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Ameliorative potential of *Katuka Musali Kashay* (polyherbal decoction of *Picrorhiza kurroa* and *Asparagus adscendens*) in methotrexate-induced hepatotoxicity in Wistar rats

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Methotrexate (MTX) is a widely used immunosuppressive and chemotherapeutic drug, but its hepatotoxic side effects restrict its clinical use. This study evaluated the ameliorative potential of *Katuka Musali Kashay* in MTX-induced hepatotoxicity in Wistar rats. The animals were divided into four groups: Normal Control (NC), Disease Control (MTX only), Standard Group (MTX + silymarin) and Test Group (MTX + *Katuka Musali Kashay*). MTX (20 mg/kg b.w., intraperitoneal) was administered for seven days to induce hepatotoxicity, followed by oral silymarin (50 mg/kg b.w.) or *Katuka Musali Kashay* (7.20 mL/kg b.w.) for the next seven days. Serum SGPT/ALT, SGOT/AST, bilirubin, ALP and total protein were measured as liver function markers, malondialdehyde (MDA) and reduced glutathione (GSH) were assessed as indicators of oxidative stress, and liver histopathology was examined. Data were analysed using one-way and two-way ANOVA in GraphPad Prism. Treatment with *Katuka Musali Kashay* significantly reduced serum SGPT, SGOT, bilirubin and ALP compared with the MTX group and improved total protein levels. Oxidative stress was reduced, as evidenced by decreased MDA levels and restored GSH. Histopathological examination revealed reduced hepatic damage in the *Katuka Musali Kashay* group, comparable to the standard silymarin group. *Katuka Musali Kashay* demonstrated significant therapeutic effects in MTX-induced hepatotoxicity, likely due to its antioxidant properties, and may be considered a potential adjunct therapy in managing MTX-induced liver toxicity.

Keywords: Hepatotoxicity, *Katuka Musali Kashay*, Methotrexate, Oxidative stress, Silymarin, Wistar rats

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The liver is a vital organ with numerous essential functions. Owing to its close connection with the gastrointestinal tract, it is a major target for xenobiotics, oxidative stress and drug toxicity. Whether exposed to high or low doses, this most important organ is responsible for breaking down every food and drug we ingest and is, consequently, more readily damaged.

Drug-induced hepatotoxicity can develop following exposure to natural or synthetic compounds and may manifest as either an acute or a chronic hepatic condition[1]. It is broadly classified as dose-dependent intrinsic injury or unpredictable idiosyncratic injury, which is not related to dose[2]. Commonly used drugs that cause hepatotoxicity include anticancer drugs, certain antitubercular agents, antibiotics, immunosuppressants and other toxic chemicals. Hepatotoxicity remains a significant cause of morbidity and mortality due to its high prevalence.

Methotrexate, originally known as amethopterin, is an immune system suppressor and chemotherapeutic drug. It is considered the first DMARD of choice in

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Abbreviations:- MTX – Methotrexate; NC – Normal Control; DC – Disease Control; SG – Standard Group; KK – *Katuka Musali Kashay*; SGOT – Serum Glutamate Oxaloacetate Transaminase; AST – Aspartate Aminotransferase; SGPT – Serum Glutamate Pyruvate Transaminase; ALT – Alanine Aminotransferase; ALP – Alkaline Phosphatase; MDA – Malondialdehyde; GSH – Reduced Glutathione; ROS – Reactive Oxygen Species; DHFR – Dihydrofolate Reductase; DMARDs – Disease-Modifying Anti-Rheumatic Drugs; WHO – World Health Organization; IAEC – Institutional Animal Ethics Committee; CPCSEA – Committee for the Purpose of Control and Supervision of Experiments on Animals; ARRIVE – Animal Research: Reporting of In Vivo Experiments; API – Ayurvedic Pharmacopoeia of India; BSA – Body Surface Area; TLC – Thin Layer Chromatography; H&E – Haematoxylin and Eosin; SD – Standard Deviation; ANOVA – Analysis of Variance; BT – Before Treatment; AT – After Treatment; i.p. – Intraperitoneal; b.w. – Body weight; IU/L – International Units per Litre; w/v – Weight per Volume; Rf – Retardation Factor.

