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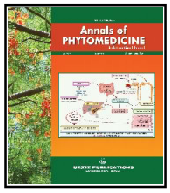

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Exploration of antigout, antioxidant, and production performance augmenting activity of *Phyllanthus niruri* L. in gout-induced broiler chicken

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Abstract

The current work was developed to determine the prophylactic antigout activity of *Phyllanthus niruri* L. in gout-induced broiler chicken. The phytochemical analysis on *P. niruri* leaves exhibited positive findings for phenolic, terpenoids, flavonoids, and alkaloid groups and GC-MS analysis revealed 30 compounds. In the experiment, one hundred and sixty one day old chicks were used and they were separated into 5 groups as normal control, Allopurinol, gout control, *P. niruri* 10 g/kg, and *P. niruri* 12.5 g/kg of feed with 8 birds per group (4 replicates) and maintained for 6 weeks. The sodium bicarbonate in water (20 g/l) was used to induce gout and the clinical signs, and production parameters were noted gross, and histopathology study was performed on dead birds. The creatinine and uric acid values in serum were noted (on days 10, 15, 18, 21, and 42) and xanthine oxidase (XO) and antioxidant enzyme activities in tissues were calculated. Uric acid progressively reduced in *P. niruri* 10 g/kg group from day 15 (16.73 ± 0.41 mg/dl) to day 21 (11.75 ± 0.28 mg/dl) and XO activity was similarly reduced (7.90 ± 0.02 u/mg protein), as that of Allopurinol (7.72 ± 0.04 u/mg protein), which validated the antigout activity mechanism. The feed conversion ratio (1.85 ± 0.01) was superior and antioxidant enzyme values returned to normal levels in the *P. niruri* 10 g/kg group than with Allopurinol. Hence, it was found that *P. niruri* @ 10 g/kg can be utilized prophylactically against gout in birds as an ideal replacement for Allopurinol.

1. Introduction

The trend of using natural compounds and herbs is increasing worldwide and herbal medicine would be an ideal option for many of the disease conditions. Gout is one of the common metabolic disorders reported in both humans and birds. This disorder is due to the deficiency and evolutionary loss of uricase enzyme activity in birds, humans, as well as primates (Oda *et al.,* 2002; Choi *et al.,* 2005) which leads to uric acid as the final product of their purine metabolism. Hence, the abnormal elevation of uric acid within blood and body fluids because of kidney dysfunction may lead to the precipitation of uric acid in visceral organs, which in turn causes gout symptoms (Lakkawar *et al.,* 2018). Generally, XO enzyme is involved in generating uric acid, and its inhibition by Allopurinol leads to anti-hyperuricemic activity. The oxidative stress is one of the adverse effects of allopurinol that might affect birds by reducing their production performance capacity (Carro *et al.,* 2010). Therefore, this study focused on natural sources expected to exhibit xanthine oxidase inhibitory activities (Kumar and Azmi *et al.,* 2014) with no side effects.

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Many Indian medicinal plants were tested for *in vitro* XO inhibitory activity and some of them showed the activity (Azmi *et al.,* 2012; Vikramachakravarthi and Selvaraju; 2017; Vikramachakravarthi *et al.,* 2022), hence they could be used to replace the synthetic XO inhibitors in gout treatment. The phytocompounds like flavonoids and polyphenols were responsible for the XO inhibitory activity (Chang *et al.,* 1993; Costantino *et al.,* 1996) and alkaloids, essential oils, and phenolic compounds also expressed antigout properties by inhibiting XO enzyme (Ling and Bochu, 2014). The lignans, tannins, and essential oils exhibited dual actions, including reducing uric acid generation and uricosuric action (Ling and Bochu, 2014). Accordingly in the current research, the *P. niruri* herb was chosen due to the incidence of flavonoids, lignans, alkaloids, and terpenoids in the leaves (Bagalkotkar *et al.,* 2006). In addition, these phytoconstituents showed anti-hyperuricemic activity (Murugaiyah and Chan, 2009) as well as antioxidant (Mazunder *et al.,* 2005) and anti-inflammatory activities (Obidike *et al.,* 2010) in rats. Hence, the current research was conducted to identify the prophylactic antigout activity of *P. niruri* and to narrow the probable mechanism of antigout activity in gout induced broiler chickens. Also, the effect of *P. niruri* supplementation on production performance and oxidative stress in gout induced broiler chickens were calculated, in comparison to allopurinol.

