Int.J.Curr.Microbiol.App.Sci (2022) *11*(09): 222-234

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 09 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1109.025

Evaluation of Ayurvedic Nanoparticle Drug *Rasamanikya* against ESBL Producing Gastrointestinal Gram-negative Bacteria *Escherichia coli* in Experimental Poultry

Bithika Halder[1*], Samar Sarkar[1], Samiran Bandapadhyay[2], Prasanta Kumar Sarkar[3], Amit Raj Gupta[1], Subhasish Batabyal[4] and KoushikPal[5]

[1]Department of Veterinary Medicine, Ethics and Jurisprudence, W.B.U.A.F.S., Kolkata-37, West Bengal, India
[2]Indian Veterinary Research Institute (IVRI), Kolkata-37, West Bengal, India
[3]Department of Rasashastra, J.B. Roy State Ayurvedic Medical College and Hospital, Kolkata, 700004, West Bengal, India
[4]Department of Veterinary Biochemistry, W.B.U.A.F.S., Kolkata-37, West Bengal, India
[5]Department of Animal Science, KVK, Ashokenagar, North 24 Parganas, 743223, West Bengal, India

**Corresponding author*

Keywords

ESBL, *Escherichia coli*, broiler, Ayurvedic nanoparticle drug, rasamanikya, toxicity

Article Info

Received:
08 August 2022
Accepted:
31 August 2022
Available Online:
10 September 2022

A B S T R A C T

The present study investigate the effects of oral administration of ayurvedic nanoparticle drug *Rasamanikya* against ESBL producing gastrointestinal gram-negative bacteria *Escherichia coli* in experimental broiler chicken and haemato-biochemical, pathological alteration due to drug administration. At the age of 21 days, the birds were divided into three groups i.e. group-C served as control group, group-E treated with Enrofloxacin which act as standard control group and group RM treated with test drug *Rasamanikya*, containing six birds in each group. On the day 22, the experiment (Drug trial) was started and continued for next 28 days. On the day 0, 7, 14, 21 and 28 of experiment fecal sample was collected by using fecal swab from each bird of three groups to examine the ESBL producing gastrointestinal gram-negative bacterial count. Blood sample also be collected on day 0, 7, 14 and 28 of experiment from wing vein with the help of tuberculin syringe in sterile vial under aseptic condition to evaluate the haemato-biochemical parameters i.e. Hb%, SGPT, SGOT, ALP, Urea and Creatinine. After 28 days of experiment, at the age of 50th day, the birds were slaughtered to collect tissue sample from heart, lung, liver and kidney. Illeo-caecal junction tissue was also collected to examine the illeo-caecal coliform count. Fecal coliform count decreased significantly in RM treated group in comparison to control group. There was no significant ($P< 0.01$) alteration in hemoglobin percentage, urea and creatinine level but serum SGPT, SGOT and ALP increased significantly in RM treated group. The illeo-caecal coliform count was decreased significantly in standard control and Rasa Manikya treated groups in both dilutions 1:50 and 1:100 in comparison to normal control group. The result indicate that the test drug *Rasamanikya* able to decrease the fecal and illeo-caecal coliform count but histopathological findings clearly showed the drug causes chronic toxicity in birds of RM treated group.

Introduction

Across the entire length, poultry gastrointestinal tract harbors a dynamic microbial community consisting of a large number of bacteria (Yeoman *et al*., 2012). The intestinal flora of broiler chicken plays an important role for growth performance and health. The gastrointestinal bacteria can be roughly classified as either pathogenic or commensal organisms. Pathogenic bacteria can harm the host by causing localized or systemic infections and intestinal lesions while commensal bacteria can benefit the host by providing nutrients, metabolic facilitation, and competitive exclusion. Among them *Escherichia coli* are normal inhabitants of the gastrointestinal tract of poultry of which some strains have become highly adapted to cause diarrhoea and a range of extra-intestinal diseases. Over the past few decades, drug resistance in *E. coli* has increased vastly worldwide due to continuous use of various class of antimicrobials in poultry, mostly through the oral route, with the aim to prevent and treat diseases, enhance growth and productivity (Page and Gautier, 2012; Landini and Albarellos, 2015). Prevalence of ESBL producting *E. coli* has been reported both in food animal isolates as well as human beings (Bhoomika *et al*., 2016). It is uncertain whether ESBL producing *E. coli* act for direct threat to poultry production but it certainly stand-in-for a major problem to human clinical medicine. However, there was no concurrence about the zoonotic activity of ESBL producing *E. coli* (Olsen *et al*., 2014). Several research has stated that, the prevalence of ESBL positive strains was reported higher in broiler farms than layer farms (Brower *et al*., 2017) and broiler farms tend to use more antimicrobials cause and harbor a higher level of resistance than layer farms. Occurrence of ESBL producing *E. coli* were reported recently in several Indian states including healthy farmed poultry as well as backyard poultry (Samanta *et al*., 2014; Samanta *et al*., 2015; Shrivastav *et al*., 2016). MacConkey agar containing cefotaxime 1.0mg/L is used in screening for extended-spectrum β-lactamase (ESBL)-producing organisms (Wilson and McCabe, 2007). To deal with this situation various nanoparticle drugs containing ultra-fined particles generally ranges from 1-100 nm in diameter (Vert *et al*., 2012) produced by nanotechnology are been studying for testing their efficacy (Singh *et al*., 2008). Many nanoparticle based drug shows potential antibacterial activity against various Gram +ve and Gram -ve bacteria including highly methicillin and carbapenem resistant strains and increasing their exposure among animals and human (Nazifi *et al*., 2015; Stebounova *et al*., 2011). In the present era nanotechnology is a rapid growing technology plays an important role on various fields of therapeutic applications and capable for solving several problems related to animal health and production (Youssef *et al*., 2019). On the other hand, ayurvedic system of medicine is one of the oldest systems of Indian traditional medicine and most of them are based on herbo-metal/mineral formulation commonly known as Bhashma having particles diameter of about 10-15nm (Farooq *et al*., 2019). Many scientific literatures support their therapeutic efficacy along with metal nanoparticle has been detected in various types of Bhashma. So it can be expected that combination of ayurveda and nanotechnology may provide the solution to resolve the antimicrobial resistance issue due to ESBL producing *E. coli* in poultry. Few research has reported that mineral rich *Rasamanikya* Nanoparticle (RMNP) which is ayurvedic herbo-metallic nanomedicine have antimicrobial potential assessed by in-vitro assay (Ruidas *et al*., 2019; Pal, *et al.*,2020; Halder *et al*., 2022).

