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Repurposing metronidazole-based antifungal drug against lung cancer in a rat model.

J.P. Haritha [1], Dr. S.Swarnalatha[2], Dr. J.Karthi[3], M.Vignesh[4]

[1]- Department of Pharmacology – Pallavan Pharmacy College, Kanchipuram
[2]- Head of the Department of Pharmacology – Vice Principal Pallavan Pharmacy College, Kanchipuram
[3]- Head of the Department of Pharmacognosy – Principal of Pallavan Pharmacy College, Kanchipuram.
[4]- Department of Pharmacology – Assistant Professor – Pallavan Pharmacy College, Kanchipuram.
Corresponding Author: J.P.Haritha
E.Mail: rohininatarajan1101@gmail.com

ABSTRACT:

Lung cancer is the leading cause of cancer death worldwide, and the high cost of newer therapies limits access to treatment in India, which makes inexpensive repurposed drugs attractive. This study evaluated whether metronidazole protects against lung carcinogenesis induced by urethane in rats, using cisplatin as the reference treatment. The animals were divided into a normal control group, a negative control group given urethane alone, a positive control group given urethane with cisplatin, and two groups given urethane with metronidazole at a low dose and a high dose. Lung tissue was assessed for lipid peroxidation (malondialdehyde), reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and the inflammatory cytokines TNF-α and IL-6, followed by histopathological examination, and the groups were compared by analysis of variance with Tukey's post hoc test. Urethane markedly increased lipid peroxidation and both cytokines, roughly doubling the cytokine levels, and sharply depleted glutathione, superoxide dismutase and catalase. Metronidazole reversed these changes in a dose-dependent manner. At the high dose, glutathione, superoxide dismutase and catalase were not significantly different from the cisplatin group, whereas lipid peroxidation and both cytokines improved considerably but still differed significantly from cisplatin. Glutathione peroxidase was raised by urethane and remained high at the high dose, so it did not follow the dose-dependent pattern. Histology showed better preservation of lung architecture at the high dose. Metronidazole therefore showed dose-dependent antioxidant and partial anti-inflammatory protective effects in this model. Tumour incidence and survival were not measured, so these findings are preliminary and need confirmation in tumour-outcome-based studies.

Keywords:

- Lung cancer
- Drug repurposing
- Metronidazole
- Urethane
- Oxidative stress
- Antioxidant enzymes
- TNF-α
- IL-6
- Cisplatin
- Chemoprevention

Introduction:

Lung cancer is the leading cause of cancer-related death worldwide. Smoking is the greatest risk factor, but the disease also occurs in people who have never smoked. In India, newer agents such as bevacizumab and pembrolizumab are unaffordable for a large share of patients, which creates a need for inexpensive alternatives. [Source figures in the PDF abstract (e.g. cost per cycle, proportion of patients unable to afford treatment) have no citations and should be referenced or removed. Drug repurposing, finding new indications for approved drugs with known safety profiles, shortens development time and reduces cost. Antifungal drugs have been repurposed on the basis of shared targets (for example SQLE with terbinafine, and cytochrome P450 enzymes with azoles) and off-target effects on proliferation, cell cycle, apoptosis and autophagy. Itraconazole, ketoconazole, clotrimazole and miconazole have all been reported to show anticancer activity in preclinical or clinical settings, including lung cancer models. Cytochrome P450 enzymes also

influence carcinogen activation and chemotherapy response, which links azole pharmacology to cancer biology.

Oxidative stress and chronic inflammation are central to the initiation and progression of lung cancer. Urethane (ethyl carbamate) is a widely used chemical carcinogen for inducing lung tumours in rodents. It is metabolically activated to reactive intermediates and reactive oxygen species that damage macromolecules, deplete antioxidant defences and sustain inflammatory signalling. Metronidazole is a nitroimidazole antibacterial and antiprotozoal agent that is inexpensive and widely available. It is a prodrug whose nitro group is reduced in anaerobic organisms to cytotoxic radicals. It is structurally related to the imidazole class but is not an antifungal. Its effect on urethane-induced lung injury had not been evaluated in the material available to us. This study therefore examined whether metronidazole (5 and 15 mg/kg) modifies oxidative stress, inflammatory cytokines and lung histology in urethane-treated rats, compared with cisplatin (1 mg/kg).