the treatment of rheumatoid arthritis and is widely used in psoriasis, soft tissue sarcoma, breast cancer, acute myeloid leukaemia, choriocarcinoma, steroid-resistant asthma, Crohn's disease and transplant rejection. Antimetabolites are chemical agents that interfere with cellular metabolism by mimicking metabolites and blocking their actions. Methotrexate, a folic acid antagonist and dihydrofolate reductase inhibitor, has immunosuppressant and anti-inflammatory properties. It is administered by intravenous, intrathecal, intraperitoneal or oral routes, with up to 90% of the oral dose excreted in urine within 12 h. While generally well tolerated, high or prolonged doses can cause hepatotoxicity, particularly if renal function or hydration is compromised. Methotrexate holds an established role in clinical therapy and is included in the WHO Model List of Essential Medicines, reflecting its importance in addressing essential healthcare needs[3]. Despite its therapeutic efficacy, hepatotoxicity remains a major limiting factor, particularly with long-term or high-dose use. This adverse effect can lead to treatment interruption, reduced patient compliance and increased morbidity. Side effects, overdose or long-term use of methotrexate may lead to toxicity, particularly when liver dysfunction or other systemic problems occur. Managing these effects requires detoxification and mitigation of the harmful impacts of the drug. Therefore, prevention and management of methotrexate-induced hepatotoxicity is of significant clinical relevance worldwide, and exploring ameliorative interventions is essential to enhance the safety profile of methotrexate and ensure its continued therapeutic utility.

Various drugs and formulations are described for their antitoxic properties. *Katuka Musali Kashay*, mentioned in *Basavarajeeyam*[4], contains *Katuka* (*Picrorhiza kurroa*) and *Safed Musali* (*Asparagus adscendens*) and is known for its antitoxic, hepatoprotective and antioxidant effects. According to *Ashtanga Hridaya Sutrasthana* (Chapter 15) and *Sushruta Samhita Sutrasthana* (Chapter 38), *Katuka* (*Picrorhiza kurroa*) is a key ingredient of *Patoladi Gana*, effective in managing poisoning and hepatic disorders[5,6]. Additionally, *Bhavaprakasa Nighantu*, *Kaiyadeva Nighantu* and *Shaligram Nighantu* highlight the antioxidant properties of *Safed Musali*, which help in treating and reversing hepatotoxicity[7-9].

Katuka (*Picrorhiza kurroa*) is a well-known herb in Ayurveda, traditionally used for treating liver disorders and upper respiratory tract disorders. It has hepatoprotective, anticholestatic, antioxidant and immune-modulating effects. *Safed Musali* (*Asparagus adscendens*) is classified as an adaptogen and is commonly believed to enhance overall body immunity and vitality and to promote liver health.

Although the individual drugs *Katuka* (*Picrorhiza kurroa*) and *Safed Musali* (*Asparagus adscendens*) are well documented for their hepatoprotective and antioxidant properties, there is limited experimental evidence evaluating their combined formulation as described in classical Ayurvedic texts. *Katuka Musali Kashay*, mentioned in *Basavarajeeyam*, is traditionally indicated for conditions associated with toxicity. However, no prior experimental study has evaluated the efficacy of this combined formulation in methotrexate-induced hepatotoxicity. Therefore, the present study aimed to evaluate the ameliorative potential of *Katuka Musali Kashay* in methotrexate-induced hepatotoxicity in Wistar rats, providing an experimental evaluation of this classical formulation and establishing evidence for its potential synergistic detoxifying effects, with a focus on its combined antitoxic actions.

Materials and Methods

Methotrexate and silymarin were purchased from an authentic pharmaceutical source.

Collection of drugs and preparation of formulation

Katuka (*Picrorhiza kurroa*) and *Safed Musali* (*Asparagus adscendens*) roots were purchased from authenticated sources and botanically verified at Sheetal Analytical Laboratory, Pune. Authentication confirmed the identity, family and plant part used. Standardisation was further supported at Sudhatatva Pharmacy, Dr. D.Y. Patil College of Ayurved and Research Centre, Pimpri Colony, Pune.

Standardisation was carried out as per Ayurvedic Pharmacopoeia of India (API) guidelines. *Katuka* (*Picrorhiza kurroa*) showed pH 5.53, loss on drying 5.65%, total ash 1.87%, acid-insoluble ash 0.40%, and extractive values of 24.35% (water-soluble) and 11.88% (alcohol-soluble). Thin layer chromatography (TLC) profiling of *Katuka* (*Picrorhiza kurroa*) demonstrated characteristic Rf values (0.05 and 0.30). Organoleptic and physicochemical parameters of *Safed Musali* (*Asparagus adscendens*) showed creamish white colour, characteristic odour, indistinct taste and creamish appearance in 10% aqueous solution, with pH 6.04, foreign matter 0.02%, loss on

drying 8.5%, total ash 9.25%, acid-insoluble ash 1.25%, and extractive values of 33.71% (water-soluble) and 23.12% (alcohol-soluble). All parameters confirmed the quality and purity of the raw drugs. The physicochemical and chromatographic evaluation ensured adequate quality control and reproducibility of the formulation.