In this research work a selected ayurvedic nanoparticle drug *Rasamanikya* have taken into consideration for studying its effects against ESBL producing gastrointestinal gram-negative bacteria *Escherichia coli* in experimental broiler chicken. This effect was size and dose dependent.

Materials and Methods

The experiment was conducted at the poultry unit of the 'Ashokenagar Krishi Vigyan Kendra', North 24 Paraganas in West Bengal state on the month of

February, 2019 and continued for 7 weeks. At that time, the climatic condition of this area is suitable for poultry farming. Total eighteen (18) numbers of day-old broiler chicks were taken for this experiment. The birds were managed on deep litter pens.

The chicks were fed broiler starter diet for four weeks and the finisher diet from 5 weeks. At the age of 2 and 4 weeks, the chicks were vaccinated against Gumboro disease and against Newcastle disease at the age of 3 and 5 weeks. At 21 days of age, the birds were divided into three groups i.e. group-C served as control group, group-E treated with Enrofloxacin which act as standard control group and group-RM treated with test drug *Rasamanikya*, containing six birds in each group. On the day 22, the experiment (Drug trial) was started and continued for next 28 days days represented in table.1.

On the day 0, 7, 14, 21 and 28 of experiment fecal sample was collected by using fecal swab from each bird of three groups to examine the gastrointestinal coliform count. In the laboratory one spatula of faecal sample was measured from each sample. Then 1000 microlitres of NSS was added to each sample and unit weight is calculated by dividing NSS with the amount of fecal sample obtained. Finally this unit weight obtained was made up to a volume of 1000 microlitres by adding equivalent amount of NSS to it. Then 120 microlitres was withdrawn from these and inoculated in MacConkey with CTX agar for bacterial culture [Fig 1.] and the petriplates were kept in incubator for overnight.

Blood sample was collected on the day 0, 7, 14 and 28 of experiment from wing vein with the help of tuberculin syringe in sterile vials under aseptic condition to evaluate the haemato-biochemical parameters i.e. Hb%, SGPT, SGOT, ALP, Urea and Creatinine. The haemoglobin percentage was estimated by using colorimeter and the biochemical tests were performed by using Ebra Chem. 5x model machine (Transition Biomedical Limited) in the biochemistry laboratory, W.B.U.A.F.S., Kolkata (West Bengal). For determination of serum SGPT, SGOT, ALP, Urea and Creatinine level specific reagents and methods were used. The reagent and sample are mixed well and aspirate. Then allow the reagents to attain 15-30°C before performing the test. To estimate the haemoglobin percentage Drabkin's solution is used.

At the age of 50th day, the birds were slaughtered for tissue sample collection from heart, lung, liver and kidney. Then preserved into 40% formalin in order to perform histopathology, embedded in paraffin, sectioned at 5 µm then stained with haematoxylin and eosin (H&E) for light microscopic examination.

Illeo-caecal junction tissue was also collected to examine the coliform count. A small part of illeo-caecal junction tissue was taken and the weight of tissue is measured. Then the tissue was dissolved in 5 ml of Normal Saline Solution (NSS). The tissue dissolved in NSS was homogenized with a homogenizer. Then the unit weight of tissue was calculated by dividing the weight of tissue with NSS with the tissue weight and the unit weight of tissue was cultured on 1:50 and 1:100 dilution in MacConkey with CTX agar plate for bacterial culture [Fig 2.]. The petriplates were kept for overnight in incubator.

Results and Discussion

Fecal coliform count was increased from 0th day to 14th day, then it was decreased in 21st day and again was increased in 28th day in control group and in RM group [Fig 1.]. In test drug group, highest count observed on 21st day and it decreased on 28th day. On 28th day, the count in RM treated group was less in comparison to control group. All the results are statistically highly significant depicted in table 2. and diagram 1.

Haemoglobin contents in test and control group have been shown in Table.2. Haemoglobin level was not altered by the control and test drug treatment. It was increased gradually in all the groups, but was statistically non significant. However, the increases

were more in standard control and test RM treated groups in comparison to the normal control group ([Diagram. 2).

Table 4 depicts effect of the control and test drug on serum ALT level. The test drug RM alter serum ALT to significant level. The ALT levels were decreased on 7th day, again were increased gradually on 14th and 28th day which clearly indicates hepatic disorder in test birds (Diagram 3).

Effects of the control and test drug on serum AST level have been shown in table 5 and diagram 4. Serum AST level was increased significantly in RM treated group may be due to hepatic damage.

Table 6. shows the results of control and test drug on serum alkaline phosphatase (ALP) level. The test drug alter serum ALP to significant level. Serum ALP level was increased day-by-day in RM treated group indicative for hepatic damage in the experimental birds (Diagram 5).

Results on serum urea level were shown in Table 6. Serum urea level was not altered to significant level by test drug treatment (Diagram 6).

Table 8. depicts effect of the test drug on serum creatinine level. The test drug didn't alter serum creatinine to significant level (Diagram 7).

Histopathological observation

The test drug, *Rasamanikya* (RM) exhibited toxicity in the vital organs which caused intercardial haemorrhage and congestion in heart [Fig 3.], haemorrhage and necrosis in lungs [Fig 4.], focal degenerative changes, congestion, necrosis [Fig 5.] with disruption of hepatic.

The illeo-caecal junction coliform count was decreased significantly in standard control and *Rasamanikya* treated groups in both the dilutions in comparison to normal controlgroup depicted in table 9 (Diagram 8).

