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Assessment of Antigout, Antioxidant and Production Performance Augmenting Activity of *Piper betle* L. in Gout Induced Broiler Chicken

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ABSTRACT

Background: The gout is commonly reported in birds and causes severe economic losses to the farmers. Hence to prevent the gout occurrence *P.betle* L. herb was selected and its antigout potential was studied in comparison with Allopurinol.

Methods: The biological experiment was conducted with 120 broiler chicks and was divided into 5 treatment groups of 8 birds each with triplicate study for six weeks. The groups were control (T_1), gout control (T_2), Allopurinol (T_3), *P.betle* L. - 10 g/kg (T_4) and *P.betle* L. - 12.5 g/kg of feed (T_5). The gout was induced using toxic dose of sodium bicarbonate in water (20 g/litre). The clinical signs and production performance were recorded and gross and histopathology studies were conducted. Serum biochemical parameters *viz.*, uric acid and creatinine were periodically estimated (day 10, 15, 18 and 42 of trial) and anti-oxidant and xanthine oxidase enzyme activity were measured.

Result: The production performances and the antioxidant activity were improved in *P.betle* L. groups and even better than Allopurinol. Also progressive reduction of uric acid and equivalent suppression of xanthine oxidase activity as that of Allopurinol were observed. Hence *P.betle* L. herb can be used effectively in the prevention of gout in birds.

Key words: Allopurinol, Broiler chicken, Gout, *P.betle L.,* Xanthine oxidase.

INTRODUCTION

The gout is one of the major metabolic disorders in broiler chicken which causes heavy economic loss to the farming community of India (Prathapkumar *et al.* 2008). Allopurinol is a xanthine oxidase enzyme inhibitor used commonly for the prevention and treatment of gout by suppressing the production of uric acid. However, its use is limited due to the side effects like oxidative stress in broiler chicken and thereby reduction in the production performance of the bird (Carro *et al*. 2010). Hence an alternative to Allopurinol synthetic drug, the natural sources could be used to inhibit the xanthine oxidase enzyme (Kong *et al*. 2000). Among the natural sources, the herbs possessing the phytocompounds such as polyphenols and flavonoids were used as xanthine oxidase inhibitors (Chang *et al.* 1993). Also the alkaloids, essential oils and phenolic compounds of herbs showed the antigout activity through their xanthine oxidase inhibitory action (Ling and Bochu, 2014). Hence the present study was designed to explore the prophylactic antigout activity of *P. betle* L. medicinal herb and to find out the mechanism of antigout action in the broiler chicken.

MATERIALS AND METHODS

P. betle L. herb local variety (Karpoori) was collected from different regions of Namakkal District of South India, Tamil Nadu and authenticated by the Botanical Survey of India (No.BSI/SRI/5/23/2017/Tech/1921) Govt. of India, Coimbatore, Tamil Nadu. The freshly collected leaves of *P.betle L.* herb were shade dried for the preparation of herbal extract and the dried leaves were finely powdered using a mechanical mixer and then collected in clean polythene bags.

The aqueous and alcoholic extracts were prepared separately using 100 grams of *P.betle* L. powder in 400 mL of distilled water and 400 mL of alcohol, respectively. Both extracts were kept in an orbital shaker for continuous agitation at 200 RPM for 72 hours. The extract was filtered through Whatman filter paper for three times after which a clear aqueous extract was obtained. The alcoholic extract was evaporated at 35°C under reduced pressure using rotary evaporator and then preserved for phytochemical analysis.

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The qualitative phytochemical screening of aqueous and alcoholic extract of *P.betle* L. leaves crude powder was carried out using the method of Harborne (1973) at the laboratory of Ethno Veterinary Herbal Research Centre for Poultry, Namakkal, Tamil Nadu. The total alkaloids content (Harborne, 1973), total phenols (Singleton *et al.* 1999) and total flavonoids (Chang *et al.* 2002) content of *P. betle* L. were estimated using double beam UV-Visible spectrophotometer. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to identify the bioactive components present in the alcoholic extract of *P. betle* L. The analysis was performed in GC-MS 5975 C Agilent System and Turbo Mass software was adopted to handle mass spectra and chromatograms (Adams, 2007).