Materials and Methods:

Drugs and chemicals

Metronidazole and cisplatin were procured from reputed suppliers. Batch number, manufacturing and expiry dates and certificates of analysis were recorded. Urethane was used as the carcinogen.

Animals and housing

Animals were obtained from a registered laboratory animal breeding facility and housed in polypropylene cages with bedding under a 12:12 h light/dark cycle, at 22 ± 2 °C and 50–60% relative humidity. They received standard pellet diet and purified water ad libitum and were acclimatised for 7 days with daily health observation. Only healthy animals were included. Procedures followed CPCSEA guidelines.

Experimental design and treatment:

Animals were randomly divided into groups of six (n = 6) planned; see for the group count and the groups reported in the results were:

Urethane induction and drug administration

The source document identifies urethane (ethyl carbamate) as the inducing agent and reports a dose of 1.5 mg/kg body weight administered

Group	Treatment
I – Normal Control	Normal saline 0.5 ml/kg, oral
II – Negative Control	Urethane 1.5 mg/kg b.w., i.p.
III – Positive Control	Urethane 1.5 mg/kg + cisplatin 1 mg/kg
IV – Low dose	Urethane 1.5 mg/kg + metronidazole 5 mg/kg
V – High dose	Urethane 1.5 mg/kg + metronidazole 15 mg/kg

intraperitoneally. Metronidazole was described as being administered at 5 and 15 mg/kg body weight, and cisplatin at 1 mg/kg. Oral administration is described for metronidazole, but the route of cisplatin administration is inconsistent in the source.

Assessment of oxidative stress and antioxidant markers

Lung tissue was identified as the sample for assessment of oxidative stress and antioxidant status. The source describes the following parameters:

Malondialdehyde (MDA):

Lipid peroxidation was assessed using a thiobarbituric acid-reactive substances-based method. The described procedure involved tissue homogenate, trichloroacetic acid and thiobarbituric acid reagents, heating, centrifugation, and spectrophotometric measurement at 532 nm.

Reduced glutathione (GSH):

GSH was assessed as an indicator of non-enzymatic antioxidant status. The final manuscript should specify the actual assay procedure, reagents, wavelength, calculation, and units used in the experiment.

Superoxide dismutase (SOD):

SOD activity was evaluated as an enzymatic antioxidant marker. The source describes an inhibition-based colorimetric principle; the exact method and calculation used should be stated from the laboratory protocol.

Catalase (CAT):

Catalase activity was described as the decomposition of hydrogen peroxide, monitored spectrophotometrically at 240 nm.

Glutathione peroxidase (GPx):

GPx activity was described using a peroxide-dependent glutathione oxidation principle. The specific assay protocol, calculation, and reporting units should be confirmed.

Assessment of inflammatory cytokines

TNF-α and IL-6 were identified as inflammatory markers. The source describes ELISA-based estimation using appropriate standards, sample incubation, washing, detection reagents, colour development, and absorbance measurement. The sample type, kit manufacturer, catalogue number, detection wavelength, and units should be confirmed from the experimental records.

Lung histopathology

Lung histopathology was included to assess tissue architecture and pathological alterations. The source describes examination of alveolar spaces and walls, bronchioles, blood vessels, inflammatory cell infiltration, congestion, and structural abnormalities. The available descriptive findings were compared across normal control, urethane-induced negative control, cisplatin-treated, and metronidazole-treated groups.

The tissue fixation procedure, staining method, section thickness, magnification, and histopathological scoring system were not adequately specified in the available text and must be added if performed.

1.8. Statistical analysis

The source reports results as mean ± SEM and describes group comparisons using analysis of variance followed by Tukey's multiple-comparison test. However, the source inconsistently identifies the analysis as one-way and two-way ANOVA and gives different sample sizes in different tables. The statistical method, number of animals included in each analysis, and significance thresholds must therefore be confirmed from the original statistical output.