Chord modified to adenomatous changes (Carcinogenic effect)

Chord modified to adenomatous changes (Carcinogenic effect) in liver [Fig 6.], atrophy and necrotic changes in kidney [Fig 7.]. *Rasamanikya* exhibited carcinogenic changes in liver. *Rasamanikya* chemically is arsenic trisulphide (As_2S_3) prepared by following ayurvedic method of purification treatment and then by melting or by sublimation.

These preparation are given for treatment purpose mixing with powder of plant drugs and with specific vehicle according to disease, called as Anupana in parlance of ayurveda. These are never prescribed as single medicine. The plant drugs and vehicles play important role to increase bioavailability and to reduce toxicity of these preparation in human being.

In this study, *Rasamanikya* was given alone without mixing with plant drugs or without any vehicle. This may be one of the reasons behind showing toxic effect by the test drug. Arsenic compounds are known as carcinogenic agents. Those act as tumor promoting agent and can induce carcinoma.

Before therapeutic application of this compounds in ayurveda, purification treatment is done. And the drug is prescribed mixing with other plant drugs powder and with specific vehicle according to diseases.

Then only this drug is used as therapeutic entity. And at therapeutic dose this drug is found to be safe. In the present case, the test drug was prepared following the ayurvedic procedures after proper purification treatment. Though the drug exhibited carcinogenic effects in liver. The possible causes of toxicity may be here the drug is given alone without mixing with plant drugs powder and no vehicle was used for application of the drug. And another cause may be the drug is not suitable as therapeutic agents in broiler chicken.

Table.1 Design of the experiment for evaluation of therapeutic potential of herbal compounds in poultry

Group	Number of birds	Used drugs	Dose and route
Group- C	6	No drug	----
Group- E	6	Enrofloxacin	@5 mg/kg b.wt., orally once daily for 28 days
Group- RM	6	*Rasamanikya*	@33.34 mg/kg b.wt., orally once daily for 28 days

Table.2 Result of fecal coliform count

Days Treatments	0day	7days	14days	21days	28days
Control	16.35±1.46^{b}	72.75±10.25^{b}	114.25±28.26^{b}	3.45±1.25^{b}	81.25±10.23^{b}
Enrofloxacin	12.25±2.35^{b}	17.45±5.26^{c}	19.95±5.62^{c}	46.25±8.95^{a}	16.45±1.65^{c}
RM	16.75±1.26^{b}	28.95±12.25^{c}	152.25±32.36^{a}	2.25±1.34^{b}	23.25±4.32^{c}

Different superscripts (a, b and c) differ significantly at 1 % level of significance (P<.01) according to Tukey's Honest Significance Difference Post hoc test.
Significant at 1 % level of significance.

Diagram.1

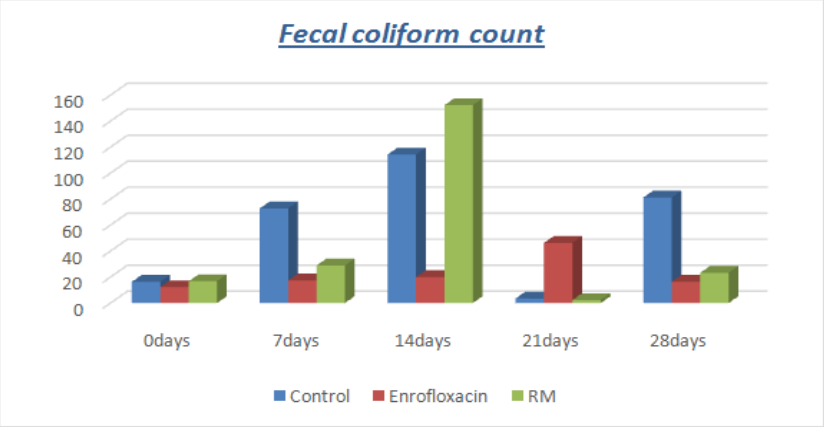


Table.3 Result of Haemoglobin% (gm/dl)

Days Treatments	0days	7days	14days	28days
Control	8.28±.46	7.96±.32	8.45±.36	9.02±.26
Enrofloxacin	8.74±.38	8.68±.21	9.21±.39	9.98±.34
RM	8.45±.24	8.52±.34	9.63±.48	9.25±.27

Mean ± Standard Error

Diagram.2

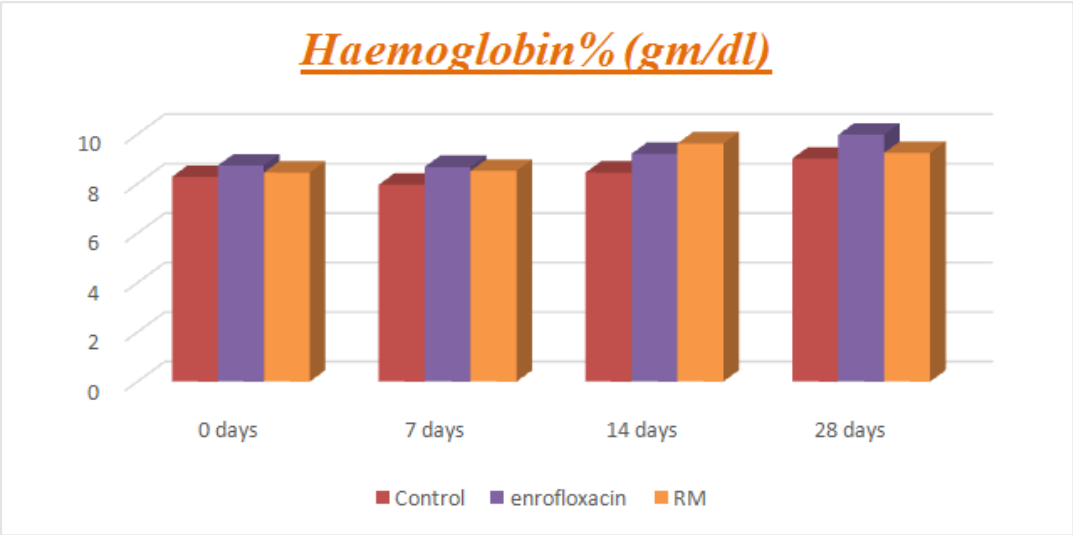


Table.4 Result of SGPT/ALT (IU/L)