Katuka Musali Kashay was prepared according to the standard Ayurvedic Pharmacopoeia of India guidelines[10]. The standard quantity of raw herbal material for the preparation of *Kashay* (decoction), comprising *Katuka* (*Picrorhiza kurroa*) and *Safed Musali* (*Asparagus adscendens*) in a 1:1 ratio, is 48 g. Sixteen parts of water are added and reduced to one-eighth, producing approximately 96 mL of decoction. However, as described in *Sharangadhara Samhita*, the recommended therapeutic dose of *Kashay* (decoction) for humans is 2 *Pala* (approximately 80 mL)[11], which also aligns with the commonly practised clinical dose. Considering classical textual references and practical clinical usage, 80 mL was selected as the standard human dose to ensure relevance, safety and uniformity. The prepared formulation was filtered and stored under appropriate conditions. Organoleptic and physicochemical analysis of the *Kashay* showed a blackish liquid with a pungent odour and bitter-astringent taste, a pH of 6.11, total solid content of 4.17% (w/v), acid value of 1.11 and specific gravity of 1.012 g/mL, indicating good quality and consistency of the formulation.

Animal experiment

The animal study was conducted in accordance with ARRIVE guidelines and followed a protocol approved by the Institutional Animal Ethics Committee (IAEC) (Project Proposal No.: SC/IAEC/2223/034; IAEC-17-015), complying with CPCSEA guidelines published in *The Gazette of India* (15 December 1998). The study was conducted at an authorised research facility using 32 male and female Wistar rats aged 6-8 weeks, with a body weight of 180 ± 20 g. The animals were housed three per cage in polypropylene cages with stainless steel grill tops, equipped with food and water facilities and corn cob bedding, and were maintained on standard pellet feed at 22 ± 3°C and 50 ± 5% humidity.

Administration of dose

Hepatotoxicity was induced by intraperitoneal administration of MTX at a dose of 20 mg/kg b.w. once daily for seven consecutive days. The dose and treatment duration were selected based on established rat models that induce significant hepatic injury while minimising mortality[12]. The dose of silymarin (50 mg/kg b.w.) was selected based on a previous study[13].

The human dose was converted to the experimental animal dose using the body surface area (BSA) conversion method described by Nair and Jacob[14]. The final dose was standardised as 7.20 mL/kg b.w. for ease of administration. The calculated dose was administered orally once daily using an oral feeding cannula throughout the study period.

Grouping of animals with route of administration

Group 1 – Normal Control (NC): n = 8 (4 male, 4 female); normal *ad libitum* diet was maintained.

Group 2 – Disease Control (DC): n = 8 (4 male, 4 female); MTX intraperitoneally for 7 days (20 mg/kg b.w.); regular diet was maintained.

Group 3 – Standard Group (SG): n = 8 (4 male, 4 female); MTX intraperitoneally for 7 days (20 mg/kg b.w.), followed by silymarin orally for the next 7 days (50 mg/kg b.w.); regular diet was maintained.

Group 4 – Test Group (KK): n = 8 (4 male, 4 female); MTX intraperitoneally for 7 days (20 mg/kg b.w.), followed by *Katuka Musali Kashay* orally for the next 7 days (7.20 mL/kg b.w.); regular diet was maintained.

Evaluation of liver function parameters

Following administration of the treatment, each animal was monitored individually, and body weight was measured weekly throughout the experimental period.

Blood samples were obtained at two defined time points: after induction of hepatotoxicity (before treatment; baseline) and at the end of the experimental period (after treatment). Serum biochemical parameters, including SGOT (AST), SGPT (ALT), bilirubin, alkaline phosphatase (ALP) and total protein, were analysed using commercially available Pathozyme kits with a ChemXpress Biochemistry Analyzer.

For oxidative stress assessment, liver tissue homogenates were analysed for malondialdehyde (MDA) levels as an index of lipid peroxidation using a Multiskan Sky High Plate Reader, and reduced glutathione (GSH) levels were estimated to evaluate antioxidant status.

Liver tissue samples were fixed in 10% neutral buffered formalin and then processed through dehydration, clearing and paraffin embedding. Sections of 3–5 µm thickness were prepared and

stained with haematoxylin and eosin (H&E) for microscopic examination.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software. All data are presented as mean ± standard deviation (SD). The Shapiro-Wilk test was used to determine whether the data distribution was normal. Two-way ANOVA (treatment × time interaction) was used to analyse body weight and blood biochemical markers measured at baseline and follow-up, followed by Bonferroni post hoc testing. One-way ANOVA was used to analyse oxidative stress markers, which were measured at a single time point, followed by Tukey's post hoc test for multiple comparisons. Statistical significance was defined as $p < 0.05$.