Results:

Table 01: Effect of metronidazole on Malondialdehyde, Glutathione, Glutathione Peroxidase test:

S.NO	GROUPS	MDA (mg/ml)	GSH (mg/ml)	GPX (µmol/g)
1	Normal control	3.402 ± 0.017	82.33 ± 0.37	3.798 ± 0.016
2	Negative Control	8.166 ± 0.012 a***	56.49 ± 0.42 a***	8.208 ± 0.025 a***
3	Positive Control	3.798 ± 0.015 a***b***	79.43 ± 0.70 a** b***	4.638 ± 0.148 a*** b***
4	Low dose 5mg	6.396 ± 0.020 a***b***c***	72.57 ± 0.63 a*** b*** c***	5.658 ± 0.026 a*** b*** c***
5	High Dose 15 mg	4.004 ± 0.016 a***b***c***	79.95 ± 0.51 a* b*** cns	7.922 ± 0.022 a*** b*** c****

Values are represented in Mean ± SEM, n=6 ns- Non significant,*p<0.05,
**p<0.01, ***p<0.001, ***p<0.0001, ****p<0.00001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (Two way ANOVA followed by Turkeys test)

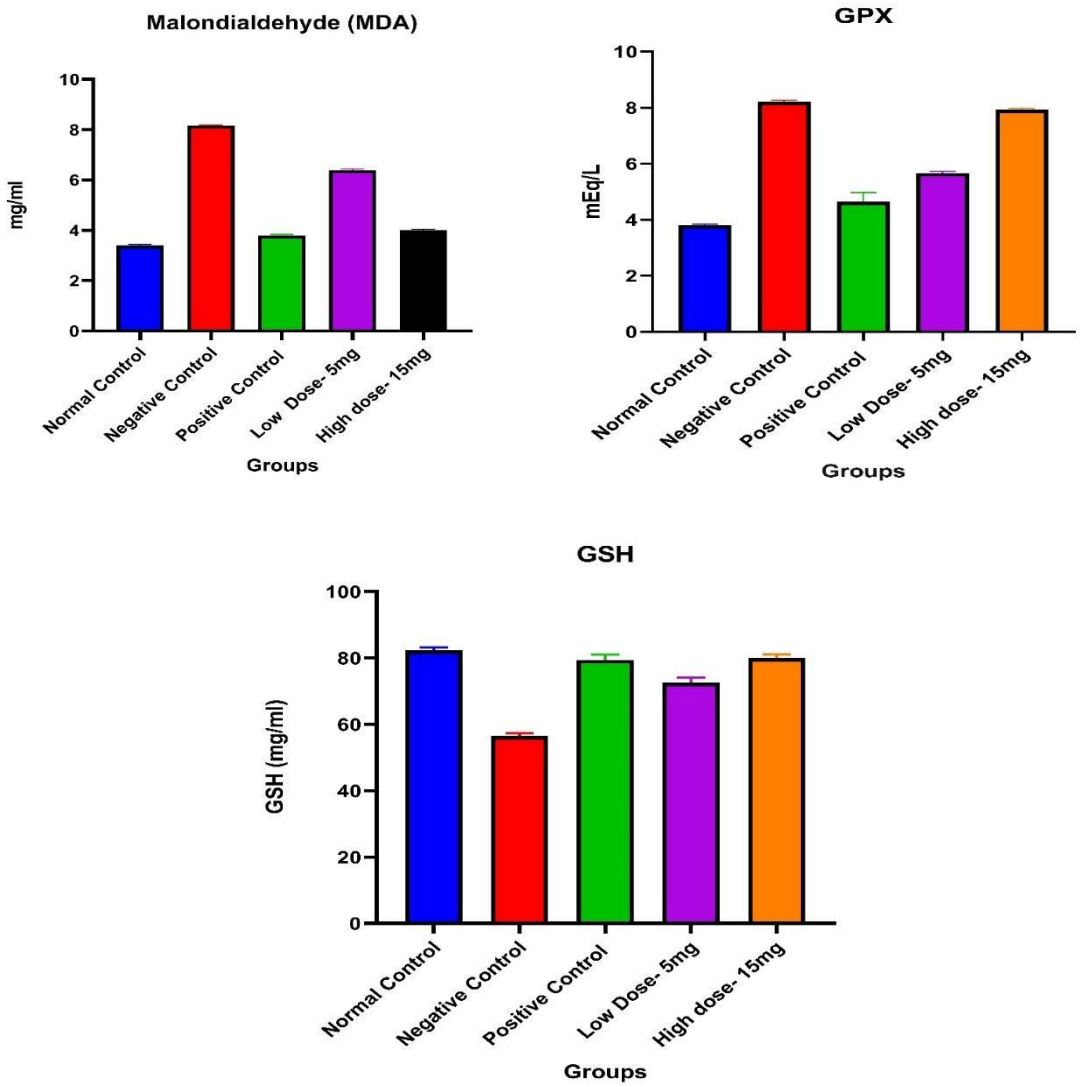


Fig 01: Effect of metronidazole on Malondialdehyde, Glutathione, Glutathione Peroxidase test