Days Treatments	0days	7days	14days	28days
Control	21.42±3.38	13.27±.62	19.25±1.25	21.48±1.84
Enrofloxacin	23.54±2.98	13.95±1.95	17.35±1.65	19.62±2.14
RM	21.78±4.25	14.67±1.25	20.75±2.25	27.25±2.25

Mean ± Standard Error

Diagram.3

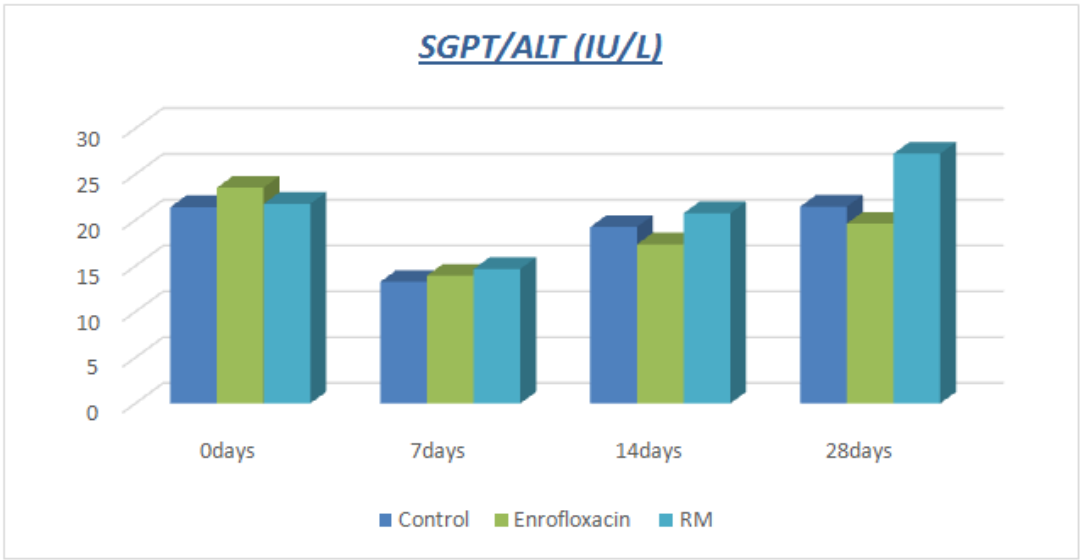


Table.5 Result of SGOT/AST (IU/L)

Days Treatments	0days	7days	14days	28days
Control	186.27±28.25	255.45±27.25	223.42±28.29	248.25±17.65
Enrofloxacin	182.45±10.35	221.25±39.25	192.55±16.45	225.15±14.25
RM	224.42±21.14	238.15±47.25	240.68±30.28	286.47±15.27

Mean ± Standard Error

Diagram.4

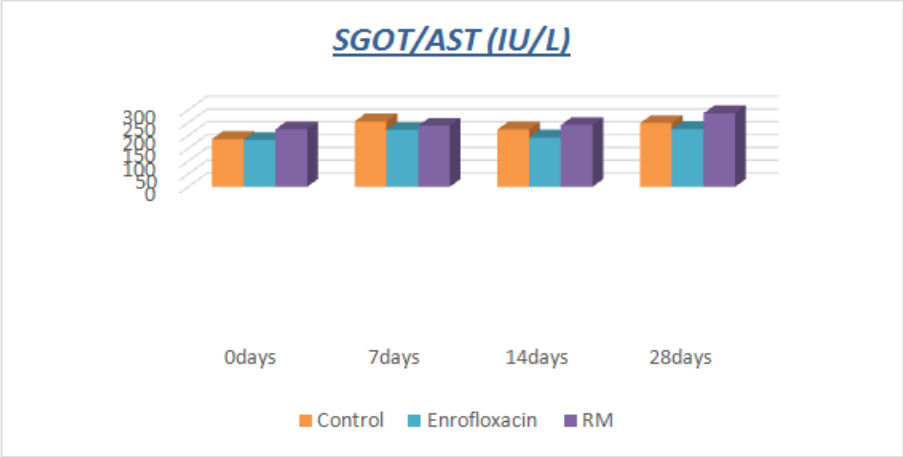


Table.6 Result of ALP (IU/L)

Days Treatments	0days	7days	14days	28days
Control	1572.28±70.15	1034.68±124.85	1928.35±178.25	2124.54±62.15
Enrofloxacin	1911.62±62.48	2218.25±168.25	2225.45±165.28	2211.48±4958
RM	2192.85±72.46	2285.25±162.45	2728.34±121.25	3563.34±52.46

Mean ± Standard Error

Diagram.5

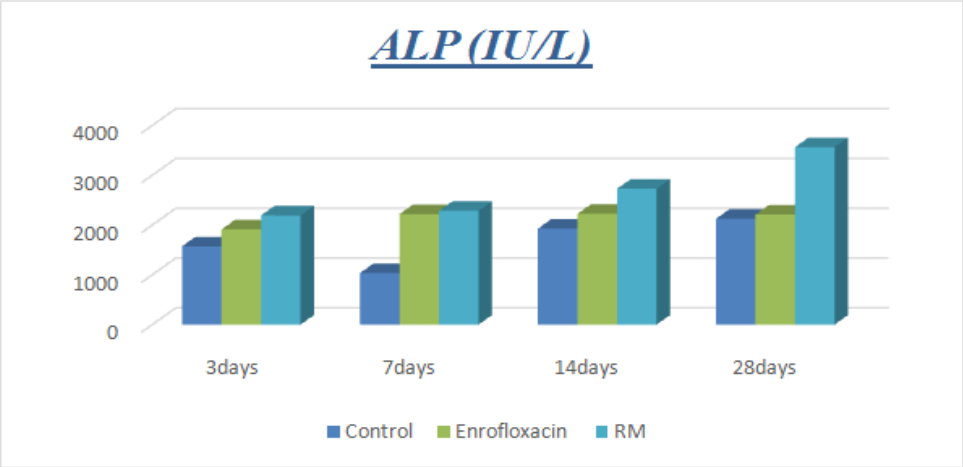


Table.7 Result of UREA (mg/dL)