2. Materials and Methods

The *P. niruri* herb was gathered from Namakkal District (Tamil Nadu), and verified by the Botanical Survey of India (No. BSI/SRI/5/23/2017/Tech/1921), GoI, Coimbatore, Tamil Nadu. The leaves

were shade-dried and finely powdered and collected in clean bags. A hundred grams of crude powders of *P. niruri* leaves were tested for major nutrient composition to analyze gross energy, ether extract, total ash, crude fibre, and crude protein at NABL-accredited Animal Feed Analytical and Quality Assurance Laboratory (AFAQAL), Namakkal, Tamil Nadu.

2.1 Phytochemical analysis

To extract the active components in the Soxhlet apparatus, 75 ml of 90% ethanol was used to make the alcoholic extract of *P. niruri* (Pradhan *et al.,* 2023) in 48 h. Then, using a rotary evaporator, the alcoholic extract was evaporated at 35°C under low pressure and kept for phytochemical analysis. The ethnoveterinary herbal research centre for poultry, Namakkal, Tamil Nadu, used the Harborne (1973) technique to perform a qualitative phytochemical study of the alcoholic and aqueous extracts. Total alkaloids (Harborne, 1973), phenols (Singleton *et al*., 1999), and flavonoids (Chang *et al.,* 2002) were quantitatively estimated using a double-beam UV-visible spectrophotometer. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to check the presence of plant-based compounds in the *P. niruri* extract (Srivani and Mohan, 2023). The testing was carried out using turbo mass software and the GC-MS 5975 C agilent system was followed to note the chromatograms and mass spectra (Adams, 2007).

2.2 Prophylactic antigout study of *P. niruri* in gout-induced broiler chicken

The prophylactic activity of *P. niruri* herb was assessed at two different doses in gout-induced broilers with eight birds per group (4 replicates). In the study, 160 one-day old chicks were kept for 42 days after being randomly assigned to one of 5 treatment groups (normal control, gout control, Allopurinol, *P. niruri* - 10 g/kg, or 12.5 g/kg of feed). The dosage was determined based on the results of earlier unpublished pilot dose experiments. The trial was conducted in the poultry farm belonging to the department and it was approved by the ethical committee (Certificate No. IAEC/13/2016, dated 28.09.2016 VCRI, Namakkal) and birds were maintained under standard environmental conditions for 6 weeks. The treatment groups have normal control (T1), gout control (T2), Allopurinol (T3), *P. niruri* - 10 g per kg (T4), and *P. niruri* - 12.5 g per kg of feed (T5).

2.2.1 Gout induction in broiler chicken

Blood samples were collected to estimate serum uric acid and creatinine on day 10 before the gout induction. Gout was induced on day 11 by administering sodium bicarbonate (Mubarak and Sharkawy, 1999) in drinking water at a dose of 20 g/l for 4 days in every treatment group, except the normal control. The broiler chicks were given a prophylactic dose of *P. niruri* on their third day of age. This was continued throughout the gout induction period and stopped on the twentieth day. Whereas, Allopurinol at the dose rate 25 mg/kg body weight (Carro *et al*., 2010) was given once daily from eighth day onwards *via* oral route and continued till 14th day of age. The production performance parameters, such as feed intake, body weight gain, and feed conversion ratio have been noted together with the clinical symptoms and mortality rate noted till 42 days of experiment.

2.2.2 Estimation of biochemical parameters, antioxidant activity, xanthine oxidase enzyme, and pathology study

The blood samples were collected before the gout induction (10th day) and after the gout induction (15th, 18th, and 21st day) for serum uric acid and creatinine analysis. Likewise, blood samples were also collected before slaughter on the 42nd day, to estimate the kidney and liver function markers aspartate aminotransferase (AST), Alanine Aminotransferase (ALT) enzymes. All birds were slaughtered on the 42nd day gross pathological changes were recorded and the organs with lesions were collected in 10% formalin for histopathology studies (Bancroft and Gamble, 2008).

The liver samples were collected to study the antioxidant activity by estimating the superoxide dismutase (SOD) content (Marklund and Marklund, 1974), catalase (CAT) content (Claiborne, 1985), and total reduced glutathione (GSH) content (Ellmans, 1959) in units/mg protein level using double beam UV-visible spectrophotometer. Also, liver tissues were frozen in liquid nitrogen for the estimation of XO activity (Settle *et al.,* 2012). Experimental data was statistically examined in a complete randomized design (Snedecor and Cochran, 2004) using the one-way ANOVA in SPSS software (version 20).

3. Results

The results of the major nutrient composition of *P. niruri* leaves powder showed gross energy @ 3515 kcal/kg, crude protein @ 14.50 %, crude fiber @ 15.85%, and total ash @ 15.15%.