Results

MTX-induced hepatotoxicity was successfully established following intraperitoneal administration for 7 consecutive days. Data are shown as mean ± SD ($n = 8$). Body weight and serum biochemical parameters were assessed statistically by two-way ANOVA (treatment × time interaction), followed by Bonferroni post hoc testing. Oxidative stress parameters were evaluated by one-way ANOVA, with subsequent Tukey's post hoc analysis. Statistical significance was set at $p < 0.05$.

Effect on body weight

Two-way ANOVA showed a significant interaction between time and treatment ($p < 0.05$) (Table 1, Supplementary Fig. S1). Administration of MTX resulted in a marked reduction in body weight gain, with the DC group exhibiting significantly lower values (4.75 ± 1.28 g) compared with the NC group (10.50 ± 1.41 g) during the induction phase (Day 7–0; $p < 0.01$), confirming successful induction of growth retardation.

Following initiation of treatment, the test group (KK) showed a pronounced recovery. During the Day 14–7 interval, body weight gain in the KK group (12.00 ± 1.83 g) was comparable to that of the standard silymarin group (12.00 ± 1.07 g), with both groups demonstrating a highly significant improvement relative to the DC group (4.80 ± 1.10 g; $p < 0.01$).

By the end of the experimental period (Day 14–0), the cumulative body weight gain in the KK group (16.71 ± 1.70 g) was comparable to that of the standard group (17.13 ± 2.10 g), without any statistically significant difference between them ($p > 0.05$). These findings suggest that *Katuka Musali Kashay* exerts a significant ameliorative effect, comparable to silymarin, in reversing MTX-induced systemic toxicity and the associated growth retardation.

Effect on serum biochemical parameters

SGOT (*serum glutamate oxaloacetate transaminase*)

Two-way ANOVA showed a significant interaction between treatment and time ($p < 0.05$) (Table 2, Supplementary Fig. S2). In the before-treatment (BT) phase, SGOT levels were significantly elevated in the DC group (144.56 ± 19.46 IU/L) compared with the NC group (81.32 ± 4.33 IU/L; $p < 0.01$), confirming successful induction of hepatotoxicity. No significant difference was observed among the DC, SG and KK groups at baseline, indicating uniform disease induction. Following treatment (after treatment, AT), SGOT levels in the DC group further increased to 152.90 ± 10.07 IU/L, whereas both treatment groups showed a significant reduction: SG decreased to 99.64 ± 8.00 IU/L and KK to 107.47 ± 6.17 IU/L ($p < 0.01$ vs DC), indicating improvement in liver function.

SGPT (*serum glutamate pyruvate transaminase*)

Two-way ANOVA showed a significant interaction ($p < 0.05$) (Table 2, Supplementary Fig. S3). In the BT phase, SGPT levels were significantly elevated in the DC group (81.19 ± 8.42 IU/L) compared with NC (36.23 ± 4.06 IU/L; $p<0.01$), confirming hepatotoxicity induction. No baseline difference was observed among the DC, SG and KK groups. In the

Table 1 — Effect of *Katuka Musali Kashay* on body weight gain (g) in MTX-induced hepatotoxicity

Parameter	NC (Group 1)	DC (Group 2)	SG (Group 3)	KK (Group 4)
Day 7–0 (g)	10.50 ± 1.41	4.75 ± 1.28##	5.13 ± 1.46	4.88 ± 1.36
Day 14–7 (g)	13.13 ± 1.64	4.80 ± 1.10##	12.00 ± 1.07**	12.00 ± 1.83**
Day 14–0 (g)	23.63 ± 0.74	9.60 ± 2.41##	17.13 ± 2.10*	16.71 ± 1.70*

Mean ± SD ($n = 8$). Two-way ANOVA followed by Bonferroni post hoc test. Day 7–0: weight gain from Day 0 to Day 7; Day 14–7: from Day 7 to Day 14; Day 14–0: from Day 0 to Day 14.
##$p < 0.01$ vs NC; *$p < 0.05$, **$p < 0.01$ vs DC.
NC – Normal Control; DC – Disease Control; SG – Standard Group (silymarin); KK – *Katuka Musali Kashay*.

AT phase, SGPT levels further increased in DC (105.93 ± 18.05 IU/L), whereas significant reductions were observed in SG (55.35 ± 5.62 IU/L) and KK (74.34 ± 3.00 IU/L) ($p < 0.01$ vs DC), indicating improved hepatocellular integrity.

Bilirubin

Two-way ANOVA showed a significant interaction ($p < 0.05$) (Table 2, Supplementary Fig. S4). In the BT phase, bilirubin levels were significantly elevated in DC (1.38 ± 0.07 mg/dL) compared with NC (0.51 ± 0.12 mg/dL; $p < 0.01$), indicating impaired hepatic function. No significant difference was observed among the DC, SG and KK groups at baseline. In the AT phase, bilirubin levels remained elevated in DC (1.39 ± 0.46 mg/dL), whereas SG (0.69 ± 0.04 mg/dL) and KK (0.78 ± 0.05 mg/dL) showed a significant reduction ($p < 0.01$ vs DC), indicating restoration of hepatic excretory function.