Days Treatments	0days	7days	14days	28days
Control	9.42±4.05	11.14±7.84	18.26±4.25	12.94±6.27
Enrofloxacin	10.17±3.35	12.25±8.25	12.27±4.95	13.85±7.16
RM	10.29±1.35	13.28±2.01	14.37±2.08	14.15±1.75

Mean ± Standard Error

Diagram.6

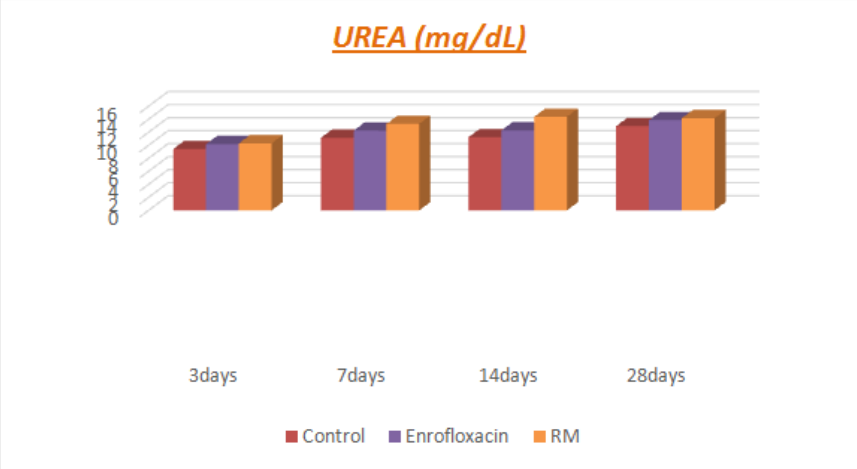


Table.8 Result of Creatinine (mg/dL)

Days Treatments	0days	7days	14days	28days
Control	.175 ±.035	.185±.014	.195±.015	.185±.034
Enrofloxacin	.195±.024	.235±.021	..225±.045	.221±.023
RM	.226±025	.225±.018	.185±.025	.187±.034

Mean ± Standard Error

Diagram.7

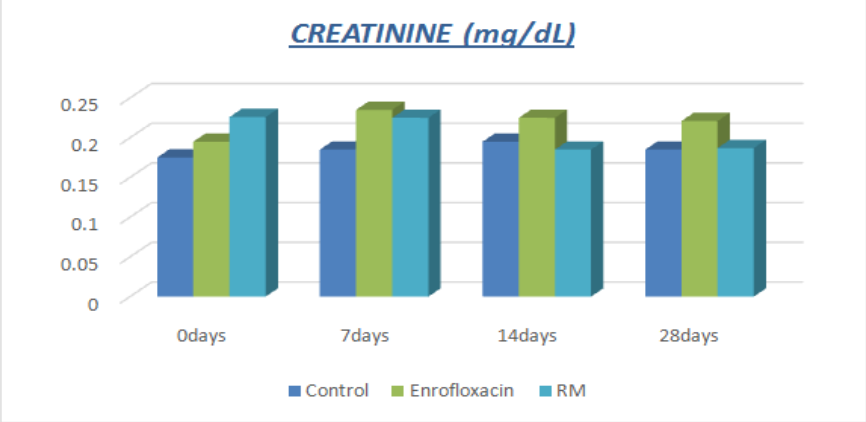
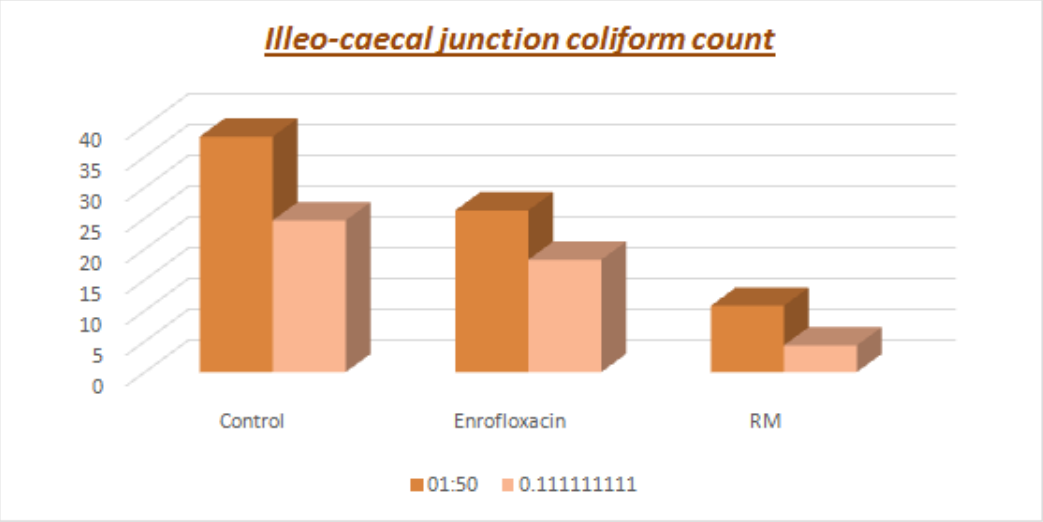


Table.9 Result of illeo-caecal junction coliform count

Dilution Treatments	1:50 Dilution	1:100 Dilution
Control	38.25±.28[c]	24.65±.85[b]
Enrofloxacin	26.26±1.78[b]	18.25±14.65[a]
RM	10.77±16.35[a]	4.35±16.63[a]

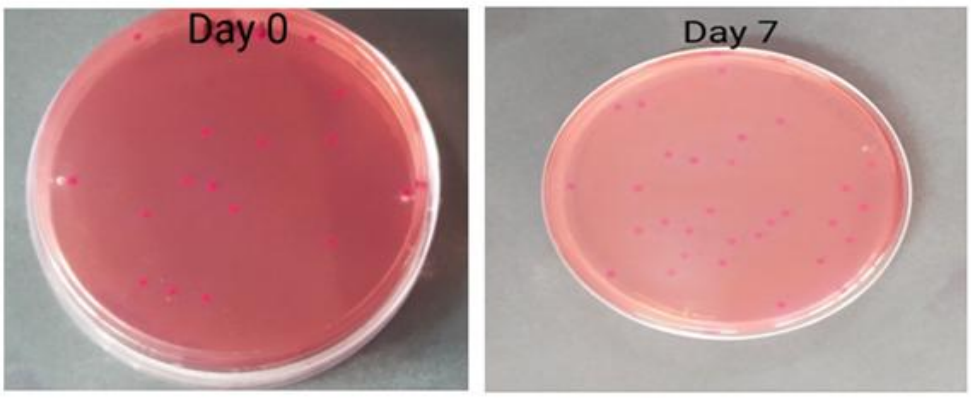
Different superscripts (a, b and c) differ significantly at 1 % level of significance (P<.01) according to Tukey's Honest Significance Difference Post hoc test. Significant at 1 % level of significance.

