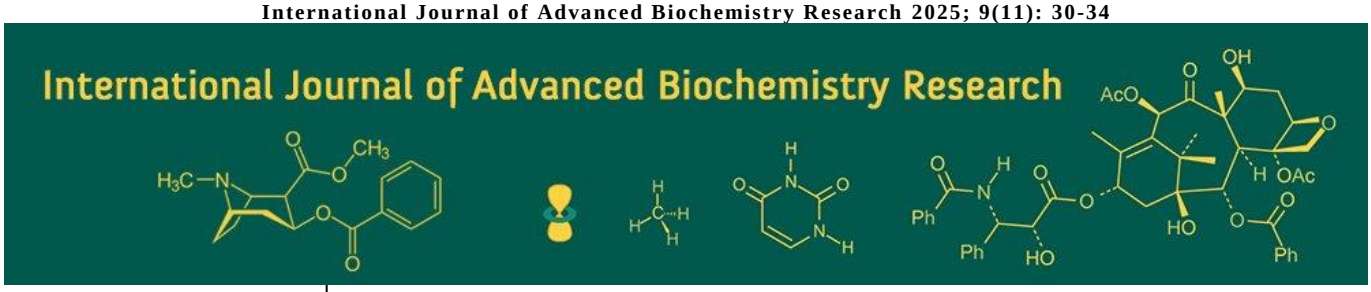


**ISSN Print:** 2617-4693
**ISSN Online:** 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(11): 30-34
www.biochemjournal.com
Received: 23-08-2025
Accepted: 26-09-2025

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# Effects of quercetin on meloxicam-induced haemato-biochemical alterations in male wistar rats

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**DOI:** https://www.doi.org/10.33545/26174693.2025.v9.i11a.6169

**Abstract**
The present study was carried out to evaluate the protective effects of quercetin (@ 50 & 100 mg/kg body weight) in meloxicam (@ 2.1 & 4.2 mg/kg body weight) induced haematological and biochemical alteration in Wistar rats (n = 56) for 28 days. In groups I to VII animals were administered with and respectively. There was a significant decrease in values of haemoglobin, packed cell volume, platelets, total erythrocyte count and significant increase in neutrophil count in group II and III (meloxicam @ 2.1 & 4.2 mg/kg) in comparison with control group (0.5 mL of carboxy methyl cellulose). Moreover, group treated with quercetin at dose rate of 100 mg/kg body weight. (groups V and VII) along different doses of meloxicam (2.1 mg/kg body weight. and 4.2 mg/kg body weight.) showed significant ($p$<0.05) increase in values of TEC, Hb, PCV, MCV, MCH, MCHC, lymphocyte and platelet count as well as decrease in values of TLC, neutrophils in comparison to group treated with high dose of meloxicam. The concentration of AST, ALT, triglyceride, cholesterol and BUN increased significantly ($p$<0.05) and total protein as well as albumin value decreased ($p$<0.05) significantly in rats of group III treated with meloxicam at dose rate of 4.2 mg/kg body weight./Day. In group II, significant ($p$<0.05) increase in concentration of AST decrease values of total protein and albumin were noted. The creatinine value did not differ significantly among all groups. In quercetin co-administered groups (groups IV, V, VI and VII), restoration in serum values of AST, ALT, triglyceride, cholesterol, BUN, total protein and albumin were noted suggesting ameliorative effect of quercetin on liver and kidney toxicities.