The experimental trail was conducted in the poultry farm belonging to department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal during the month of June-July 2018. The effect of *P. betle* L. herb was studied at the dose rate of 10 g/kg and 12.5 g/kg of feed against gout induced broiler chicken. One hundred and twenty (120) day old broiler chicks were divided into five groups of eight birds each with triplicate experiment and they were maintained under standard environmental condition for six weeks. The groups were control (T_1), gout control (T_2), Allopurinol (T_3), *P. betle* L. -10 g/kg (T_4) and *P.betle* L. - 12.5 g/kg of feed (T_5).

Gout was induced by sodium bicarbonate (Mubarak and Sharkay, 1999) dissolved in drinking water @ 20 g/L from 11th day to 14th day (four days) of age in all groups, except control. The herbal dosing was commenced prophylactically on 3rd day of age of broiler chicks and continued during the gout induction period and withdrawn on 20th day, whereas Allopurinol dosing was commenced on 8th day of age at the dose rate of 25 mg/kg body weight (Carro *et al.* 2010) and given for one week.

The clinical signs and mortality rate were noted and the production performance parameters like feed intake, body weight gain were recorded and the feed conversion ratio was calculated. 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 42nd day to estimate the kidney and liver function parameters (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) enzymes). All birds were slaughtered on 42nd day and gross pathological changes were recorded and the organs showing lesions were collected in 10% neutral buffered formalin for histopathology studies (Bancroft and Gamble, 2008).

The liver samples were collected to study the antioxidant activity by estimating the superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (CAT) (Claiborne, 1985) and total reduced glutathione (GSH) content (Ellmans, 1959) in double beam UV/ Visible spectrophotometer. Also liver tissues were frozen in liquid nitrogen for the estimation of xanthine oxidase activity (Settle *et al*. 2012). Experimental data was statistically analyzed in complete randomized design (Snedecor and Cochran, 2004)

RESULTS AND DISCUSSION

Phytochemical analysis

The results of qualitative phytochemical analysis of aqueous and alcoholic extracts of *P.betle* L. revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins and terpenoids. Further quantitative phytochemical analysis revealed the presence of alkaloids (62.00 ± 0.57 mg/g), flavonoids (7.68 ± 0.36 mg of Rutin/g) and phenols (130.00 ± 1.15 mg of Gallic Acid Equivalent/g) which has antigout and antioxidant activity (Ling and Bochu, 2014). The phytocompounds detected in the GC-MS analysis are shown with their retention time (RT) and the area percentage (Table 1 and Fig 1). The major biologically active phytocompounds like acetoxy chavichol, chavibetol, eugenol, beta and gamma tocopherol, phytol and beta sitosterol were detected in the leaves of *Piper betle* L.

Biological experiment

A detailed biological experiment was conducted to study the antigout and antioxidant activity of *P.betle* L. 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.

Production performance parameters estimation

The results of production performance parameters (Table 2) showed the equivalent body weight with less feed intake of *P. betle* L. groups than control and better feed efficiency than Allopurinol. These findings might be due to the presence of antistress and antioxidant phytochemicals like ûavonoids, polyphenols, terpenoids and alkaloids in *P. betle* leaves (Awang, 1988). But the production performance was declined in the allopurinol treatment group which might be due to the oxidative stress (Carro *et al*. 2010). The influence of antioxidants on the body weight was very well correlated with the higher values of tissue SOD, CAT and GSH level in the *P. betle* leaves group. The presence of Vitamin E in *P. betle* L. herb might have reduced the stress due to gout induction in broiler chicken and prevented the predisposition to death and also improved the appetite and body weight in broiler chicken (Traber and Atkinson, 2007).

