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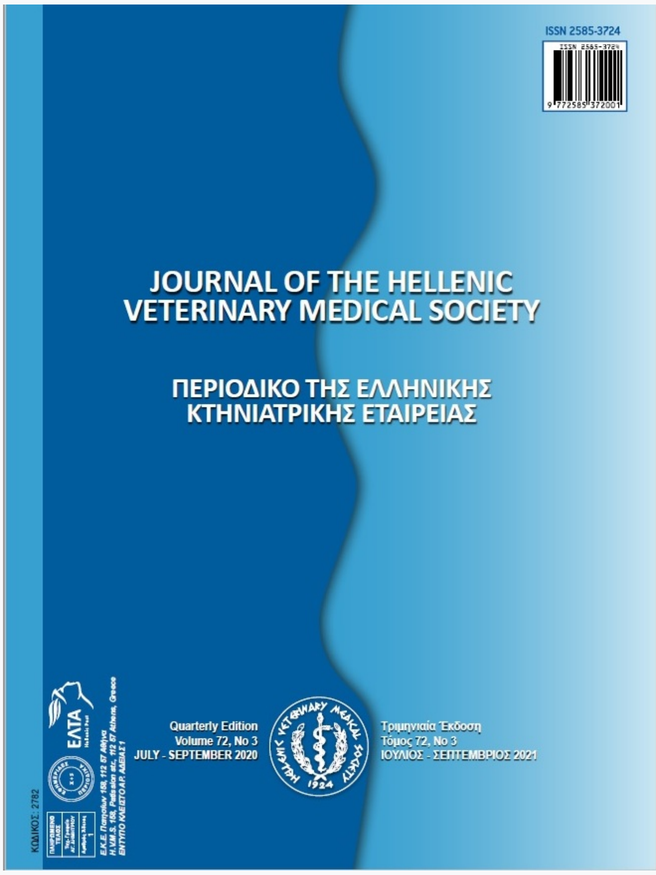

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Research article
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Anticoccidial potential of *Ageratum conyzoides* and its effect on Blood parameters of experimentally infected Broiler Chickens

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ABSTRACT: Coccidiosis is an infectious parasitic disease of poultry which causes heavy economic losses to commercial poultry industry. Coccidiosis has been generally controlled by using different synthetic anticoccidial drugs but, due to development of resistance to these drugs this method is losing its effectiveness. Exploration of novel and alternative compounds against avian coccidiosis is need of time now a days. In this regard, medicinal plants can serve as substitute to these synthetic anticoccidials. Thus, to find out alternative novel agents, current research was designed to evaluate the anticoccidial potential of *Ageratum conyzoides* extract (ACE). For *in vivo* experiment, 105 broiler chicks were purchased and further divided into 7 sub-groups (15 birds in each group). At 7th day of experiment, Groups A, B and C were fed with plant extract at 100, 200 and 300 mg/kg respectively. Group D was supplemented with Vitamin-E while, Group E and F remained as infected medicated and infected un-medicated control groups and served with standard medicine (Baycox®) and Phosphate Buffer Saline (PBS) respectively. Moreover, Group G served as normal control group. At day 14th of experiment, all groups except rom Group G were orally infected with 60,000 sporulated oocysts of mixed *Eimeria* species. Anticoccidial potential of ACE was evaluated on the basis of lesion scores, fecal scores, oocyst scores and feed conversion ratio. Furthermore, the effect of ACE on the serum chemistry was also evaluated to check toxicity of plant extract if any. The results were compared with standard medicine (Baycox®). On the basis of results, ACE showed anticoccidial activity by reducing fecal, lesion and oocyst scores in infected chicks ($P<0.05$). ACE also improved FCR of infected chicks. Moreover, ACE exhibited positive effects on serum chemistry of broiler chickens ($P<0.05$).