Diagram.8 Illeo-caecal junction coliform count in test drug (RM) poultry group



The illeo-caecal junction coliform count was decreased significantly in standard control and Rasa Manikya treated groups in both the dilutions in comparison to normal control group.

Fig.1 Fecal coliform count on day 0, 7, 14, 21 and 28 in test drug *Rasamanikya* (RM)



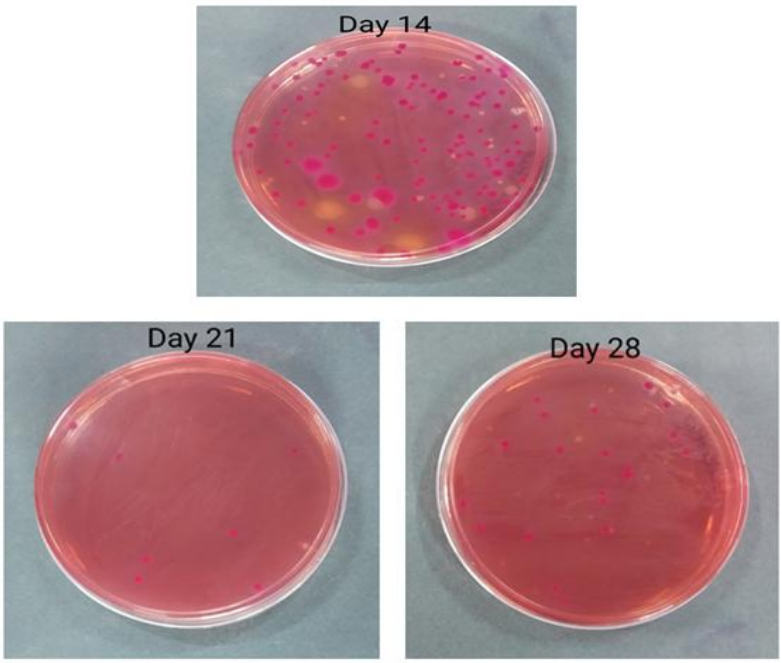


Fig.2 Illeo-caecal junction coliform count in test drug RM group

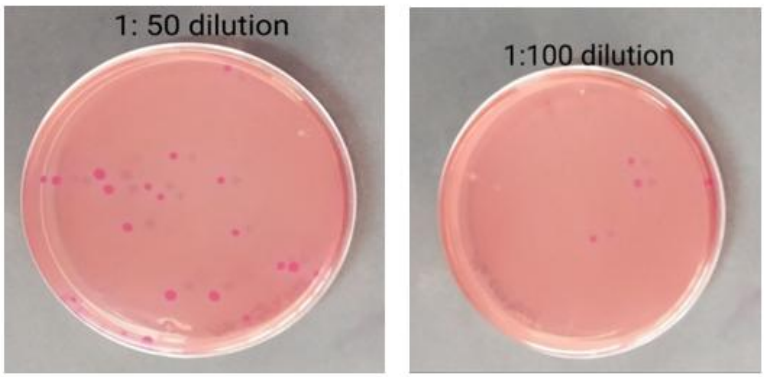
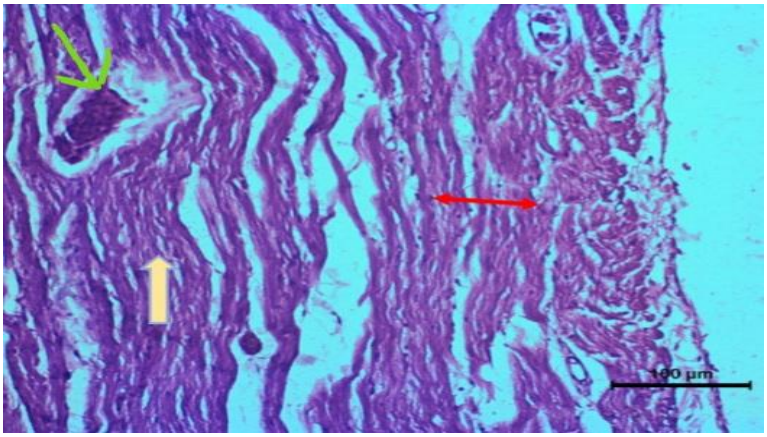
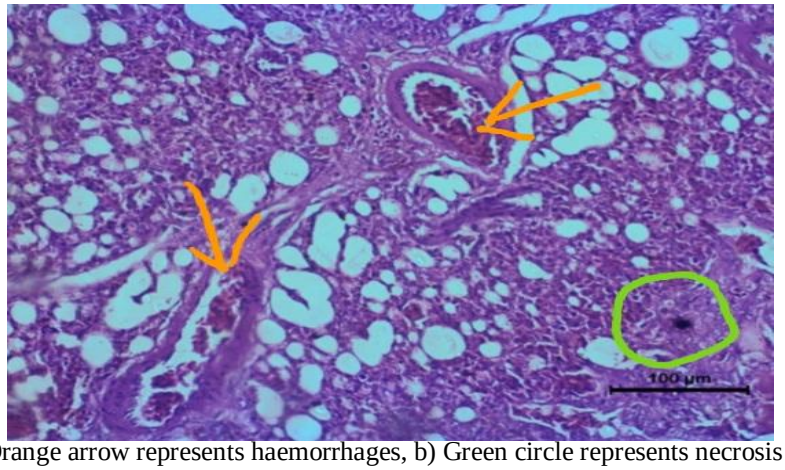