Table 02: Effect of metronidazole on superoxide dismutase, Catalase, TNF-α test:

S.NO	GROUPS	SOD (mg/ml)	CAT (units / mg protein)	(units / mg protein)
1	Normal control	11.56 ± 0.107	77.61 ± 0.399	39.53 ± 0.08
2	Negative Control	2.970 ± 0.189 a***	31.21 ± 1.735 a***	75.66 ± 0.09 a***
3	Positive Control	9.830 ± 0.293 a*** b***	65.67 ± 1.104 a*** b***	46.00 ± 0.26 a*** b***
4	Low dose 5mg	5.798 ± 0.251 a*** b*** c***	44.91 ± 1.051 a*** b*** c***	67.56 ± 0.21 a*** b*** c***
5	High Dose 15 mg	8.906 ± 0.226 a*** b*** cns	62.48 ± 0.656 a*** b*** cns	58.64 ± 0.19 a*** b*** c***

Values are represented in Mean ± SEM, n=6 ns- Non significant,*p<0.05, **p<0.01, ***p<0.001, ***p<0.0001, ****p<0.00001

Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (Two way ANOVA followed by Turkeys test)

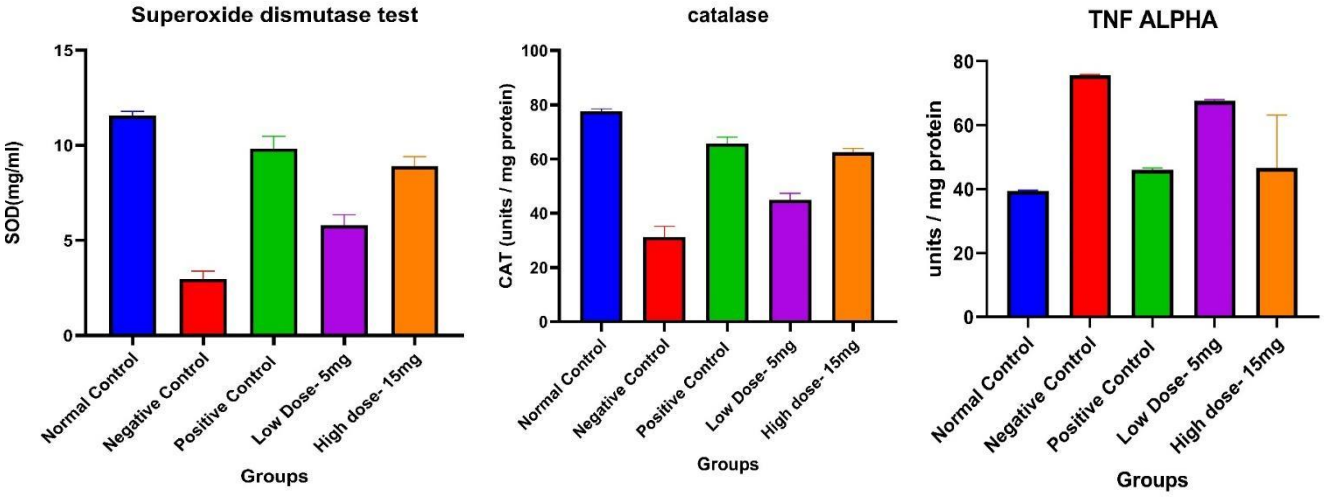


Fig 02: Effect of metronidazole on superoxide dismutase, catalase, and TNF-α

Table 03: Effect of metronidazole on IL-6

S.No	Groups	(units / mg protein)
1.	Normal control	20.62 ± 0.10
2.	Negative Control	44.49 ± 0.14 a^{****}
3.	Positive Control	23.75 ± 0.08 a^{****} b^{****}
4.	Low dose – 5mg	36.38 ± 0.12 a^{****} b^{****} c^{****}
5.	High Dose – 15 mg	30.52 ± 0.10 a^{****} b^{****} c^{****}

Values are represented in Mean ± SEM, n=5 ns- Non significant, *p<0.05,
**p<0.01, ***p<0.001, ****p<0.0001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Tukey's test).