***Keywords*:** Resistance, Sporulated oocyst, *Eimeria*, Medicinal plant

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INTRODUCTION

Avian coccidiosis is caused by single cell parasitic protozoa of genus *Eimeria*, which belongs to Phylum Apicomplexa having seven types with multiple life cycle stages (Abbas *et al.*, 2015, 2019). Among all species pathogenic ones are *E. tenella* and *E. necatrix* that cause cecal coccidiosis in chickens. *Eimeria* species affect intestine of the bird and cause bloody diarrhea, poor Feed Conversion Ratio (FCR) and mortality which lead to heavy economic losses to poultry industry (Chapman, 2014; Shahid *et al.,* 2020; Khater *et al*., 2020). The coccidiosis infection starts with the ingestion of sporulated oocysts by the chicken from contaminated feed and water (Qamar *et al.,* 2020; Zhang *et al.,* 2020). Coccidian oocysts produce millions of oocysts by asexual and sexual multiplication within the host. So, it is not easy to protect the birds from infection (Fall *et al*., 2016). Synthetic anticoccidial drugs are generally used to control coccidiosis. Sulfanilamide was the first anticoccidial drug that was used against coccidiosis in poultry birds. Later on, a variety of anticoccidial drugs has been developed to control and treat coccidiosis. However, due to the irrational use of anticoccidial drugs, resistance has been developed in *Eimeria* species (Abbas *et al.*, 2019). There is need of time to find out and explore some other novel agents and compounds for the sustainable effective control of coccidiosis. In the last decade, many plants and their compounds have been reported to have excellent potential as anticoccidials and immunomodulators (Khan *et al.,* 2017; Yi *et al.,* 2020). Use of antioxidants rich plants have gained special importance (Ijaz *et al.,* 2020; Masood *et al.,* 2020; Alshamiri *et al.,* 2021), therefore, plants rich in antioxidants (phenols, flavonoids, tannins and saponins) are being used as an alternative strategy to treat coccidiosis (Abbas *et al.* 2019). *Ageratum conyzoides* also called as goat weed bears various pharmacological properties against different parasitic and bacterial diseases of humans and livestock due to its various antioxidants and other useful compounds (Nweze and Obiwulu, 2009). On the basis of presented literature including antioxidant properties of various botanicals, present study was planned to assess the anticoccidial potential of *Ageratum conyzoides* against experimental *Eimeria* infection in chickens.

MATERIALS AND METHODS

Plant material

Leaves of *Ageratum conyzoides* were procured from local market. The plants material was dried out and then with the help of electric mill converted into powder form. Aqueous methanolic extract of *Ageratum conyzoides* (ACE) was prepared using Soxhlet's apparatus (Velp Italy) following method described by Abbas *et al.* (2017). The suspension was evaporated in rotary evaporator (Heidolph Germany) at 50°C. The prepared ACE was stored at 4°C in refrigerator after freeze drying until further use.

Parasite

Parasitic material (*Eimeria* oocysts) was collected from guts of naturally infected chicken and outbreak cases in Faisalabad. Sporulation of *Eimeria* oocysts was done in Potassium dichromate solution (2.5%) at 25-29°C under 60-80% humidity and examination was done following Ryley *et al.* (1976). The sporulated oocysts were counted by Modified McMaster Technique. The chambers of McMaster slide were filled with the sample and allowed to stand undisturbed for 2-3 minutes so that the sporulated oocysts float and come to same focus level. The slides were examined under microscope at low (10x) and high magnification (40x).

Experimental design

A total of 105 day old chicks broiler chicks were purchased and reared by adopting proper management practices. At seven days of age chicks were divided in 7 equal groups (A, B, C, D, E, F and G) having 15 birds in each group. Groups A, B and C were given (orally) different doses of ACE @ 100, 200 and 300 mg/kg of body weight. At 14th day of experiment, all groups were given (oral route) infection with 50,000 oocysts of mixed *Eimeria* species except group G which remained as un-infected, un-medicated control group. Group D was fed with Vitamin E (Aquasol E®) which was administered in water @ 87 mg/kg of body weight. Group E served as Baycox® treated group and Group F served as infected un-treated group. Baycox® (A&K Pharmaceuticals, Faisalabad) was administered in group E @ 1ml/l of water.

Evaluation of Anticoccidial Activity

Anticoccidial potential of ACE was evaluated on the basis of parameters such as FCR, lesion score, (Johnson and Reid 1970), oocyst score (Hilbrich 1978) and fecal score (Youn *et al.*, 1993).

Analysis of Serum Chemistry

To access toxicity of plant extract in infected chicks serum enzymes levels including Alanine transferase (ALT), Lactate dehydrogenase (LDH) and creatinine levels were carried out using the commercially available diagnostic kits (Merck, Germany) according to manufacuturer instructions. (Abbas *et al.*, 2017, 2019).

Statistical Analysis

Statistical analysis was done by ANOVA and DMR tests using SAS (statistical analysis software) (SAS, 2004). The data were considered statistically significant with *P* value <0.05.