3.1 Phytochemical analysis

The positive results for alkaloids, carbohydrates, flavonoids, phenols, saponins, and terpenoids were observed for *P. niruri* extract. Further quantitative phytochemical analysis showed the concentration of alkaloids (81.00 ± 0.57 mg/g), flavonoids (7.43 ± 0.36 mg of rutin/g), and phenols (110.00 ± 1.00 mg of gallic acid equivalent/g).

The phytocompounds identified in the GC-MS study were shown based on their retention time as well as area percentage (Table 1 and Figure 1). The major biologically active phytocompounds like octadecatrienoic acid, phenol, 4-methoxy, 2,3,6 -trimethyl, vitamin E, phytol, isophytol, 2-methyl octadecadienol, diepoxy hexadecane and benzoquinoline were the biologically active compounds detected in the analysis.

3.2 Prophylactic antigout study of *P. niruri* in gout-induced broiler chicken

A detailed biological experiment was carried out to find out the antigout and antioxidant activity of *P. niruri* herb in different doses (10 g/kg and 12.5 g/kg feed) in comparison with a standard antigout drug; namely, allopurinol in gout-induced broiler chicken.

3.2.1 Gout induction in broiler chicken

The clinical signs such as watery droppings, dullness, depression unthriftiness, and subsequent mortality (four birds) occurred in the gout control group only. The clinical signs and mortality did not occur in normal control and all the *P. niruri* and Allopurinol treatment groups, till the end of the trial.

The findings of production performance parameters are depicted in Table 2. The results showed a higher body weight and better feed conversion ratio of the *P. niruri* treatment groups than Allopurinol and gout control groups.

Table 1: Major compounds identified in the GC-MS study of *P. niruri* alcoholic extract

S. No.	Component name	Retention time (min)	Area detected (percentage)
1.	a. Phytol/Isophytol	18.827	1.87
2.	a. 9,12,15-Octa decatri enoic acid, ethylester, (Z,Z,Z)		
	b. 2-Methyl-Z, Z-3,13 octadecadienol	19.315	1.41
3.	a. 9-Octa decadienal, (Z)		
	b. 1,2-15, 16-Diepoxyhexadecane		
	c. 2-Methyl-Z,Z-3,13-octadecadienol	21.981	5.53
4.	a. Benzenamine, N–2-(3,4-dimethoxy phenyl) ethyl 2–nitro.		
	b. 2 (3H) - Oxazolone , 3-(3-4-dimethoxy phenyl) methyl - 4,5–diphenyl		
	c. Benzeneethamine N-(3,4-dimethoxy phenyl) methyl-3,4-dimethoxy	25.029	31.04
5.	a. Beta tocopherol,		
	b. Isoquinoline,6,7 dimethoxy-1- methyl 4- (3- 4-dimethyl phenyl)	25.10	4.92
6.	a. Vitamin Ec. Beta tocopherol, O-methyl	25.528	13.18
7.	a. 3H-Imidazo (4 – 5 – b - Pyridine), 2" (2-Ethylhexyl sulianyl)		
	b. Benzimidazol - 2 - amine, N -(2,4-dimethoxy benzyl)	25.982	15.54
8.	a. 1,4 Dimethoxy,2,3 dimethyl benzene		
	b. Phenol, 4- methoxy,2,3,6 - trimethyl	26.063	18.45