The gout syndrome is commonly occurs at the age of 10-14 days in broiler chicks (Prathapkumar *et al.* 2008) hence in the present study, the gout was induced on 10th day of age using toxic dose of sodium bicarbonate. The clinical signs *viz.* watery droppings, dullness, depression and unthriftiness and subsequent mortality (four birds) occurred in gout control group only. The hyperuricimia and higher creatinine level in gout control group bird's causes gout leading to development of clinical signs (Sodhi *et al.* 2008). The clinical signs and mortality did not occur in control and all the treatment groups, till the end of the trial which showed the protective effect of Allopurinol and *P. betle* L. treatment groups in gout induced broiler chicken.

Biochemical parameters, Antioxidant and Xanthine oxidase estimation

The progressive reduction of serum uric acid by the *P. betle* L. treatment groups from day 15 to day 42 (Table 3) showed the prevention activity of herb against the development of gout in broiler chicken. It is also authenticated by the reduced xanthine oxidase enzyme activity (Table 5), which one is major culprit for hyperuricemia. Since the xanthine oxidase (XO) enzyme is a key mediator in uric acid production in liver and well known therapeutic target for many allopathic hypouricemic agents (Borges *et al.* 2002), the estimation of hepatic xanthine oxidase in the gout induced broiler chicken could be a vital parameter to explore the antigout activity. The equivalent suppression of xanthine oxidase enzyme activity showed by *P. betle* L. groups as that of allopurinol indicated the probable mechanism of antihyperuricemic

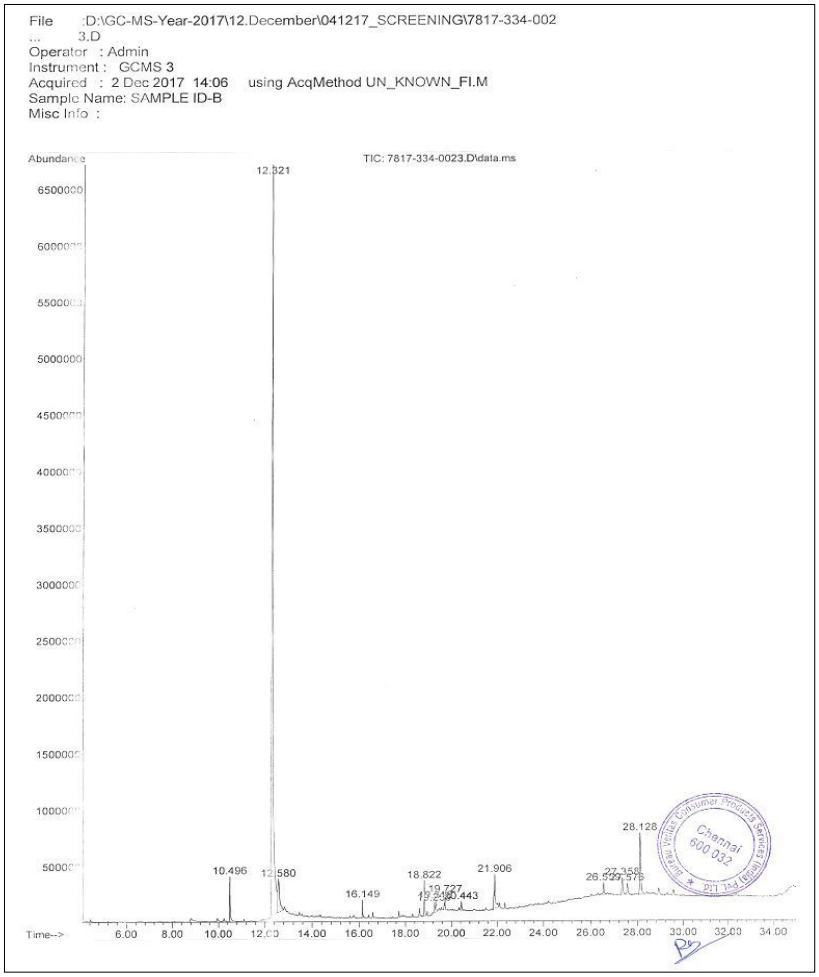

Fig 1: GC-MS chromatogram of *P. betle* L. alcoholic extract.