RESULTS

All the groups administered with ACE showed the improvement in FCR in dose dependent manner. There was no statistical analysis for FCR due to group feeding of birds. The group which was administered with higher dose of ACE exhibited better FCR as shown in Table 1.

Lesion scores of different groups have been shown in Table 2. The groups treated with ACE showed the reduced lesion score in infected groups in dose dependent manner. Lesion score of group treated with higher dose was non significantly different to Baycox® and Vitamin E (P>0.05) and were significantly different to infected non medicated group (P<0.05).

Table 1: Feed Conversion Ratio (FCR) of different treated groups

Groups	Feed consumed	Weight gain	FCR
A. conyzoides @ 100 mg/kg	1632.11	620.11	2.63
A. conyzoides @ 200 mg/kg	1660.20	660.30	2.51
A. conyzoides @ 300 mg/kg	1644.18	706.10	2.32
Vitamin E	1602.19	761.13	2.10
Baycox®	1594.15	780.12	2.04
Infected, non-medicated	1690.14	581.70	2.90
Non-infected, non-medicated	1801.12	900.42	2.00

*Because of group feeding statistical analysis was not achievable

Table 2: Lesion score of different treated groups (n=6)

Groups	0	+1	+2	+3	+4	Mean±SD
A. conyzoides@ 100 mg/kg	-	1	2	2	1	2.50±0.5[b]
A. conyzoides@ 200 mg/kg	-	1	2	3	-	2.33±0.5[b]
A. conyzoides@ 300 mg/kg	-	2	2	2	-	2.00±0.5[c]
Vitamin E	-	3	1	2	-	1.83±0.5[c]
Baycox®	-	3	2	1	-	1.66±0.5[c]
Infected, non-medicated	-	-	1	2	3	3.33 ±0.5[a]
Non- infected, non-medicated	-	-	-	-	-	-

Means with the different letters (a, b, c) are significantly different (*P*<0.05)

All ACE administered groups showed minimal oocyst score, however, the better results were observed in group treated with higher dose of ACE which were non significantly different to Baycox® and Vitamin E (P>0.05) as shown in Table 3.

Fecal scores of different groups are shown in Table 4. Minimal fecal score was observed in ACE treated groups. The fecal score of groups treated with higher dose of ACE was non significantly different to Baycox® and Vitamin E (P>0.05) and were significantly different to infected non medicated group (P<0.05).

Table 3: Oocyst score of different treated groups (n=6)

Groups	0	+1	+2	+3	+4	+5	Mean±SD
A. conyzoides@ 100 mg/kg			1	2	2	1	3.5±0.5^{ab}
A. conyzoides@ 200 mg/kg		1	1	2	2		2.83 ±0.75^{b}
A. conyzoides@ 300 mg/kg	1	1	3	1			1.6±0.5^{c}
Vitamin E	1	3	1	1			1.33±0.5^{c}
Baycox®	2	2	2				1.00±0.5c^{c}
Infected, non-medicated				2	2	2	4.00±0.50^{a}
Non- infected, non-medicated	-	-	-	-	-	-	-

Means with the different letters (a, b, c) are significantly different (*P*<0.05)

Table 4: Fecal score of different treated groups (n=6)

Groups	4th day	5th day	6th day
A. conyzoides@ 100 mg/kg	3.21±0.8^{b}	3.30±0.7^{a}	2.89±0.6^{b}
A. conyzoides@ 200 mg/kg	3.15±0.5^{b}	3.22±0.7^{a}	1.90±0.5^{bc}
A. conyzoides @ 300 mg/kg	3.00±0.5^{bc}	3.08±0.75^{bc}	1.50±0.6^{c}
Vitamin E	2.01±0.8^{bc}	2.00±0.5^{bc}	1.52±0.5^{c}
Baycox®	1.71±0.8^{c}	1.66±0.5^{c}	1.37±0.5^{c}
Infected, non-medicated	3.80±0.44a	3.89±0.5^{a}	3.00±0.88^{a}
Non- infected, non-medicated	0.0±0.1^{d}	0.0±0.0^{d}	0.0±0.0^{d}

Means with the different letters (a, b, c) are significantly different (*P*<0.05)

ACE treated groups showed the improvement in serum chemistry of infected groups. Mean serum enzyme values i.e. ALT, LDH and Creatinine of ACE treated groups at higher dose were non significantly different to Baycox® and Vitamin E (P>0.05) and were significantly different to infected non medicated group (P<0.05) as shown in Table 5. In current study, lower serum enzyme values ALT, LDH and creatinine were observed in ACE treated groups as compared to control groups suggesting that the plant extract was nontoxic. Stabilization of ALT and LDH levels may indicate that the extracts are not hepatotoxic. The significant decrease in serum creatinine levels across different doses may suggest that extract are not nephrotoxic.