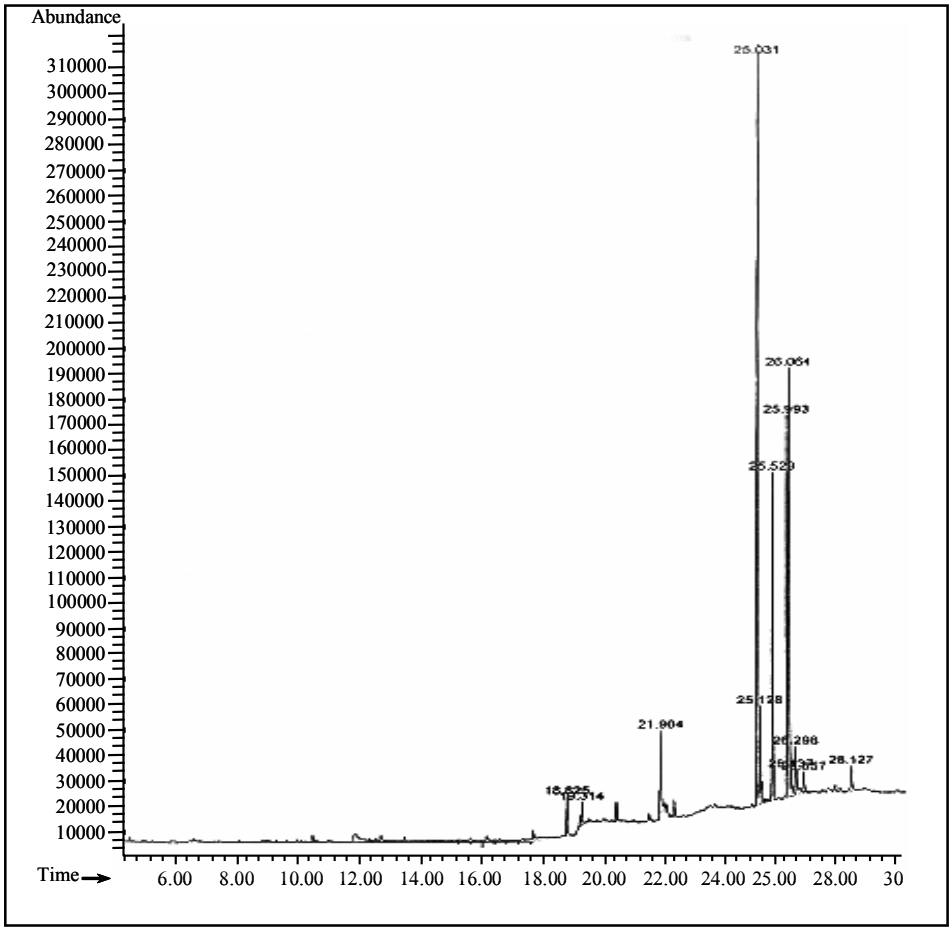

Figure 1: GC-MS chromatogram analysis *P. niruri* alcoholic extract.

Table 2: Results of production performance parameters of treatment groups in gout-induced broiler chicken at the end of the experiment (n = 8) (Mean ± SE)

Parameter	Normal control (T_1)	Gout control (T_2)	Allopurinol 25 mg/kg (T_3)	*P. niruri* 10 g/kg(T_4)	*P. niruri* 12.5 g/kg (T_5)
Body weight (g)	2188.67^{d} ± 14.79	1324.33^{a} ± 31.32	2126.33^{c} ± 14.99	2146.30 ^{b} ± 9.30	2147.83 ^{b} ± 11.71
Feed intake (g)	4030.00^{c} ± 5.16	2896.00^{a} ± 20.92	4020.00^{c} ± 6.83	3980.00 ^{b} ± 3.41	3985.00 ^{b} ± 2.58
Feed conversion ratio	1.84^{a} ± 0.01	2.18 ^{d} ± 0.05	1.89^{c} ± 0.01	1.85 ^{b} ± 0.01	1.85 ^{b} ± 0.01

Columns bearing general superscript did not change significantly at 5% (p<0.05) level.

Table 3: Results of serum uric acid (mg/dl) and creatinine (mg/dl) level of treatment groups in gout induced broiler chicken (n = 8) (Mean ± SE)

Age	Parameter	Normal control (T_1)	Gout control (T_2)	Allopurinol 25 mg/kg (T_3)	*P. niruri* 10 g/kg (T_4)	*P. niruri* 12.5 g/kg (T_5)
Day 10	Uric acid	9.81^{a} ± 0.19	9.61^{a} ± 1.00	9.75^{a} ± 0.21	9.73^{a} ±0.41	9.68^{a}±0.24
	Creatinine	0.47^{a} ± 0.02	0.45^{a} ± 0.04	0.48^{a} ± 0.00	0.48^{a} ±0.02	0.46^{a} ±0.02
Day 15	Uric acid	9.61^{a} ± 0.19	28.21^{d} ± 1.00	10.15^{b} ± 0.21	16.73^{c}±0.41	16.98^{c}±0.24
	Creatinine	0.45^{a} ± 0.19	0.86^{b} ± 0.07	0.45^{a} ± 0.01	0.45^{a}±0.01	0.47^{a}±0.01
Day 18	Uric acid	9.21^{a} ± 0.39	25.05^{d} ± 0.70	9.30^{a} ± 0.24	14.91^{b}±0.27	14.90^{b}±0.38
	Creatinine	0.45^{a} ± 0.02	0.79^{b} ± 0.00	0.48 ^{a} ± 0.01	0.48 ^{a}±0.01	0.49 ^{a}±0.00
Day 21	Uric acid	9.37^{a} ± 0.40	24.02^{c} ± 0.64	9.54^{a} ± 0.24	11.75^{b}±0.28	11.80^{b}±0.35
	Creatinine	0.44^{a} ± 0.02	0.74^{b} ± 0.01	0.43^{a} ± 0.02	0.43^{a}±0.02	0.47 ^{a}±0.02
Day 42	Uric acid	6.61^{a} ± 0.20	21.97^{c} ± 0.13	6.51^{a} ± 0.20	7.56^{b}±0.29	7.59^{b}±0.28
	Creatinine	0.42^{a} ± 0.02	0.78^{b} ± 0.01	0.43^{a} ± 0.01	0.43^{a}±0.02	0.47 ^{a}±0.02

Columns bearing general superscript did not change significantly at 5% (p<0.05) level.