Table 1: Results of major phytoactive compounds detected in the GC-MS analysis of *P. betle L.* extract.

S. No	Name of the component	Retention time value (Minutes)	Area %
1	a. Chavibetol / 3 allyl 6 methoxy phenol	10.497	2.70
	b. Eugenol		
2	a. Acetoxy chavichol / 4, allyl-1,2-diacetoxy benzene	12.322	70.97
3	a. 4 Chromanol	12.578	6.31
4	a. Phytol	18.821	2.18
5	a. Vitamin E,	26.557	1.17
	b. Gamma tocopherol		
	c. O-methyl dl alpha tocopherol		
6	a. Gamma sitosterol	28.126	6.20
	b. Beta sitosterol		

activity. The xanthine oxidase inhibitory activity might be due to presence of acetoxy chavichol (Tanaka *et al.* 1997) in *P. betle* L. which might have reduced the uric acid generation. The diuretic activity of phytol (Krishnamoorthy and Subramaniam, 2014) compound present in *P. betle* L. might have helped in the excretion of excess uric acid formed during gout induction.

Also, serum creatinine levels of *P. betle* L. did not differ with control group at any stage of experiment (Table 3). This finding revealed that the renal functions were not affected due to gout induction in the *P. betle* L. groups. Further, the results of serum biochemistry showed better total protein, albumin, globulin and albumin: globulin ratio level and reduced levels of ALT and AST (Table 4). Hence, the prophylactic dosing of herbs protected the visceral organs *viz*. kidney, liver and heart from the gout induced damage as evidenced by Behtari and Feizi (2015).

The antioxidant activity *P. betle* L. is comparable with that of control group (Table 5). The values of SOD, CAT and GSH indicated the better antioxidant activity of *P.betle* L. groups than allopurinol. The occurrence of oxidative stress in allopurinol treatment group was evidenced by the declined values of SOD, CAT and GSH than control and *P. betle* L. groups (Carro *et al*. 2010). The presence of antioxidant phytochemicals like flavonoids, polyphenols, terpenoids and alkaloids in *P. betle* leaves (Awang, 1988) might have contributed the antioxidant activity when compared to allopurinol.

Gross and histopathology findings

pathological examination of gout control group birds showed severe chalky white deposits over heart and liver (Fig 2) and severe mottling of kidney with urate deposits and dilated ureter (Fig 3). Histopathology findings also revealed that

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	Control (T_1)	Gout Control (T_2)	Allopurinol 25 mg/kg (T_3)	*P. betle* 10 g/kg (T_4)	*P. betle* 12.5 g/kg (T_5)
Body weight (g)	2188.67^{cd}±14.79	1324.33^{a}±31.32	2126.33^{b}±14.99	2154.17^{c}±30.59	2151.83^{c}±14.74
Feed intake (g)	4030.00^{d}±5.16	2896.00^{a}±20.92	4020.00^{d}±6.83	3950.00^{b}±5.16	3945.00^{b}±5.16
Feed Conversion ratio	1.84^{b}±0.01	2.18 $^{=d}$±0.05	1.89^{c}±0.01	1.83^{ab}±0.02	1.83^{ab}±0.01