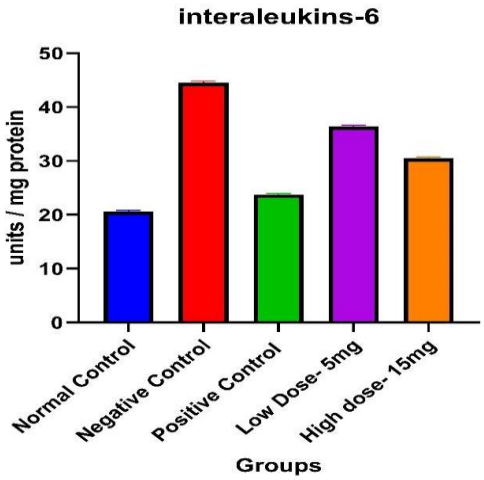


Fig 03: Effect of metronidazole on IL-6

Discussion:

This study examined whether metronidazole protects the lung against urethane-induced carcinogenic injury. Urethane produced the expected pattern of oxidative and inflammatory stress: lipid peroxidation increased, reduced glutathione, superoxide dismutase and catalase were depleted, TNF-α and IL-6 rose, and lung histology was disrupted. This is consistent with the metabolic activation of urethane to reactive intermediates and reactive oxygen species. Metronidazole reduced lipid peroxidation in a dose-dependent manner, with the high dose approaching the cisplatin group, which indicates less membrane lipid damage. Glutathione, superoxide dismutase and catalase recovered progressively, and at the high dose all three were statistically indistinguishable from cisplatin, suggesting that both the non-enzymatic thiol pool and the enzymatic detoxification of superoxide and hydrogen peroxide were preserved. Reduced generation of reactive oxygen species or support of glutathione synthesis and recycling could explain this, but neither was tested here, so these remain hypotheses. Glutathione peroxidase behaved differently. It was raised by urethane, possibly as a compensatory response to peroxide load, and it stayed high at the high dose while the low dose reduced it more, so this is not a dose-dependent normalisation, and the compensatory explanation is untested. TNF-α and IL-6 both fell dose-dependently with metronidazole, but even at the high dose they remained significantly different from cisplatin, so the anti-inflammatory effect is real but weaker than the antioxidant effect and weaker than that of the reference drug. The data cannot show whether the cytokine changes are a direct effect or a consequence of reduced oxidative injury. The histology agreed with the biochemistry: the high dose most closely resembled normal lung and the low dose showed only partial recovery, although the assessment was descriptive and unscored, which limits its weight. Overall, high-dose metronidazole matched cisplatin for glutathione, superoxide dismutase and catalase but not for lipid peroxidation, glutathione peroxidase, TNF-α or IL-6. This raises the possibility of repurposing an inexpensive, widely used drug as an adjunct antioxidant or chemopreventive agent. However, tumour incidence, multiplicity and survival were not measured, only two dose levels were reported, and there was no metronidazole-alone or vehicle control group. Inconsistencies in species, units, sample size and statistics also remain to be resolved. The findings therefore describe a protective biochemical and histological signature rather than proven anticancer efficacy, and tumour-outcome-based confirmation is needed before any clinical extrapolation.

Conclusion:

Metronidazole at 15 mg/kg lowered lipid peroxidation, restored GSH, SOD and CAT to levels statistically comparable with cisplatin, reduced TNF-α and IL-6 (though not to cisplatin levels), and improved lung histology in urethane-treated rats. The 5 mg/kg dose gave partial effects. GPx did not follow a dose-dependent improvement. The results support further investigation of metronidazole as a protective or adjunct agent, with tumour-outcome measures, a wider dose range, longer treatment and mechanistic studies, before any clinical extrapolation.

Compliance with ethical standards:

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Disclosure of conflict of interest:
There is no conflict of interest.

Author Contribution:
Dr.S.Swarnalatha and Dr.J.Karthi had principal responsibility for the conception and design of the study and drafting of the manuscript. M.Vignesh was responsible for the revision of the manuscript for important intellectual content and contributed towards reviewing of the literature. All authors agreed on the final version of the manuscript.

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