3.2.2 Estimation of biochemical parameters, antioxidant activity, XO enzyme activity, and pathology study

The creatinine and serum uric acid levels for different treatment groups from day 15 to day 42 are depicted in Table 3. The progressive reduction in the serum uric acid level was found in the Allopurinol and *P. niruri* treatment groups on different days. Also, serum creatinine levels of *P. niruri* did not differ from the control group at any stage of the experiment.

Table 4: Findings of serum total protein, globulin, albumin, albumin: globulin ratio, ALT and AST of treatment groups in gout-induced broiler chicken at the end of the experiment (n = 8) (Mean ± SE)

Parameter	Normal control (T_1)	Gout control (T_2)	Allopurinol 25 mg/kg (T_3)	*P. niruri* 10 g/kg (T_4)	*P. niruri* 12.5 g/kg (T_5)
Total protein (g/dl)	3.40^{a}± 0.12	4.02^{b}± 0.04	3.42^{a}± 0.11	3.36 ^{a}± 0.09	3.33 ^{a}± 0.09
Albumin (g/dl)	1.69^{a}± 0.06	2.51^{b}± 0.05	1.71^{a}± 0.10	1.67 ^{a}± 0.08	1.65 ^{a}± 0.08
Globulin (g/dl)	1.71^{b}± 0.10	1.50^{a}± 0.03	1.71^{b}± 0.12	1.69 ^{b}± 0.09	1.68 ^{b}± 0.09
Albumin globulin ratio	1.01^{a}± 0.08	1.66 ^{b}± 0.05	1.04 ^{a}± 0.12	1.00 ^{a}± 0.08	1.00 ^{a}± 0.08
ALT (u/l)	26.08^{a}± 1.33	59.11^{b}± 1.75	29.47^{a}± 2.01	26.10 ^{a}± 1.85	26.30 ^{a}± 2.06
AST (u/l)	182.83^{a}± 4.14	342.35^{b}± 8.82	189.00^{a}± 4.26	182.80 ^{a}± 3.59	183.10 ^{a}± 3.82

Columns bearing general superscript did not change significantly at 5% (p<0.05) level.

The SOD, catalase, and reduced glutathione levels of various treatment groups are revealed in Table 5. A significant difference in the antioxidant enzyme levels was found between gout control as well as other treatment groups (Allopurinol and *P. niruri* treatment groups). The *P. niruri* treatment groups showed significantly higher antioxidant activity than the Allopurinol group. Likewise, XO activity (Table 5) of Allopurinol and *P. niruri* treatment groups significantly differed from the gout control group. A significant difference in xanthine oxidase activity was not noted between the control and Allopurinol and *P. niruri* treatment groups.

Table 5: Results of SOD, CAT, GSH, and xanthine oxidase enzyme activity of treatment groups in gout-induced broiler chicken at the end of the experiment (n = 8) (Mean ± SE)

Parameter	Normal control (T_1)	Gout control (T_2)	Allopurinol 25 mg/kg (T_3)	*P. niruri* 10 g/kg (T_4)	*P. niruri* 12.5 g/kg (T_5)
SOD (Units/mg protein)	1.15 ^{c}± 0.01	0.78 ^{a}± 0.01	0.98 ^{b} ± 0.01	1.13 ^{c}± 0.02	1.17 ^{c}± 0.03
CAT (Units/mg protein)	9.27^{c} ± 0.21	6.37^{a} ± 0.22	8.58^{b} ± 0.26	9.26 ^{c}± 0.04	9.26 ^{c}± 0.19
GSH (Units/mg protein)	10.60 ^{c}± 0.10	8.04 ^{a}± 0.01	9.96^{b}± 0.03	10.46 ^{c}± 0.11	10.45 ^{c}± 0.04
XO activity (Units/mg protein)	7.85^{abc} ± 0.04	8.70^{d} ± 0.05	7.72^{a} ± 0.04	7.90^{c} ± 0.02	7.87^{bc} ± 0.02

Columns bearing general superscript did not change significantly at 5% (p<0.05) level.