Fig.3 Histopathological findings of heart in RM group of poultry



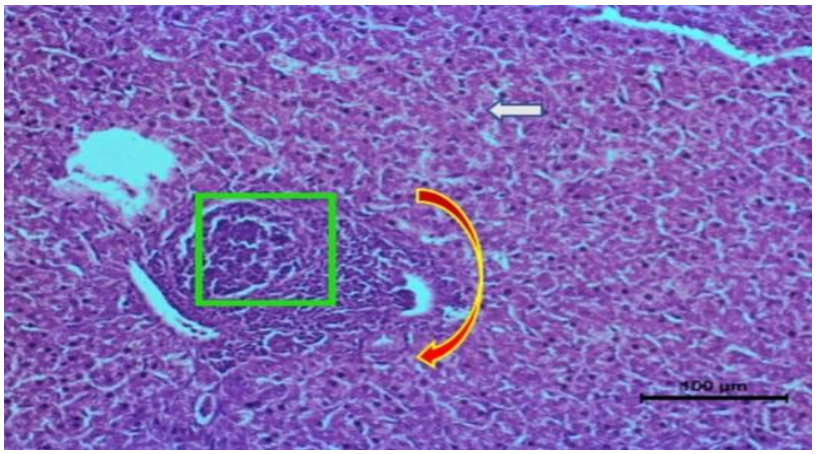
a) Red arrowrepresents haemorrhages, b) Green arrow represents congestion, c) Yellow arrow represents hyaline degeneration

Fig.4 Histopathological findings of lung in RM group of poultry



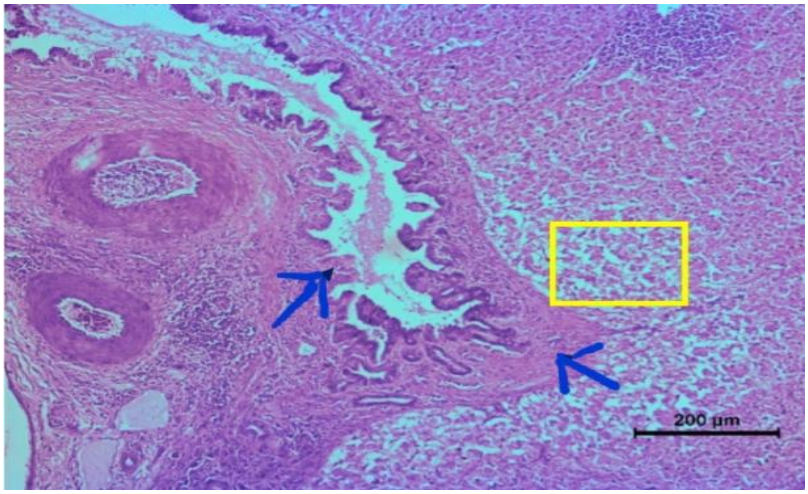
a) Orange arrow represents haemorrhages, b) Green circle represents necrosis

Fig.5 Histopathological findings of liver in RM group of poultry



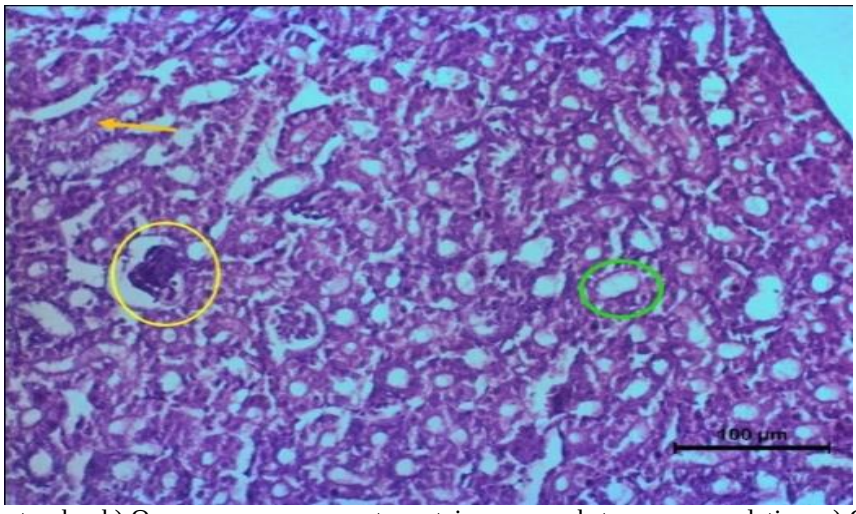
a) Green box represents congestion, b) Red arrow represents lymphoma, c) White arrow represents necrosis

Fig.6 Histopathological findings of liver in RM group of poultry



a) Yellow box represents degeneration, b) Blue arrow represents adenomatous change (carcinogenic effect)

Fig.7 Histopathological findings of kidney in RM group of poultry



a) Yellow circle represents atrophy, b) Orange arrow represents proteinaceous substance accumulation, c) Green circle represents discontinuation of epithelial cells.

It can be concluded that ayurvedic nanoparticle drug *Rasamanikya* effective against ESBL producing gastrointestinal gram-negative bacteria *Escherichia coli* and reduces the fecal and illeo-caecal coliform count.

But the test drug produces biochemical and pathological alteration in experimental poultry which is correlated to each other and indicates the chronic toxicity in birds of RM treated group.

Acknowledgement

The author would like to acknowledge Dr. Prasanta Kumar Sarkar, Professor and H.O.D, Department of Rasashastra, J.B. Roy State Ayurvedic Medical College and Hospital, Kolkata (W.B.) for providing the test drug *Rasamanikya*, Dr. Samiran Bandapadhyay, Senior scientist, Indian Veterinary Research Institute (IVRI), Kolkata to allow in the institute laboratory for the experiment under his supervision, Dr. Subhasish Batabyal, Professor and Head, Department of Veterinary Biochemistry, W.B.U.A.F.S., Kolkata for co-operation in conducting biochemical tests, Dr. Samiran Mondal, Associate Professor, Department of Veterinary Pathology, W.B.U.A.F.S., Kolkata for helping in histopathological studies and all the lab technicians for helping during the whole experiment period.