Table 5: Serum enzymes values of different treated groups (n=4)

Groups	ALT	LDH	Creatinine
A. conyzoides@ 100 mg/kg	14.90 ± 1.23^{b}	575.76 ±16.74^{b}	0.36 ±0.03^{a}
A. conyzoides@ 200 mg/kg	12.90 ± 1.71^{b}	556.91±18.30^{b}	0.28 ±0.04^{b}
A. conyzoides@ 300 mg/kg	12.80 ±1.14^{c}	533.03 ±24.35^{c}	0.26 ±0.03^{c}
Vitamin E	12.01 ±1.04^{c}	524.51 ±21.13^{c}	0.18 ±0.03^{c}
Baycox®	11.50 ±1.11^{c}	518.50 ±20.13^{d}	0.17 ±0.01^{c}
Infected, non-medicated	25.61 ± 2.32^{a}	889.94 ±22.16^{a}	0.54 ±0.03^{a}
Non- infected, non-medicated	8.63.08±1.8^{d}	494.43±12.66^{d}	0.11±0.07^{d}

Means with the different letters (a, b, c) are significant different (*P*>0.05) ALT (Alanine transaminase), LDH (Lactate dehydrogenase)

DISCUSSION

Several scientific reports have shown that plants and their antioxidant compounds (phenols, flavonoids, tannins and saponins) are being used as an alternative strategy for improving the health status of the animals (Jang *et al.*, 2007; Molan *et al.,* 2009; Abbas *et al.,* 2019; Raheel *et al.*, 2019; Elghobashy *et al.*, 2020; Hassan *et al.*, 2020; Juman *et al.*, 2020; Khaskheli *et al.*, 2020; Ragab *et al.*, 2020; Zhang *et al.,* 2020). On the basis of results of current research it can be concluded that *Ageratum conyzoides* has anticoccidial activity as positive effects were seen on different parameters including lesion, oocysts, fecal score and. Results were non-significantly different to standard medicine (Baycox®) and Vitamin E (*P*>0.05). ACE also improved FCR of infected chicks. Results of this study revealed that ACE @ 100, 200, 300 mg/kg showed anticoccidial activity in dose dependent manner against mixed *Eimeria* infection. Similar type of anticoccidial effects have also been reported in previous studies on the evaluation of anticoccidial potential of different botanical extracts (Dkhil *et al.,* 2011; Yang *et al.,* 2015; Wang *et al.*, 2016). In one study, ethanolic extract from *Carica papaya* (leaves) showed remarkable positive effect on the weight gain in broiler chickens infected with *Eimeria* (Nghonjuyi *et al*., 2015).

In another study, ACE extract was administered orally to chicks and administration of ACE showed positive effect on red blood cells count, white blood cells and packed cell volume in challenged treated birds. Thus ultimately oocyst excretion and level of infection was also reduced (Nweze and Obiwulu, 2009). Likewise, the *in vivo* anticoccidial potential of *Trachyspermum ammi* (ajwain) and its effect on serum chemistry has also been reported in broiler chickens. Supplementation of *Trachyspermum ammi* into the feed of broiler reduced lesion, oocyst and fecal scores, and improved serum chemistry of birds (Abbas *et al.,* 2019). Abbas *et al.* (2017) has also reported similar type of anticoccidial effects of *Beta vulgaris* (sugar beet) extract in broiler chicks. *Beta vulgaris* extract reduced the lesion score, oocysts count and also improved hematological parameters of birds.

In another recent study, oral treatment with methanolic leaf extract of *Lannea schimperi* reduced the oocysts shedding and growth of *Eimeria tenella* in experimenatlly infected chickens and concluded that *Lannea schimperi* possess anticoccidial potential against cecal coccidiosis (Mikail *et al.,* 2019).

Ageratum conyzoides is enriched with various antioxidant compounds which include phenols, flavenoids, conyzorium, methexnebilitin and quercetin. Antioxidants compounds of *A. conyzoides* may decrease the oxidative stress level which is produced due to *Eimeria* parasite (Nweze and Obiwulu 2009). The anticoccidial potential of *A. conyzoides* on different paramters can be due to the action of different active and antioxidant compounds contained *A. conyzoides* which can reduce *Eimeria* infection level by interfering with lipid peroxidation process.