Gross pathological examination of gout control group birds showed chalky white deposits over the heart and liver (Figure 2) and severe mottling of the kidney with urate deposits (Figure 3). Histopathology findings also revealed the disruption of cardiac myofibers and fibrous tissue infiltration in the heart (Figure 4) and urate crystals, massive tubular necrosis, and intertubular hemorrhage in the kidney (Figure 5) in the gout control group. Whereas, the gross pathological examination of Allopurinol and *P. niruri* 10 g/kg (Figure 6) and *P. niruri* 12.5 g/kg groups showed normal architecture of visceral organs (heart, liver, and kidney) at the end of experiment analysis. Likewise, appreciable lesions of gout induction like disruption of cardiac fibers, tubular hemorrhage and necrosis in kidney were not observed in histopathology study, instead the normal appearance of heart and liver and normal tubular structure of kidney (Figure 7) was observed.

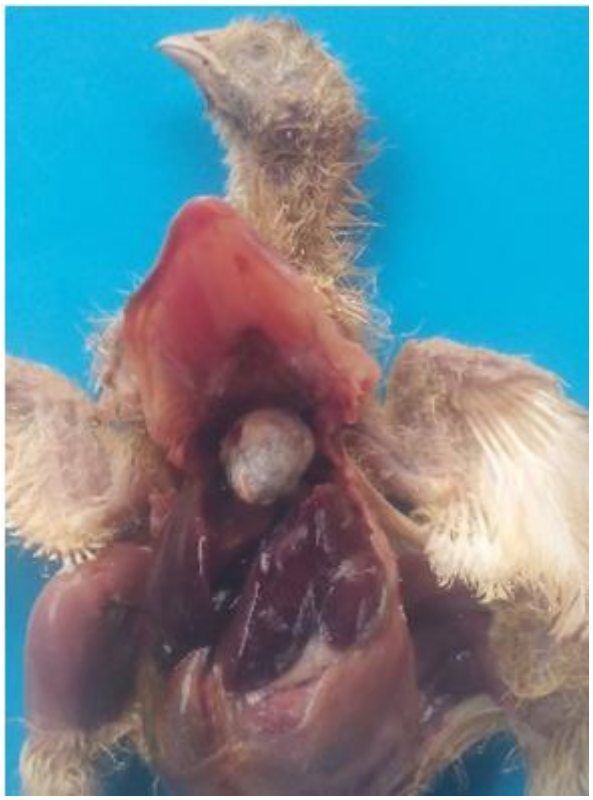

Figure 2: Gout control group (T_2) day 14 - chalky white urate deposits over the heart and in the liver.

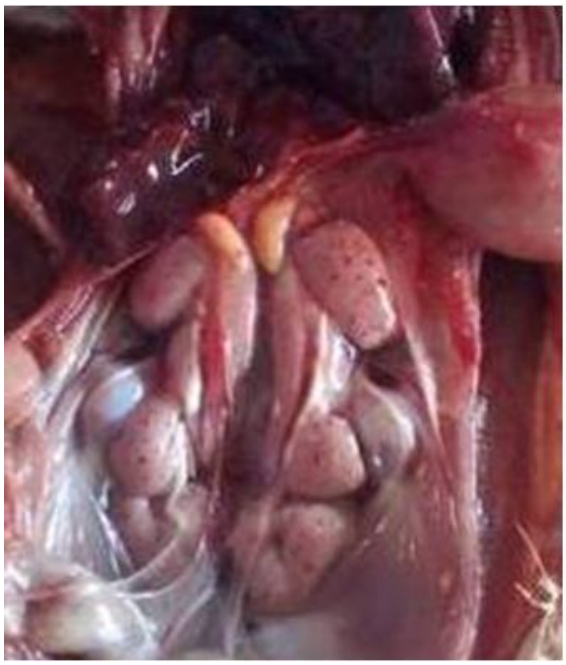

Figure 3: Gout control group (T2) day 14 - severe mottling of kidney with urate deposits.

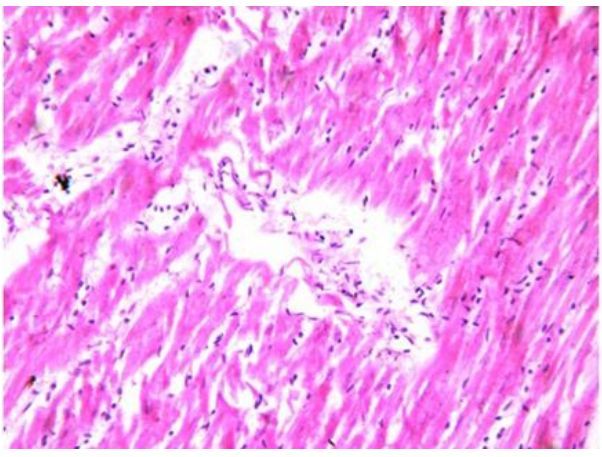

Figure 4: Gout control group (T2) day 14 - heart showing disruption of cardiac myofibres and fibrous tissue infiltration.