Total protein

Two-way ANOVA showed a significant interaction ($p < 0.05$) (Table 2, Supplementary Fig. S5). In the BT phase, total protein levels were significantly elevated in DC (9.83 ± 0.79 g/dL) compared with NC (5.96 ± 0.62 g/dL; $p < 0.01$), indicating altered hepatic function. No baseline difference was observed among the DC, SG and KK groups. In the AT phase, total protein remained elevated in DC (10.67 ± 0.77 g/dL), whereas SG (6.79 ± 0.56 g/dL) and KK (7.36 ± 0.69 g/dL) showed significant normalisation ($p < 0.01$ vs DC), indicating improvement in hepatic synthetic capacity.

***Alkaline phosphatase* (*ALP*)**

Two-way ANOVA revealed a significant interaction ($p < 0.05$) (Table 2, Supplementary Fig. S6). In the BT phase, ALP levels were significantly elevated in DC (112.86 ± 9.87 IU/L) compared with NC (41.24 ± 3.55 IU/L; $p < 0.01$), confirming hepatotoxicity. No significant difference was observed among the DC, SG and KK groups at baseline. In the AT phase, ALP levels remained elevated in DC (114.75 ± 6.94 IU/L), whereas SG (73.24 ± 6.66 IU/L) and KK (77.10 ± 4.88 IU/L) showed a significant reduction ($p < 0.01$ vs DC), indicating restoration of hepatic function.

Effect of *Katuka Musali Kashay* on oxidative stress parameters

As oxidative stress parameters were measured at a single post-treatment time point, they were evaluated using one-way ANOVA with Tukey's post hoc test (Table 3, Supplementary Fig S7 & S8).

***MDA* (*Malondialdehyde*)**

MDA levels were significantly elevated in the DC group (26.82 ± 1.75 µM/mg tissue) compared with the NC group (10.61 ± 0.97 µM/mg tissue; $p < 0.01$), indicating enhanced lipid peroxidation and oxidative

Table 2 — Effect of *Katuka Musali Kashay* on serum biochemical parameters

Parameter	NC	DC	SG	KK
SGOT (BT) (IU/L)	81.32 ± 4.33	144.56 ± 19.46##	141.50 ± 18.87	141.48 ± 17.65
SGOT (AT) (IU/L)	79.28 ± 3.66	152.90 ± 10.07##	99.64 ± 8.00**	107.47 ± 6.17**
SGPT (BT) (IU/L)	36.23 ± 4.06	81.19 ± 8.42##	83.05 ± 6.27	82.79 ± 6.49
SGPT (AT) (IU/L)	37.49 ± 4.48	105.93 ± 18.05##	55.35 ± 5.62**	74.34 ± 3.00**
Bilirubin (BT) (mg/dL)	0.51 ± 0.12	1.38 ± 0.07##	1.36 ± 0.07	1.36 ± 0.11
Bilirubin (AT) (mg/dL)	0.58 ± 0.11	1.39 ± 0.46##	0.69 ± 0.04**	0.78 ± 0.05**
Total protein (BT) (g/dL)	5.96 ± 0.62	9.83 ± 0.79##	9.90 ± 1.23	9.91 ± 1.37
Total protein (AT) (g/dL)	6.43 ± 0.47	10.67 ± 0.77##	6.79 ± 0.56**	7.36 ± 0.69**
ALP (BT) (IU/L)	41.24 ± 3.55	112.86 ± 9.87##	113.59 ± 8.60	113.73 ± 9.09
ALP (AT) (IU/L)	42.63 ± 3.00	114.75 ± 6.94##	73.24 ± 6.66**	77.10 ± 4.88**

Mean ± SD (n = 8). Two-way ANOVA followed by Bonferroni post hoc test. BT – Before Treatment; AT – After Treatment.
##$p < 0.01$ vs NC; **$p < 0.01$ vs DC.
NC – Normal Control; DC – Disease Control; SG – Standard Group (silymarin); KK – *Katuka Musali Kashay*; SGOT – Serum Glutamate Oxaloacetate Transaminase; SGPT – Serum Glutamate Pyruvate Transaminase; ALP – Alkaline Phosphatase.