References

Bhoomika, Shakya S., Patyal A., Gade N. E. 2016. Occurrence and characteristics of extended-spectrum β-lactamases producing *Escherichia coli* in foods of animal origin and human clinical samples in Chhattisgarh, India. Vet. World, 9: 996-1000.

Brower C. H., Mandal S., Hayer S., Sran M., Zehra A., Patel S. J., Kaur R., Chatterjee L., Mishra S., Das B. R., Singh P., Singh R., Gill J. P. S., Laxminarayan R. 2017. The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India. Environ. Health Perspect., 125:077015.

Farooq S. and Mahmood Z. 2019. Nanoparticles in Ayurvedic Medicine: Potential and Prospects. New look Phytomedicine, Academic Press, 22:581-596.

Halder B., Sarkar S., Bandapadhyay S., Sarkar P. K., Gupta A. R., Batabyal S. 2022. Evaluation of selected Ayurvedic nanoparticle drug *Rasamanikya* against MDR pathogen in mice model. The Pharma Innovation Journal, 11(6S): 2745-2748.

Landoni M. F., Albarellos G. (2015). The use of antimicrobial agents in broiler chickens. Vet. J., 205: 21-27.

Nazifi S., Fatemeh N., Mehdi F., Yalda Z., Mohammadinezhad S. 2015. Evaluation of Protective Effect of Penicillamine on Silver Nanoparticles-Induced Oxidative Stress in BALB/c Mice. İstanbul Üniv. Vet. Fak. Derg. / J. Fac. Vet. Med. Istanbul Univ, 41(2):205-211.

Olsen R. H., Bisgaard M., Löhren U., Robineau B., Christensen H. 2014. Extended-spectrum β-lactamase-producing *Escherichia coli* isolated from poultry: a review of current problems, illustrated with some laboratory findings. Avian Path., 43: 199-208.

Page S. W.,Gautier P. 2012. Use of antimicrobial agents in livestock. Rev. Sci. Tech., 31:145–88.

Pal S., Dey S., Batabyal K., Banerjee A. Joardar S. N., Samanta I. and Isore D. P. 2020. Prevalence and Characterization of Extended Spectrum Beta Lactamase Producing *Escherichia coli* from Broilers. Int.J.Curr.Microbiol.App.Sci. 9(03): 594-602.Doi:https://doi.org/10.20546/ijcmas.2020.903.070

Ruidas B. B., Som Chaudhury S., Pal K., Sarkar P., Das Mukhopadhyay C. 2019. A novel herbometallic nanodrug has the potential for antibacterial and anticancer activity through oxidative damage. Nanomedicine (Lond.), 14(9):1173-1189.

Samanta I., Joardar S. N., Das P. K., Sar T. K. 2015. Comparative possession of Shiga toxin, intimin, enterohaemolysin and major extended spectrum beta lactamase (ESBL) genes in Escherichia coli isolated from backyard and farmed poultry. Iranian J Vet.Res.,16: 90-93.

Samanta I., Joardar S. N., Das P. K., Das P., Sar T. K., Dutta T. K., Bandyopadhyay, S., Batabyal S., Isore D. P. 2014. Virulence repertoire, characterization and antibiotic resistance pattern analysis of *Escherichia coli* isolated from backyard layers and their environment in India. Avian Dis.,; 58: 39-45.

Shrivastav A, Sharma R. K., Sahni Y. P., Shrivastav N., Gautam V., Jain S. 2016. Study of antimicrobial resistance due to extended spectrum beta-lactamase-producing *Escherichia coli* in healthy broilers of Jabalpur. Vet. World, 9: 1259-1263.

Singh M., Singh S., Prasad S. and Ganibhir, I. S. 2008. Nanotechnology in medicine and antibacterial effect of Silver Nanoparticles. Digest journal of nanomaterials and Biostructures Vol. 3, No. 3, P: 115-122.

Stebounova L. V., Adamcakova-Dodd A., Kim J.S, Park H., O'Shaughnessy P. T., Grassian V. H., Thorne P. S. 2011. Nanosilver induces minimal lung toxicity or inflammation in a subacute murine inhalation model, Particle and Fibre Toxicology, 8:5.

Vert, M.; Doi, Y.; Hellwich, K. H.; Hess, M.; Hodge, P.; Kubsia, P.; Rinaudo, M. and Schue, F. O. (2012). Terminology for Biorelated Polymers and Application (IUPAC Recommendation. Pure and Applied Chemistry, 84 (2); 377410, doi: 10/ 1351 PAC- REC-10-12-4.

Wilson, G. and McCabe, D. (2007).The use of antibiotic-containing agars for the isolation of extended-spectrum β-lactamase-producing organisms in intensive care units. Clinical Microbiology and Infection, 13 (4); 451-453,https://doi.org/10.1111/j.1469-0691.2006.01667.x.

Yeoman C. J., Nicholas Chia, Patricio Jeraldo, Maksim Sipos, Nigel D Goldenfeld, Bryan A White. 2012. The microbiome of the chicken gastrointestinal tract. Animal health and research reviews, 89-99, https://doi.org/10.1017/S1466252312000138.

Youssef F S, El-Banna H A, Youssef Elzorba H, Mohamed Galal A. Application of some nanoparticles in the field of veterinary medicine. International journal of veterinary science and medicine, 2019, 78-93.

How to cite this article:

Bithika Halder, Samar Sarkar, Samiran Bandapadhyay, Prasanta Kumar Sarkar, Amit Raj Gupta, Subhasish Batabyal and Koushik Pal. 2022. Evaluation of Ayurvedic Nanoparticle Drug *Rasamanikya* against ESBL Producing Gastrointestinal Gram-negative Bacteria *Escherichia coli* in Experimental Poultry. *Int.J.Curr.Microbiol.App.Sci.* 11(09): 222-234. doi: https://doi.org/10.20546/ijcmas.2022.1109.025