**Keywords:** Meloxicam, haematological, biochemical, quercetin, wistar rats

## Introduction
Meloxicam is an oxicam derivatives, belongs to the enol-acid group of non-steroidal anti-inflammatory drug (NSAID). Administration of anti-inflammatory drugs to decrease signs of inflammation is a standard therapeutic approach. Non-steroidal anti-inflammatory drugs are frequently used to treatment of arthritic and post-operative conditions to reduce pain and inflammation. The three major activities of NSAIDs are analgesic, antipyretic and anti-inflammatory (Phillips and Currier, 2004) [16]. However, besides its clinical efficiency, it has severe toxicities and side effects such as cardiovascular thrombotic events, gastrointestinal ulcers, hepatic and renal impairment (Sostres *et al.*, 2013; Burukoglu *et al.*, 2016; Mohammed *et al.*, 2019) [21, 6, 14].
Nowadays, it is assumed that natural flavonoids occurring in fruits and plant derived-foods are relevant, not only for organoleptic properties or technological reasons, but also because of their potential health-promoting effects, as suggested by the available experimental and epidemiological studies. Quercetin is one of the most natural flavonoid present frequently studied bioflavonoid in the subclass of flavonols and has more biopharmacological properties (Renugadevi and Prabu, 2010) [18]. Epidemiological studies have suggested that the intake of food containing flavonoids may be associated with risk of coronary heart disease, hyper cholesterolemia, atherosclerosis and heart failure (Stoclet *et al.*, 2004). It is marketed as a diet supplement with anti-histamine, anti-inflammatory, anti-viral, immunomodulatory and antioxidant properties (Ross and Kasum, 2002) [19]. It also possesses anti-tumoral, anti-fungal, vasorelaxation activity on hippocampal neurons (Pu *et al.*, 2007) [17]. It also scavenges superoxide in ischemia reperfusion injury (Huk *et al.*, 1998) [9].

## Materials and Methods

This present study was designed to investigate the protective effects of quercetin on meloxicam induced haematological-biochemical alterations in rat model for a period of 28 days at the small animal house facilities of the College of Veterinary Science & Animal Husbandry, Kamdhenu university, Navsari (India). The Institutional Animal Ethics Committee approved the protocol vide No. 131-VCN-VPP-2024.

### Design of the Experiment

A total of 56 adult female Wistar rats weighing 200-350 gms were used as experimental animals. The rats aged between 6-8 weeks were procured from the Ribosome Research Centre, Kim, Surat, Gujarat. Rats were maintained in an environment-controlled room at a temperature of 22˚C and relative humidity of 40 to 70 percent. The photoperiod was 12 hours' light and 12 hours' dark. Prior to the commencement of an experiment, rats were acclimatized in an animal house for 7 days. Clean, dry and autoclaved rice husk was used as bedding material for experimental animals. Bedding material was changed every two days. Animals were fed a standard pellet (size 12mm) diet. Rats were provided with *ad libitum* pellet feed which was procured from M/S VRK Nutrition Solution, Pune, Maharashtra. Rats were provided unlimited drinking water (RO water) in polypropylene bottles throughout the period of the experiment. After acclimatization the rats were randomly divided into seven different groups viz.,

After acclimatization the rats were randomly assigned into seven different groups *viz*., Group I to VII with 8 rats in each. The total experimental study duration was 28 days. In Group I (Vehicle control Group), animals were administered 0.5 mL of carboxy methyl cellulose daily for 28 days by oral gavage. In group II and III rats were given meloxicam at a dose rate of 2.1 mg/kg and 4.2 mg/kg body weight daily for 28 days by orally respectively. In group IV and V rats were administered meloxicam at a dose rate of 2.1 mg/kg body weight. along with different doses of quercetin (50 and 100 mg/kg body weight) oral gavage for the period of 28 days. In group VI and VII rats were given meloxicam at dose rate of 4.2 mg/kg body weight along with different doses of quercetin (50 and 100 mg/kg body weight) orally for the period of 28 days.

### Sample Collection and Haemato-Biochemical Analysis

Blood samples were taken from the rats of all the groups on the end of the experiment (29$^{th}$ day) in sterile vials containing $K_3$EDTA at 1 mg/ml as well as plain vacutainers from the retro-orbital plexus for haematological parameters and serum biochemical analysis, respectively. Bood samples were analysed for detailed haematological indices using a haematological-analyser (Exigo EOS Vet, Sweden). The plain vacutainers containing blood samples were initially kept at room temperature for 30 min and centrifuged at 112 g for 10 min to obtain serum, which was stored at-20 °C until further analyses. Serum samples were analysed for the enzymatic activities of aspartate amino transferase (AST), alanine amino transferase (ALT), blood urea nitrogen (BUN), serum creatinine, total protein (TP), albumin, cholesterol and triglycerides by using standard procedures and assay kits (Q-Line Biotech Private Ltd.) on biochemical parameters analyzer (Merck Instrument, Model: Microlab 300, India).