Table 3 — Effect of *Katuka Musali Kashay* on oxidative stress parameters after treatment

Parameter	NC (Group 1)	DC (Group 2)	SG (Group 3)	KK (Group 4)
MDA (µM/mg tissue)	10.61 ± 0.97	26.82 ± 1.75##	12.88 ± 1.32**	17.92 ± 1.74*
GSH (µM/mg tissue)	25.43 ± 2.01	13.27 ± 2.23##	21.84 ± 1.65**	18.51 ± 2.46*

Mean ± SD (n = 8). One-way ANOVA followed by Tukey's post hoc test.
##$p < 0.01$ vs NC; *$p < 0.05$, **$p < 0.01$ vs DC.
NC – Normal Control; DC – Disease Control; SG – Standard Group (silymarin); KK – *Katuka Musali Kashay*; MDA – Malondialdehyde; GSH – Reduced Glutathione.

stress following MTX administration. Treatment with silymarin (SG) resulted in a significant reduction in MDA levels to 12.88 ± 1.32 µM/mg tissue ($p < 0.01$ vs DC). Similarly, the KK group demonstrated a significant decrease in MDA levels to 17.92 ± 1.74 µM/mg tissue ($p < 0.05$ vs DC). Although the reduction in MDA levels was more pronounced in the SG group, the effect observed in the KK group was substantial, indicating effective attenuation of lipid peroxidation and significant antioxidant potential.

***GSH* (*reduced glutathione*)**

GSH levels were significantly decreased in the DC group (13.27 ± 2.23 µM/mg tissue) compared with the NC group (25.43 ± 2.01 µM/mg tissue; $p < 0.01$), indicating depletion of antioxidant reserves due to oxidative stress. Treatment with silymarin (SG) significantly restored GSH levels to 21.84 ± 1.65 µM/mg tissue ($p < 0.01$ vs DC). The KK group also exhibited a significant increase in GSH levels to 18.51 ± 2.46 µM/mg tissue ($p < 0.05$ vs DC), indicating recovery of antioxidant capacity. Even though restoration was more evident in the SG group, the KK group displayed considerable attenuation of oxidative stress, suggesting stimulation of endogenous antioxidant mechanisms.

Histopathological evaluation of liver tissue

Histological examination of haematoxylin and eosin (H&E)-stained liver sections (Fig. 1) revealed