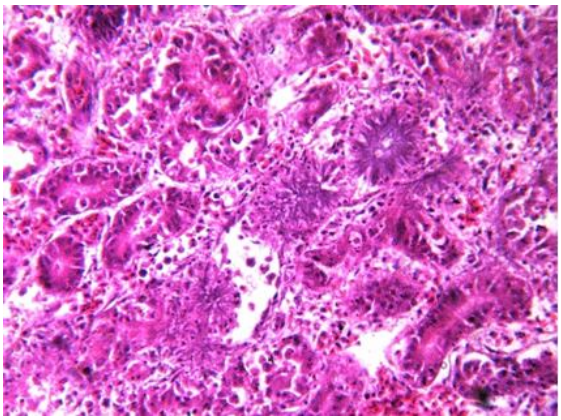

Figure 5: Gout control group (T2) day 14 - kidney showing urate crystals, massive tubular necrosis, and intertubular hemorrhage.

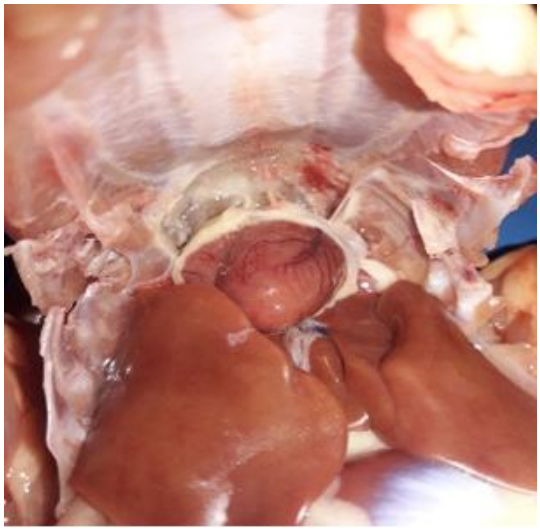

Figure 6: *P. niruri* 10 g (T5) day 42 - normal appearance of heart and liver without urate deposits.

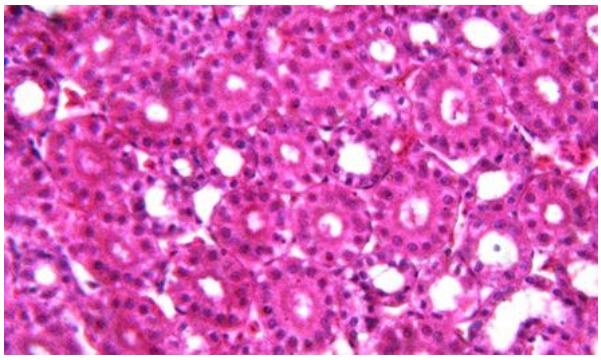

Figure 7: *P. niruri* 10 g (T5) day 42- kidney showing normal tubular structure.

4. Discussion

The major nutrient composition study of *P. niruri* leaf powder showed better gross energy and crude protein levels, whereas the presence of higher crude fiber and total ash limits its usage on a larger scale as a feed additive.

4.1 Phytochemical analysis

The major antigout activity of herbs was attributed to the incidence of alkaloids, lignans, phenol, and flavonoids (Ling and Bochu, 2014). Hence, the detection of all of these phytochemicals in *P. niruri* extract could contribute to the expression of prophylactic antigout activity.

4.2 Prophylactic antigout study of *P.niruri* in gout-induced broiler chicken

4.2.1 Gout induction in broiler chicken

The gout syndrome commonly occurs at the age of 10-14 days in broiler chicks (Prathapkumar *et al.*, 2008), hence in the present analysis; the gout was induced on the 10th day of age using the toxic dose of sodium bicarbonate. The results showed hyperuricemia and higher creatinine contents in gout control group birds, which might lead to the development of clinical signs of gout (Sodhi *et al.,* 2008). The clinical signs and mortality did not occur in control and all the *P. niruri* and Allopurinol treatment groups, till the end of the trial which showed the protective effect of Allopurinol and *P. niruri* treatment groups in gout-induced broiler chicken.