### Statistical Analysis

The data were subjected to statistical analysis using SPSS 20.0 statistical software. A one-way analysis of variance followed by Duncan's multiple range test was performed to determine between the group differences. The values were presented as mean±standard error (SE). The criterion for statistical significance was $p<0.05$.

## Results and Discussion

### Haematological alteration

The mean±SE values of the haematological and biochemical parameters observed in different treatment groups of rats on day 29$^{th}$ of the experiment are presented in Tables 1 and 2, respectively.

**Table 1:** Details of haematological values of different treatment groups of rats

| Parameters | Group I | Group II | Group III | Group IV | Group V | Group V | Group VII |
|---|---|---|---|---|---|---|---|
| TEC (x $10^6$/µL) | $7.25^{a}\pm0.12$ | $6.67^{b}\pm0.15$ | $6.06^{c}\pm0.09$ | $7.11^{ab}\pm0.16$ | $7.17^{ab}\pm0.05$ | $7.00^{ab\pm}0.32$ | $7.12^{ab\pm}0.57$ |
| Hb (g/dL) | $15.51^{a}\pm0.18$ | $14.33^{b}\pm0.18$ | $12.34^{c\pm}0.41$ | $14.68^{ab\pm}0.43$ | $14.86^{ab\pm}0.30$ | $14.05^{b\pm}0.16$ | $14.35^{b\pm}0.27$ |
| PCV (%) | $43.33^{a}\pm0.73$ | $36.79^{b}\pm0.72$ | $31.75^{c}\pm0.93$ | $40.76^{a}\pm0.43$ | $41.08^{a}\pm0.51$ | $37.64^{b\pm}0.56$ | $39.13^{ab\pm}1.21$ |
| MCV (fL) | $52.32^{a}\pm0.53$ | $51.40^{a}\pm0.47$ | $50.53^{b}\pm0.17$ | $51.60^{a}\pm0.34$ | $52.01^{a}\pm0.60$ | $51.06^{a\pm}0.80$ | $51.33^{a\pm}0.57$ |
| MCH (Pg) | $20.01^{a\pm}0.16$ | $19.47^{abc\pm}0.14$ | $18.91^{c\pm}0.23$ | $19.72^{ab\pm}0.13$ | $19.80^{ab\pm}0.12$ | $19.28^{bc\pm}0.22$ | $19.30^{bc\pm}0.21$ |
| MCHC (g/dL) | $38.26^{a}\pm0.25$ | $36.61^{cd}\pm0.33$ | $35.76^{d}\pm0.31$ | $37.39^{abc}\pm0.71$ | $37.93^{ab}\pm0.27$ | $37.33^{abc\pm}0.39$ | $37.60^{abc\pm}0.25$ |
| TLC (x $10^3$/µL) | $8.38^{a\pm}1.02$ | $10.80^{ab\pm}1.40$ | $13.71^{c\pm}0.44$ | $9.23^{ab\pm}0.50$ | $8.58^{a\pm}0.65$ | $11.49^{bc}\pm0.47$ | $11.09^{b}\pm0.59$ |
| Neutrophils (%) | $30.56^{a}\pm1.39$ | $42.83^{c}\pm2.80$ | $43.91^{c}\pm0.89$ | $34.48^{ab}\pm1.21$ | $33.47^{ab}\pm4.20$ | $43.68^{c\pm}2.31$ | $39.36^{bc\pm}2.92$ |
| Eosinophils (%) | $1.13^{a}\pm0.29$ | $1.38^{a}\pm0.32$ | $1.50^{a}\pm0.26$ | $1.38^{a}\pm0.32$ | $1.13^{a}\pm0.35$ | $1.38^{a\pm}0.31$ | $1.25^{a\pm}0.73$ |
| Basophils (%) | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Lymphocytes (%) | $61.05^{a}\pm1.41$ | $55.89^{a}\pm1.48$ | $47.71^{b}\pm1.97$ | $57.02^{a}\pm1.87$ | $60.11^{a}\pm1.45$ | $55.83^{a\pm}0.78$ | $56.76^{a\pm}1.94$ |
| Monocytes (%) | $4.77^{a}\pm0.37$ | $4.52^{a}\pm0.26$ | $4.36^{a}\pm0.24$ | $4.53^{a}\pm0.27$ | $4.66^{a}\pm0.39$ | $4.53^{a\pm}0.27$ | $4.62^{a\pm}0.25$ |
| PLT (x $10^3$/µL) | $847.13^{a}\pm38.43$ | $798.50^{a}\pm32.67$ | $780.0^{a}\pm49.14$ | $817.88^{a}\pm33.91$ | $826.63^{a}\pm44.83$ | $796.38^{a\pm}26.73$ | $813.50^{a\pm}37.07$ |

