributed to the preparation of the original manuscript draft. Aparna Misra and Sheetal Verma critically reviewed and revised the manuscript for important intellectual content. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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AI USE STATEMENT

No artificial intelligence (AI) tools or AI-assisted technologies were used in the preparation, writing, editing, citation formatting, or figure preparation of this manuscript. The manuscript was prepared and reviewed entirely by the authors.

REFERENCES

1. Hamooya BM, Siame L, Muchaili L, Masenga SK, Kirabo A. Metabolic syndrome: epidemiology, mechanisms, and current therapeutic approaches. Front Nutr. 2025 Sep 3;12:1661603. doi: 10.3389/fnut.2025.1661603.
2. Byaruhanga MP. Exploring the role of oxidative stress in the pathophysiology of metabolic syndrome: implications for therapeutic targeting. Res Invention J Res Med Sci. 2025;4(3):26-32. doi:10.59298/RIJRMS/2025/432632.
3. Berbudi A, Khairani S, Tjahjadi AI. Interplay between insulin resistance and immune dysregulation in type 2 diabetes mellitus: implications for therapeutic interventions. Immunotargets Ther. 2025;14:359-82. doi:10.2147/ITT.S499605.
4. Bryan S, Baregzay B, Spicer D, Singal PK, Khaper N. Redox-inflammatory synergy in the metabolic syndrome. Can J Physiol Pharmacol. 2013;91(1):22-30. doi:10.1139/cjpp-2012-0295.
5. Khan AW, Jandeleit-Dahm KAM. Atherosclerosis in diabetes mellitus: novel mechanisms and mechanism-based therapeutic approaches. Nat Rev Cardiol. 2025;22(7):482-96. doi:10.1038/s41569-024-01115-w.
6. Petryn TS, Nagalievska MR, Sybirna NO. Comparison of high-fat and high-carbohydrate diets for obtaining an experimental model of metabolic syndrome. Studia Biologica. 2021;15(1):3-14. doi:10.30970/sbi.1501.642.
7. Panchal SK, Poudyal H, Iyer A, Nazer R, Alam MA, Diwan V, et al. High-carbohydrate, high-fat diet-induced metabolic syndrome and cardiovascular remodeling in rats. J Cardiovasc Pharmacol. 2011;57(5):611-24. doi:10.1097/FJC.0b013e31821b1379.
8. Dupas J, Feray A, Goanvec C, Guernec A, Samson N, Bougaran P, et al. Metabolic syndrome and hypertension resulting from fructose enriched diet in Wistar rats. Biomed Res Int. 2017;2017:2494067. doi:10.1155/2017/2494067.
9. Zolfaghari M, Faramarzi M, Hedayati M, Ghaffari M. The effect of resistance and endurance training with ursolic acid on atrophy-related biomarkers in muscle tissue of diabetic male rats induced by streptozotocin and a high-fat diet. J Food Biochem. 2022;46(8). doi:10.1111/jfbc.14202.
10. Chen C, Cohrs CM, Stertmann J, Bozsak R, Speier S. Human beta cell mass and function in diabetes: recent advances in knowledge and technologies to understand disease pathogenesis. Mol Metab. 2017;6(9):943-57. doi:10.1016/j.molmet.2017.06.019.
11. Racine KC, Iglesias-Carres L, Herring JA, Wieland KL, Ellsworth PN, Tessem JS, et al. The high-fat diet and low-dose streptozotocin type-2 diabetes model induces hyperinsulinemia and insulin resistance in male but not female C57BL/6J mice. Nutr Res. 2024;131:135-46. doi:10.1016/j.nutres.2024.09.008.
12. Kaydash OA, Ivanov VV, Vengerovsky AI, Buyko EE, Schepetkin IA. The experimental model of type 2 diabetes mellitus caused by a high-fat diet with low-dose streptozotocin in rats. Bull Siberian Med. 2020;19(2):41-7. doi:10.20538/1682-0363-2020-2-41-47.
13. Vatandoust N, Rami F, Salehi AR, Khosravi S, Dashti G, Eslami G, et al. Novel high-fat diet formulation and streptozotocin treatment for induction of prediabetes and type 2 diabetes in rats. Adv Biomed Res. 2018;7:107. doi:10.4103/abr.abr_8_17.
14. Brito AK, Mendes AV, Timah Acha B, Santos Oliveira AS, Lopes Macedo J, Suzuki Cruzio A, et al. Experimental models of type 2 diabetes mellitus induced by combining hyperlipidemic diet (HFD) and streptozotocin administration in rats: an integrative review. Biomedicines. 2025;13(5):1158. doi:10.3390/biomedicines13051158.
15. Petchi RR, Vijaya C, Parasuraman S. Antidiabetic activity of polyherbal formulation in streptozotocin - nicotinamide induced diabetic wistar rats. J Tradit