Columns bearing common superscript did not vary 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	Control (T_1)	Gout Control (T_2)	Allopurinol 25 mg/kg (T_3)	*P. betle* 10 g/kg (T_4)	*P.betle* 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	9.77^{a} ± 0.15	9.66^{a} ± 0.13
	Creatinine	0.47^{a} ± 0.02	0.45^{a} ± 0.04	0.48^{a} ± 0.00	0.47^{a} ± 0.04	0.46^{a} ± 0.00
Day 15	Uric acid	9.61^{a} ± 0.19	28.21^{e} ± 1.00	10.15^{a} ± 0.21	15.07^{c} ± 0.15	15.16^{c} ± 0.13
	Creatinine	0.45^{a} ± 0.19	0.86^{b} ± 0.07	0.45^{a} ± 0.01	0.46^{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	13.09^{b} ± 0.51	13.42^{b} ± 0.63
	Creatinine	0.45^{a} ± 0.02	0.79^{b} ± 0.00	0.48 ^{a} ± 0.01	0.45^{a} ± 0.02	0.47^{a} ± 0.00
Day 21	Uric acid	9.37^{a} ± 0.40	24.02^{c} ± 0.64	9.54^{a} ± 0.24	9.60^{a} ± 0.34	9.64^{a} ± 0.21
	Creatinine	0.44^{a} ± 0.02	0.74^{b} ± 0.01	0.43^{a} ± 0.02	0.44^{a} ± 0.02	0.45^{a} ± 0.02
Day 42	Uric acid	6.61^{a} ± 0.20	21.97^{c} ± 0.13	6.51^{a} ± 0.20	6.61^{a} ± 0.32	6.65^{a} ± 0.36
	Creatinine	0.42^{a} ± 0.02	0.78^{b} ± 0.01	0.43^{a} ± 0.01	0.44^{a} ± 0.01	0.43^{a} ± 0.01

Columns bearing common superscript did not vary significantly at 5% (P < 0.05) level.

Table 4: Results of serum total protein, albumin, globulin, 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	Control (T_1)	Gout Control (T_2)	Allopurinol 25 mg/kg (T_3)	*P. betle* 10 g/kg (T_4)	*P. betle* 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} ± 0.08	3.37^{a} ± 0.13
Albumin (g/dL)	1.69^{a} ± 0.06	2.51^{b} ± 0.05	1.71^{a} ± 0.10	1.63^{a} ± 0.08	1.61^{a} ± 0.08
Globulin (g/dL)	1.71^{b} ± 0.10	1.50^{a} ± 0.03	1.71^{b} ± 0.12	1.73^{b} ± 0.08	1.75^{b} ± 0.16
Albumin Globulin Ratio	1.01^{a} ± 0.08	1.66 ^{b} ± 0.05	1.04 ^{a} ± 0.12	0.96 ^{a} ± 0.08	1.0^{a} 2.0 ± 0.16
ALT (U/L)	26.08^{a} ± 1.33	59.11^{b} ± 1.75	29.47^{a} ± 2.01	27.53^{a} ± 1.98	27.32^{a} ± 0.43
AST (U/L)	182.83^{a} ± 4.14	342.35^{b} ± 8.82	189.00^{a} ± 4.26	182.28^{a} ± 5.50	185.40^{a} ± 4.46

Columns bearing common superscript did not vary significantly at 5% (P < 0.05) level.

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	Control (T_1)	Gout Control (T_2)	Allopurinol 25 mg/kg (T_3)	*P. betle* 10 g/kg (T_4)	*P. betle* 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	1.14^{c} ±0.01	1.14^{c} ±0.02
CAT(Units/ mg protein)	9.27^{c} ±0.21	6.37^{a} ±0.22	8.58^{b} ±0.26	9.33^{c} ±0.15	9.39^{c}±0.17
GSH(Units/ mg protein)	10.60 ^{c}±0.10	8.04 ^{a}±0.01	9.96^{b}±0.03	10.38^{c} ±0.11	10.38 ^{c}±0.04
XO activity(Units/ mg protein)	7.85^{abc}±0.04	8.70^{d} ±0.05	7.72^{a} ±0.04	7.75^{ab} ±0.04	7.75 ^{ab}±0.06

Columns bearing common superscript did not vary significantly at 5% (P < 0.05) level.

Fig 2: Gout control group (T_2) day 14 - severe chalky white urate deposits over heart and in liver leads to loss of hepatic architecture.

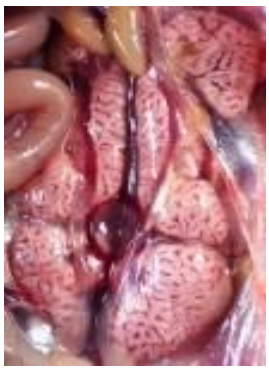

Fig 3: Gout control group (T_2) day 14 - severe mottling of kidney with urate deposits and dilated ureter.