Note: Means bearing different superscripts in a rows differ significantly ($p<0.05$) between the groups.
Group I: Vehicle control-0.5%CMC @ 0.5 ml, *per os*
Group II: Meloxicam @ 2.1 mg/kg body weight, *per os*
Group III: Meloxicam @ 4.2 mg/kg body weight, *per os*
Group IV: Meloxicam @ 2.1 mg/kg body weight, *per os* and Quercetin @ 50 mg/kg body weight, *per os*
Group V: Meloxicam @ 2.1 mg/kg body weight, *per os* and Quercetin @ 100 mg/kg body weight, *per os*
Group VI: Meloxicam @ 4.2 mg/kg body weight, *per os* and Quercetin @ 50 mg/kg body weight, *per os*
Group VII: Meloxicam @ 4.2 mg/kg body weight, *per os* and Quercetin @ 100 mg/kg body weight, *per os*
N = Number of animals

In present study, significant ($p$<0.05) reduction was noted in TEC, Hb, PCV, MCH, MCV, MCHC and lymphocyte count in meloxicam treated rats at dose rate 4.2 mg/kg body weight. (group III). While significantly ($p$<0.05) increase counts of neutrophil and TLC were also noted in high dose meloxicam (group III) treated animals. In group II also significant reduction in TEC, Hb, PCV and MCHC was noted. While non significant decrease in value of MCV, TLC, platelets and lymphocyte were noted in group II. In groups V and VII showed significant ($p$<0.05) increase in values of TEC, Hb, PCV, MCV, MCH, MCHC, lymphocyte and platelet count as well as decrease in values of TLC, neutrophils in comparison to group treated with high dose of meloxicam. In group IV animals non-significant increase in level of TEC, Hb, PCV, MCHC, monocyte counts and significantly ($p$<0.05) decrease in neutrophil counts as compared to group II rats. In group VI, significant ($p$<0.05) increased levels of TEC, Hb, PCV, MCV, MCHC and non-significant decreased levels of TLC as well as neutrophil counts as compared to group III animals. The decrease level of TEC, Hb and packed cell volume suggested the development of anemia. The calculation of MCV helps in morphological type classification of anaemia. In this study decreased MCV suggests the reduce in the size of erythrocytes and the anemia was microcytic anemia. The decreased value of Heamoglobin suggests the hypochromic anaemia (Vihol, 2010) [25]. increased levels of TEC, Hb, PCV, MCV, MCHC and non-significant decreased levels of TLC as well as neutrophil counts as compared to group III animals. The decreased value of Hb suggests the hypochromic anaemia (Vihol, 2010) [25].

In present study, ameliorative effects of quercetin on different blood parameters were recorded. The quercetin at the dose rate of 50 mg/kg body weight did not show any noticeable protective effect however, the results showed quercetin at high dose of 100 mg/kg body weight. ameliorates the changes in various studied haematological parameters developed by meloxicam administration at two doses i.e. 2.1 and 4.2 mg/kg body weight/day. Our results were in accordance with the findings noted by earlier researchers who have also reported comparative ameliorative effects of quercetin against various toxicants (Yousef *et al.*, 2010; Selvakumar *et al.*, 2012; Uzun and Kalender, 2013; Kasmi *et al.*, 2018; Baraiya *et al.*, 2023) [26, 20, 24, 11, 5].