Complement Med. 2014 Apr;4(2):108-17. doi: 10.4103/2225-4110.126174

16. Patel VR, Gupta AK, Saini S, Dwivedi R, Aziz F. Herbal medicines: an extensive analysis of polyherbal treatments for metabolic syndrome. Era J Med Res. 2025;12(1):27-34. doi:10.24041/ejmr.2025.6.
17. Samant SS, Kolambkar SV, Hurkadale PJ, Jagtap VA, Beerwala FA. Unlocking the medicinal potential of Anthocephalus cadamba: a phytochemical and pharmacognostic perspective. In: The Chemistry inside Spices & Herbs: Research and Development. Vol 6. Bentham Science Publishers; 2026. p. 59-77. doi:10.2174/9798898813062126060007.
18. Attar MMK, Gidde MND, Bhosale MMJ, Jadhav MBD, Pawar MHY, Satpute DSS, et al. Phytochemical constituents and pharmacological potential of Neolamarckia cadamba: a comprehensive review. Adolesc Saude. 2026;21(6 Suppl):577-98. doi:10.67440/ahj.v21i6s.1657.
19. Januario E, Barakat A, Rajsundar A, Fatima Z, Palienkar VN, Bullapur AV, et al. A comprehensive review of pathophysiological link between non-alcoholic fatty liver disease, insulin resistance, and metabolic syndrome. Cureus. 2024;16(12). doi:10.7759/cureus.75677.
20. Patel VR, Gupta AK, Dwivedi J, Lal N, Abidi A, Misra A. *A. indicus* and *T. arjuna* combination ameliorates hematological and histopathological changes in a rat model of metabolic syndrome. Cureus. 2025;17(9). doi:10.7759/cureus.93523.
21. Mishra SB, Maurya M, Srivastava V. Heartwood extract of Neolamarckia cadamba ameliorates oxidative stress linked type II diabetes in Wistar albino rats. Res J Pharm Technol. 2023;16(3):1121-6. doi:10.52711/0974-360X.2023.00187.
22. Yadav JP, Patel DK, Dubey NK, Mishra MK, Verma A, Grishina M, et al. Wound healing and antioxidant potential of Neolamarckia cadamba in streptozotocin-nicotinamide induced diabetic rats. Phytomedicine Plus. 2022;2(2):100274. doi:10.1016/j.phyplu.2022.100274.
23. Sawarkar A, Bhapkar T, Dubey S, Umap S, Borekar V. The anti-pyretic activity of aqueous and alcoholic extract of stem bark of Neolamarckia cadamba on brewer's yeast-induced Wistar rats. Int J Adv Biochem Res. 2024;8(Suppl 8):388-90. doi:10.33545/26174693.2024.v8.i8Sf.1835.
24. Bhujel M, Kandanur SGS, Sripada L, Golakoti NR. A comprehensive review of the medicinal properties of *T. arjuna* and its major constituent arjunolic acid: a natural product source for new drug leads. RSC Adv. 2026;16(25):23217-56. doi:10.1039/D6RA02419J.
25. Nerkar AG, Chinkure S. Investigation of antibacterial activity of syrup prepared from extracts of cinnamon (Cinnamomum zeylanicum) and Arjuna (*T. arjuna*). Curr Trends Pharm Pharm Chem. 2025;7(4):120-2. doi:10.18231/j.ctppc.13317.1764403304.
26. Rajaram V, Namasivayam A, Shanmugam R, Mahendra J, Sudhakar U, Munusamy T, et al. *T. arjuna*: an overview of its magical properties. Bioinformation. 2024;20(12):2080-5. doi:10.6026/9732063002002080.
27. Amalraj A, Gopi S. Medicinal properties of *T. arjuna* (Roxb.) Wight & Arn.: a review. J Tradit Complement Med. 2017;7(1):65-78. doi:10.1016/j.jtcme.2016.02.003.
28. Prakash V, Goel N, Giri KR, Goel A. Clinical study evaluating antihyperglycemic efficacy and safety of *T. arjuna* versus sitagliptin in type-2 diabetes mellitus patients. Bioinformation. 2024;20(12):1862-8. doi:10.6026/9732063002001862.
29. Khaliq F, Parveen A, Singh S, Gondal R, Hussain ME, Fahim M. Improvement in myocardial function by *T. arjuna* in streptozotocin-induced diabetic rats: possible mechanisms. J Cardiovasc Pharmacol Ther. 2013;18(5):481-9. doi:10.1177/1074248413488831.
30. Patel VR, Saini S, Dwivedi J, Gupta AK, Shrivastava AK, Misra A. Exploring the concept and scope of polyherbal formulations: a comprehensive review. Int J Herb Med. 2025;13(2):9-16. doi:10.22271/flora.2025.v13.i2a.973.
31. Ramesh P, Palaniappan A. *T. arjuna*, a cardioprotective herbal medicine-relevancy in the modern era of pharmaceuticals and green nanomedicine-a review. Pharmaceuticals (Basel). 2023;16(1):126. doi:10.3390/ph16010126.