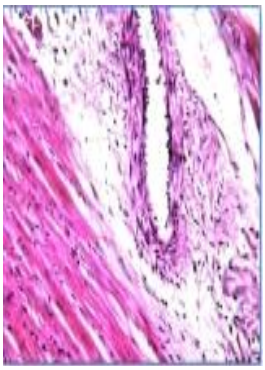

Fig 4: Gout control group (T_2) day 14-heart showing disrupted myofibers and fibrous tissue hyperplasia.

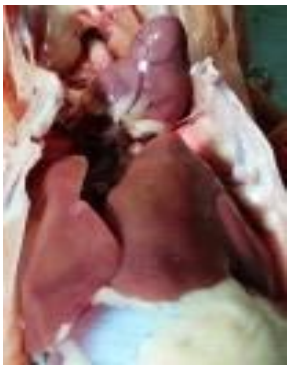

Fig 6: *P. betle* L. -10 g/kg (T_4) day 42-normal heart and liver due to herb supplementation.

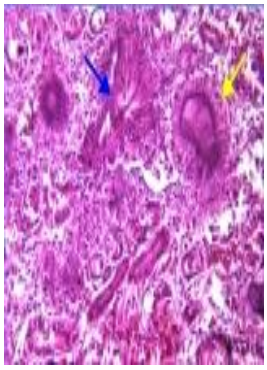

Fig 5: Gout control group (T_2) day 14 - kidney showing solidary (yellow arrow) and feathery pattern (blue arrow) of urate crystals and tubular necrosis.

the severe disruption of cardiac myofibers along with fibrous tissue hyperplasia in heart (Fig 4) and solid and feathery pattern of urate crystals and necrosis of tubular epithelium in renal tubules (Fig 5) in gout control group. Whereas the Allopurinol and *P.betle* L. 10 g/kg (Fig 6 and 7) and *P.betle* L. 12.5 g/kg group did not show any appreciable gross lesions and maintenance of normal architecture of visceral organs (heart, liver and kidney) in histopathology studies. 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 property of acetoxy chavichol, gamma and beta sitosterol (Saeidnia *et al*. 2014) and eugenol (Lee *et al*. 2007) of *P.betle* L. might have alleviated the gouty inflammation in broiler chicken. These

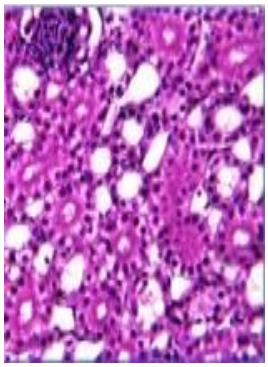

Fig 7: *P. betle* L. - 10 g/kg (T_4) day 42 - kidney revealing normal tubular and glomerular pattern due to herb supplementation.

findings are also very well supported by the earlier authors (Wang *et al.* 2016).

The results of present study showed that significant differences were not observed between *P. betle L.* treatment groups. Hence the most effective dose of *P. betle* L. *viz.* 10 g/kg feed could be preferred as an economic point of view than 12.5 g/kg group for prophylactic use.

CONCLUSION

The antigout activity of *P.betle* L. was due to the xanthine oxidase enzyme inhibitory activity as evidenced by the present study. The significant antioxidant activity exhibited by *P.betle L*. 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.betle* L. herb might be due to presence of pharmacologically active phytocompounds like acetoxy chavichol, eugenol, beta and gamma tocopherol, phytol and beta sitosterol which were detected in GCMS analysis.

Since there were no significant differences between the two different prophylactic dose level *viz.* 10 g/kg and 12.5 g/kg of feed, *P.betle* L. 10 g/kg feed dose can be used efficiently as an alternative medicine to Allopurinol for the prevention of gout syndrome in broiler chicken.

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