**Biochemical alteration**

In the present experiment, blood biochemical parameters such as AST, ALT, urea, serum creatinine, total protein, albumin, cholesterol and triglycerides were measured and analysed. Details of the same are presented in the Table 2.0.

**Table 2:** Serum biochemical values reported in different experimental groups

| Parameter | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|---|---|---|---|---|---|---|---|
| AST (U/L) | $154.04^{a\pm}3.14$ | $170.34^{cd\pm}2.38$ | $184.38^{e\pm}3.3$ | $165.63^{bc\pm}4.1$ | $158.95^{ab}\pm4.0$ | $178.48^{de\pm}3.43$ | $163.29^{abc\pm}3.07$ |
| ALT (U/L) | $62.11^{a\pm}3.33$ | $68.96^{ab}\pm1.22$ | $71.42^{b}\pm3.44$ | $66.57^{ab}\pm3.00$ | $64.65^{ab}\pm1.00$ | $70.06^{ab\pm}2.76$ | $65.12^{ab\pm}1.47$ |
| TP (g/dL) | $12.61^{a}\pm0.61$ | $10.09^{cd}\pm0.41$ | $9.09^{d}\pm0.20$ | $11.00^{bc}\pm0.87$ | $12.19^{a}\pm0.37$ | $9.70^{cd}\pm0.36$ | $11.53^{ab}\pm0.30$ |
| Albumin (g/dL) | $4.31^{a}\pm0.17$ | $3.53^{bc}\pm0.13$ | $2.69^{d}\pm0.22$ | $3.71^{abc\pm}0.09$ | $4.02^{ab}\pm0.15$ | $3.38^{c}\pm0.18$ | $3.81^{abc}\pm0.17$ |
| Creatinine (mg/dL) | $0.72^{a\pm}0.059$ | $0.94^{a}\pm0.14$ | $1.04^{a}\pm0.10$ | $0.90^{a}\pm0.16$ | $0.75^{a}\pm0.83$ | $0.97^{a\pm}0.11$ | $0.77^{a\pm}0.039$ |
| BUN (mg/dL) | $21.50^{a}\pm0.55$ | $23.10^{a}\pm0.50$ | $25.83^{b}\pm1.51$ | $22.59^{a}\pm0.74$ | $21.70^{a}\pm0.59$ | $23.23^{a}\pm0.81$ | $22.55^{a}\pm0.76$ |
| Cholesterol (mg/dL) | $171.50^{a}\pm4.61$ | $197.63^{ab}\pm3.64$ | $204.25^{b}\pm2.52$ | $181.13^{ab}\pm5.08$ | $174.00^{a}\pm3.31$ | $199.25^{ab}\pm4.41$ | $177.38^{ab}\pm4.24$ |
| Triglycerides (mg/dL) | $77.13^{a}\pm2.75$ | $85.00^{ab}\pm1.14$ | $96.00^{c}\pm3.33$ | $82.00^{ab}\pm3.80$ | $78.25^{ab}\pm1.77$ | $88.00^{bc}\pm2.47$ | $80.25^{ab}\pm1.84$ |

Note: Means bearing different superscripts in rows differ significantly ($p$<0.05) between the groups.
Group I: Vehicle control-0.5%CMC @ 0.5 ml, *per os*
Group II: Meloxicam @ 2.1 mg/kg body weight, *per os*
Group III: Meloxicam @ 4.2 mg/kg body weight, *per os*
Group IV: Meloxicam @ 2.1 mg/kg body weight, *per os* and Quercetin @ 50 mg/kg body weight, *per os*
Group V: Meloxicam @ 2.1 mg/kg body weight, *per os* and Quercetin @ 100 mg/kg body weight, *per os*
Group VI: Meloxicam @ 4.2 mg/kg body weight, *per os* and Quercetin @ 50 mg/kg body weight, *per os*
Group VII: Meloxicam @ 4.2 mg/kg body weight, *per os* and Quercetin @ 100 mg/kg body weight, *per os*
N = Number of animals

The concentration of AST, ALT, triglyceride, cholesterol and BUN increased significantly ($p$<0.05) and total protein as well as albumin value decreased ($p$<0.05) significantly in rats of group III treated with meloxicam at dose rate of 4.2 mg/kg body weight/day. In group II, significant ($p$<0.05) increase in concentration of AST decrease values of total protein and albumin were noted. The creatinine value did not differ significantly among all groups. In quercetin co-administered groups (groups IV, V, VI and VII), restoration in serum values of AST, ALT, triglyceride, cholesterol, BUN, total II protein and albumin were noted suggesting ameliorative effect of quercetin on liver and kidney toxicities.