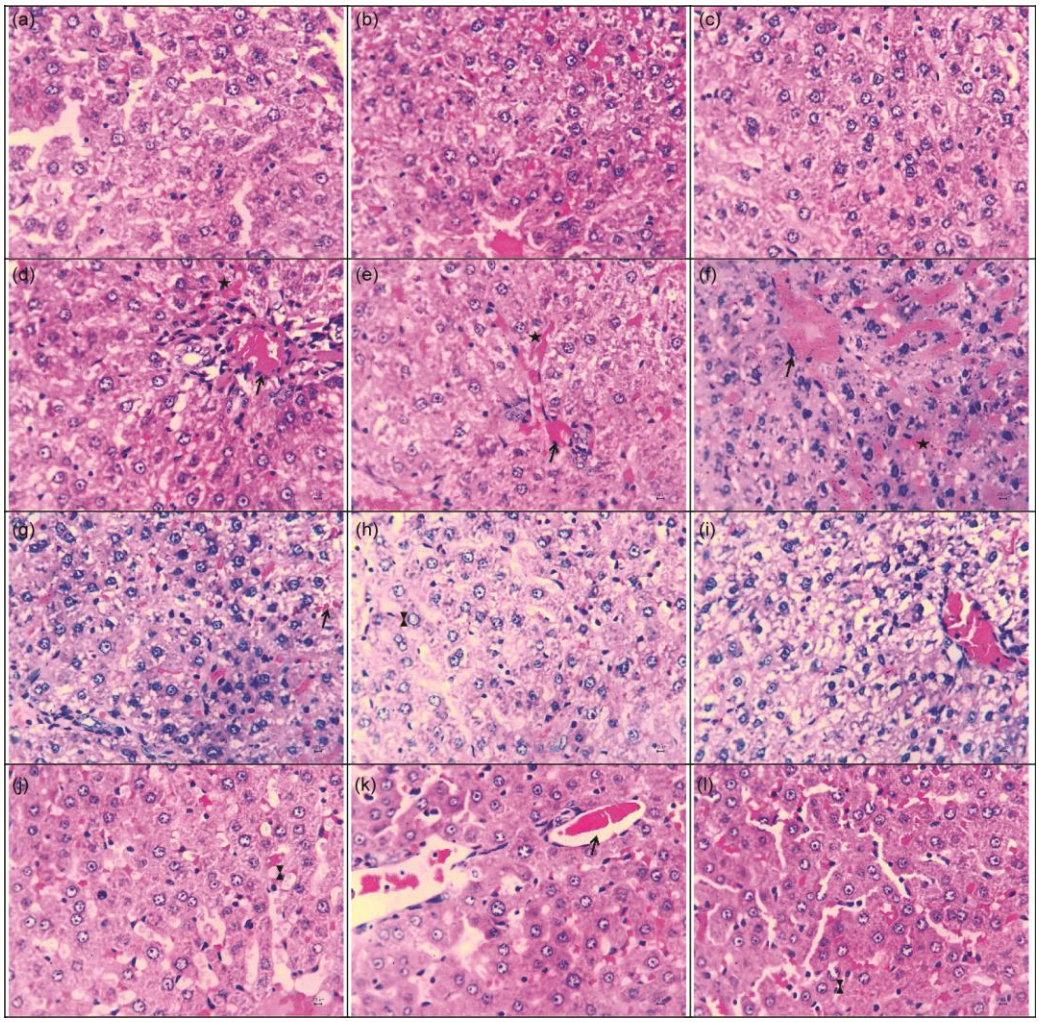


Fig. 1 — Histopathological examination of liver tissue sections stained with haematoxylin and eosin (H&E) (×400). Group 1 (a-c): Normal Control (NC) group showing normal hepatic architecture with well-arranged hepatocytes and intact central vein. Group 2 (d-f): Disease Control (DC) group showing hepatocellular degeneration, vacuolisation, congestion, haemorrhage and inflammatory cell infiltration. Group 3 (g-i): Standard Group (SG; silymarin) showing near-normal hepatic architecture with mild cellular alterations and reduced pathological changes. Group 4 (j-l): Test Group (KK; *Katuka Musali Kashay*) showing preserved hepatic architecture with minimal pathological alterations compared with the DC group. Arrow: congestion; star: haemorrhage; hourglass: cellular swelling

normal hepatic architecture in the NC group, characterised by normal-appearing hepatocytes, an intact central vein and portal tract, with no evidence of vacuolisation or cellular swelling. In contrast, the DC group showed significant degeneration of hepatic histoarchitecture with marked vacuolisation and cellular swelling of hepatocytes, vascular congestion, haemorrhage and increased inflammatory cell infiltration, indicating moderate hepatic damage.

The SG group demonstrated considerable restoration of normal liver architecture with only mild pathological alterations. Mild eosinophilic cytoplasm around the portal area, minimal inflammatory cell infiltration, mild vascular congestion and minimal cellular swelling were observed in some sections, while other sections exhibited nearly normal hepatic architecture without vacuolisation. Similarly, the KK group showed preservation of hepatic histoarchitecture with minimal to mild pathological changes, including mild eosinophilic cytoplasm, minimal inflammatory cell infiltration and minimal cellular swelling, suggesting hepatoprotective activity against hepatic injury. These findings highlight the therapeutic effect of *Katuka Musali Kashay* against MTX-induced hepatotoxicity.

Discussion

The changes in serum biochemical and oxidative stress parameters and the histopathological alterations in liver cells after MTX administration indicate that the drug exerted severe and aggressive effects, leading to oxidative stress and inflammation. The pathogenesis of MTX-induced hepatotoxicity is primarily initiated by the formation of intracellular polyglutamates, which prolong the residence time of the drug and facilitate sustained inhibition of dihydrofolate reductase (DHFR)[15]. This metabolic disruption results in profound folate depletion and impaired nucleic acid synthesis, which is further exacerbated by the localised accumulation of adenosine[16]. The consequence of this biochemical shift is activation of hepatic stellate cells, which is the fundamental cause of MTX-related fibrosis and progressive liver injury[17,18].

MTX-induced hepatotoxicity caused a reduction in body weight as a result of the overall adverse effects on metabolism. It can affect the entire digestive system and is frequently accompanied by cramps, nausea and stomach pain[19]. Malabsorption, weight loss and medication discontinuation are frequently caused by these symptoms.

These findings corroborate earlier experimental studies on methotrexate- and other drug-induced hepatotoxicity, where herbal and antioxidant interventions have demonstrated significant hepatoprotective effects by mitigating oxidative stress, reducing serum transaminase levels and improving histopathological outcomes. Similar protective effects have been reported with *Katuka* (*Picrorhiza kurroa*), which shows significant amelioration of oxidative stress-induced liver damage, and with *Safed Musali* (*Asparagus adscendens*), whose root extracts exhibit notable antioxidant activity and free radical scavenging potential in experimental models[20,21].