The results of production performance parameters showed the equivalent body weight with less feed intake of *P. niruri* groups than control and better feed efficiency than Allopurinol. These results may be explained by the presence of antioxidant and antistress phytochemicals in *P. niruri* leaves, such as alkaloids, terpenoids, polyphenols, and flavonoids (Awang, 1988; Sharma and Alam, 2022). Nevertheless, the Allopurinol treatment group's production performance decreased, which might have been caused by oxidative stress (Carro *et al.,* 2010).

The presence of vitamin E in the *P. niruri* herb might have reduced the stress due to gout induction in broiler chickens prevented the predisposition to death and also improved the body weight and appetite in broiler chickens (Traber and Atkinson, 2007). Also, the 9,12,15-octa decatrienoic acid detected in *P. niruri* showed hepatoprotective activity (Krishnamoorthy and Subramaniam, 2014) which could have protected the liver from gout-induced cell damage and contributed to a better feed conversion ratio.

4.2.2 Estimation of biochemical parameters, antioxidant activity, xanthine oxidase enzyme, and pathology study

The progressive drop of serum uric acid and serum creatinine showed the prevention activity of herbs against the development of gout in broiler chickens. The diuretic activity of the phytol (Krishnamoorthy and Subramaniam, 2014; Singh *et al.,* 2021) compound present in *P. niruri* might have helped in the excretion of excess uric acid formed during gout induction.

The finding of serum creatinine revealed that renal functions were not affected due to gout induction in the *P. niruri* groups. Further, the results of serum biochemistry showed better albumin, globulin, albumin, as well as total protein: globulin ratio levels and decreased levels of ALT and AST. Hence, the prophylactic dosing of herbs

protected the visceral organs like kidney, liver, and heart from gout-induced damage as evidenced by Behtari and Feizi (2015).

P. niruri antioxidant activity is similar to that of the control group. Evidence of oxidative stress in the Allopurinol treatment group was observed through decreased values of SOD, CAT, and GSH compared to the control and *P. niruri* groups (Carro *et al.,* 2010; Begum *et al.,* 2022). The presence of antioxidant phytochemicals like flavonoids, polyphenols, terpenoids, alkaloids, and in *P. niruri* leaves (Awang, 1988; Malik *et al.,* 2020) might have contributed to the antioxidant activity when compared to allopurinol.

The drop in serum uric acid levels is also correlated and authenticated by the reduced xanthine oxidase enzyme activity in the *P. niruri* treatment groups which is the major reason for hyperuricemia. Since the XO enzyme is a key mediator in uric acid production in the liver and well well-known therapeutic target for many allopathic hypouricemic agents (Borges *et al.,* 2002), the estimation of hepatic XO in the gout-induced broiler chicken could be a vital parameter to explore the antigout activity. The comparable suppression of xanthine oxidase enzyme activity in *P. niruri* groups, similar to that of allopurinol, suggests a potential mechanism for antihyperglycemic activity.

The gross and histopathology pictures clearly illustrated that the prophylactic dosing of herbs prevented the gout-induced damage in visceral organs and the effect is equivalent to Allopurinol. The anti-inflammatory properties of phenol, 4-methoxy,2,3,6-trimethyl (Hadi *et al.,* 2016), isoquinoline (Shanmugapriya and Kalavathi, 2012), phytol (Krishnamoorthy and Subramaniam, 2014) and diepoxy hexadecane (Hameed *et al.,* 2016) of *P. niruri* might have alleviated the gouty inflammation in broiler chicken. These findings are also very well supported by the earlier authors (Wang *et al.,* 2016). The findings of the current research indicated that significant differences were not observed between *P. niruri* treatment groups. Hence, the most effective dose of *P. niruri* at 10 g/kg feed could be preferred from an economic point of view over the 12.5 g/kg group for prophylactic use.

5. Conclusion

The antigout activity of *P. niruri* was due to the xanthine oxidase enzyme inhibitory activity as evidenced by the present study. The significant antioxidant activity exhibited by the *P. niruri* herb in gout-induced chicken was also reflected in the production performance than Allopurinol treated group. The antigout, antioxidant, and production performance augmenting activities of *P. niruri* herb might be due to the presence of pharmacologically active phytocompounds identified in the GC-MS analysis like octadecatrienoic acid, phenol compound, vitamin E, phytol, isophytol, 2-methyl octadecadienol and diepoxy hexadecane. Since there were no significant differences between the two different prophylactic dose levels at the rate of 10 g/kg and 12.5 g/kg of feed, *P. niruri* at 10 g/kg feed dose can be used efficiently as an ideal replacement agent to Allopurinol for the prevention of gout syndrome in broiler chicken.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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