In present experiment, there were significant increased levels of AST and ALT due to meloxicam administration at dose rate 2.1 mg/kg body weight and 4.2 mg/kg body weight. Our findings were in agreement with results recorded by previous workers (Al-Rekabi *et al.*, 2009; Ahmed *et al.*, 2015) [2, 1]. Elevated levels of serum alanine aspartate and alanine transferase are indicative markers for liver dysfunction. In this study, these markers were found to be elevated in the groups treated with only meloxicam at different doses. Interestingly, quercetin treatment at 100mg/kg body weight. dose in groups V and VII which were also administered with meloxicam at low and high dose respectively revealed decrease in these enzymes and interestingly results of groups I, V and VII comparable.

After the end of experiment, the values of total protein and albumin were found to be decreased at both doses of meloxicam. The present results correlate with the findings of previous authors (Al-Rekabi *et al.*, 2009; Jadav *et al.*, 2014; Al Wahed *et al.*, 2017; Amin *et al.*, 2017) [2, 10, 3, 4]. The elevated level of reactive oxygen species (ROS), degrades DNA, damages mitochondria and the endoplasmic reticulum which results in altered synthesis of protein and gene expression, which subsequently inhibits mRNA

translation, protein unfolding and denatures damaged proteins. Quercetin treatment at dose rate of 100 mg/kg body weight. improved enzymatic activities of total protein and albumin which suggests its protective effect on meloxicam induced toxicity. However, at low dose of quercetin (50 mg/kg body weight.) ameliorative effects were non-significant were noted and these results supported observations of previous workers (Usadadiya *et al.*, 2021; Baraiya *et al.*, 2023) [23, 5].

In current study, significantly increased BUN concentration was noted in group III which was administered with high dose of meloxicam. Moreover, creatinine was also found to be non-significantly increased in low and high dose of meloxicam treated rats. Present results were in accordance with the reports of earlier researchers (Mahaprabhu *et al.*, 2011; Amin *et al.*, 2017) [12, 4]. Quercetin has potent anti-oxidant capacity so it reduces the production of ROS which in turn helps in maintaining the normal level of GFR and ultimately decrease the serum creatinine and BUN levels. In current experiment, restoration of urea and creatinine concentrations by quercetin were noted which supported the findings of earlier workers (El-Ela & Abdel-Aziz, 2019; Mansour *et al.*, 2022) [7, 13].

Presently, cholesterol level was noted to be significantly increased in group of rats treated with high dose (4.2 mg/kg body weight.) of meloxicam. Our results were in accordance with the results of previous researchers (Al-Wahed *et al.*, 2017; Amin *et al.*, 2017) [3, 4]. However, in group treated with low dose of meloxicam (2.1 mg/kg body weight.) results were found to be non-significantly increased in comparison with the control group. The results indicated that severity of toxicity increase in dose dependent manner upon administration of meloxicam. It is believed that the antioxidant property could also play a major role in protection of lipid membranes from free radicals. Quercetin attenuates the abnormal dispersion of membrane lipids in circulation as well as reduces the excessive generation of more toxic lipid peroxides which results in less harmful effects on cells and tissues (Padma *et al.*, 2012) [15].

## Conclusions

The findings of the present study showed that meloxicam administration for 28 days altered haemato-biochemical alterations mainly affecting which were normalized following treatment with quercetin at dose rate of 100 mg/kg body weight. Quercetin pretreatment revealed good haemato protective, hepato-protective, and nephro-protective effects against meloxicam induced organ toxicities in a dose dependant manner.

## Acknowledgement

We are highly thankful to Principal, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Navsari, Gujarat for providing the necessary facility.

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