Katuka (*Picrorhiza kurroa*) enhances digestive functions, which improves metabolism and nutrient absorption. It helps in eliminating toxins and enhancing nourishment to tissues. It is rich in active compounds such as picrosides (picroside I and II), kutkoside and apocynin. Kutkin, the principal active fraction of *Picrorhiza kurroa*, comprises kutkoside and picrosides and has been reported to possess hepatoprotective activity. The plant also contains other bioactive constituents, including drosin and apocynin. Apocynin, a catechol compound, may exert antioxidant effects by modulating the oxidative response of neutrophils[22]. Picrosides have demonstrated notable antioxidant activity, which may help scavenge reactive oxygen species (ROS) and reduce oxidative stress and subsequent hepatic injury. These compounds stabilise cell membranes and prevent the leakage of liver enzymes such as SGOT and SGPT into the bloodstream, thereby lowering their levels. It enhances bile production and stimulates the flow of bile from the gallbladder into the intestines. Therefore, it helps decrease bilirubin levels by improving the ability of the liver to eliminate excess bilirubin through bile. It possesses detoxifying properties that contribute to the maintenance of hepatic function and metabolic homeostasis, which play a crucial role in reducing elevated ALP and protein levels in the blood. Its anti-inflammatory property helps to alleviate hepatic inflammation by downregulating pro-inflammatory cytokines and mitigating oxidative stress, thereby reducing hepatocellular injury and preventing further liver cell damage. Kutkoside exerts antioxidant activity
by scavenging free radicals and ROS, thereby reducing oxidative stress. These compounds

scavenge harmful free radicals such as superoxide anions and hydroxyl radicals, which contribute to oxidative stress and cellular damage. It also enhances the activity of antioxidant enzymes and protects essential thiol compounds such as glutathione, further decreasing oxidative damage. Methotrexate-induced hepatotoxicity is mediated by oxidative stress and excessive ROS generation. Picrosides mitigate this effect by enhancing antioxidant enzymes such as superoxide dismutase and catalase and preserving glutathione levels, thereby reducing oxidative damage[23]. By preventing lipid peroxidation and stabilising cellular structure, it reduces oxidative damage to cell membranes and aids in healing, particularly in inflammatory conditions. Together, these actions make *Katuka* (*Picrorhiza kurroa*) a highly effective agent.

Safed Musali (*Asparagus adscendens*) exhibits rejuvenating action; it supports nutrient absorption and enhances metabolism, which indirectly supports overall nourishment and body weight gain. It is recognised for its saponins, alkaloids and polysaccharides, which enhance vitality and resistance to stress, including oxidative stress. These constituents support liver function by improving antioxidant defences and promoting liver cell regeneration. Its ability to scavenge free radicals and enhance antioxidant enzymes such as catalase and superoxide dismutase maintains the oxidant–antioxidant balance and reduces oxidative damage[24]. These bioactive constituents also promote cellular regeneration and improve hepatocellular function, supporting hepatic detoxification and metabolic homeostasis. Its diuretic property may further facilitate the elimination of toxic metabolites and bilirubin, which could contribute to the normalisation of altered serum biochemical parameters such as ALP and total protein levels.

Histopathological evaluation revealed that MTX induced marked hepatic alterations, including vacuolisation, vascular congestion and inflammatory cell infiltration, which may be mediated through excessive ROS generation, lipid peroxidation and inflammatory responses. Treatment with *Katuka Musali Kashay* significantly attenuated these pathological changes and restored near-normal hepatic architecture, comparable to the standard treatment group. The ameliorative effect may be attributed to its phytoconstituents, which enhance endogenous antioxidant activity, reduce oxidative stress-mediated hepatocellular damage, stabilise cellular membranes and promote hepatic regeneration.

Conclusion

The present study evaluated the ameliorative potential of *Katuka Musali Kashay* in methotrexate-induced hepatotoxicity in Wistar rats. MTX caused significant hepatic injury, evidenced by altered serum biochemical parameters, increased lipid peroxidation (elevated MDA), reduced antioxidant status (decreased GSH) and histopathological alterations. Treatment with *Katuka Musali Kashay* significantly improved these changes, with effects comparable to the standard drug silymarin, indicating ameliorative activity through reduced oxidative stress and restored hepatic histoarchitecture. Further studies incorporating additional molecular markers are required to elucidate the mechanisms and establish clinical applicability.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_25(10)(2026)975-983_SupplData.pdf

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Author Contributions

THU: Conceptualization, Methodology, Data collection, Analysis, Writing – original draft; SHD: Supervision, Writing – review & editing, Final approval of manuscript.

Ethics Approval

Approved by the Institutional Animal Ethics Committee (IAEC) under Project No.: SC/IAEC/2223/034, in compliance with CPCSEA guidelines.

Informed Consent

Not applicable (animal experiment).

Conflict of Interest

No conflicts of interest are disclosed by the authors.

Use of Artificial Intelligence (AI)

The authors certify that the scientific content of this publication was not created, developed, interpreted or

altered using artificial intelligence (AI) methods. The authors accept full responsibility for the originality, accuracy and integrity of the manuscript and have examined and validated all AI-assisted content.

Data Availability

The data that support the findings of this study can be obtained from the corresponding author upon reasonable request.

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