In current study, stability in different tested serum enzyme values indicates that the extract has non toxic effects on liver and kidney of birds. ALT, LDH and creatinine refer to hepatotoxicity while serum creatinine and urea refer the nephrotoxicity. So, on the basis of above mentioned studies it may be concluded that plants derived extracts like *Ageratum conyzoides* may be helpful in controlling coccidiosis and toxicity in chicken.

CONCLUSION

Present study reports the anticoccidial activities of *A. conyzoides* extract in broiler chickens. *A. conyzoides* showed anticoccidial activity against coccidiosis in dose dependent manner. It suggests that *A. conyzoides* derived compounds may be helpful in treatment and controlling coccidiosis in chicken. Further studies, are needed to characterize the active compounds of *A. conyzoides* which are involved in enhancing the anticoccidial potential against avian coccidiosis.

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REFERENCES

Abbas A, Abbas RZ, Khan MK, MA Raza, Mahmood MS, Saleemi MK, Hussain T, Khan JA Sindhu ZUD (2019) Anticoccidial effects of *Trachyspermum ammi* (Ajwain) in broiler chickens. Pak Vet J 39: 301-304.

Abbas A, Iqbal Z, Abbas RZ, Khan MK, Khan JA (2017) Immunomodulatory activity of Pinus radiata extract against coccidiosis in broiler chicken. Pak Vet J 37: 145 - 149.

Abbas A,Iqbal Z, Abbas RZ (2015) *In-vitro*anticoccidial potentaial of *Sacharrum officiarum* extract against *Eimeria* oocysts. BolLatinoam Caribe Plant Med Aromat14:456-61.

Abbas RZ, Iqbal Z, Khan MN, Zafar MA, Zia MA (2010) Anticoccidial activity of *Curcumalonga* L. in broiler chickens. Braz Arch Biol Techno l53: 63-67.

Abbas RZ, Colwell DD, Gilleard J (2012) Botanicals An alternative approach for the control of avian coccidiosis. Worlds PoultSci J 68: 203-215.

Abbas RZ, Iqbal Z, Akhtar MS, Khan MN, Jabbar A,Sandhu ZD (2006) Anticoccidial screening of *Azadirachtaindica* (Neem) in broilers. Pharmacologyonline 3:365-71.

AbbasA, Iqbal Z, Abbas RZ, Khan MK,KhanJA,Sindhu ZD, MahmoodMS,Saleemi MK (2017) *In vivo* anticoccidial effects of *Beta vulgaris* (sugar beet) in broiler chickens. Microb Pathog.111:139-144.

Alshamiri MMA, Ali SAM, Abdalla HO and Ahmed HB (2021). The effect of supplementing different levels of phytase enzyme on performance, some carcass properties and economics of broiler chickens. Agrobiol Records 4: 14-22.

Chapman HD (2014) Milestones in avian coccidiosis research.A review. PoultSci93:501-511.

DkhilMA, Abdel-Baki AS, Wunderlich F, Sies H, Al-Quraishy S (2011) Anticoccidial and anti-inflammatory activity of garlic in murine *Eimeria papillata* infections. Vet Parasitol 175: 66-72.

Elghobashy KA, Eldanasoury MM, Elhadary AA, Farid M (2020) Phytochemical constituent, HPLC profiling and antioxidant activity of *Passiflora incarnata* and *Arctium lappa* leaves extracts. Int J Vet Sci 9: 42-49.

Fall AD, Gbati OB, DiattaW,Lapo RA, Diatta-Badji K, Dieng M, Ibra S, Dieng SIM, Bassene E Pangui LJ (2016) Anticoccidian activity of ethanol roots extract of *Cassia sieberiana*DC in chickens. European J Med Plants11: 1-7.

Hassan NMF, Sedky D, Abd El-Aziz TH, Shalaby HA, Abou-Zeina HAA (2020) Anthelmintic potency and curative effect of pomegranate peels ethanolic extract against *H. contortus* infection in goats. Int J Vet Sci 9: 210-216.

Hilbrich, P (1978)Krankheiten des geflugelsunterbesonderedberucksichitigung der haltung und futterung. Hermann kuhn kg. Schwenningen am neckar Germany.

Jang IJ, Jun M,Lillehoj MHS, Dalloul RA, Kong IK, Kim S, Min W2007: Anticoccidial effect of green tea-based diets against *Eimeria maxima*. Vet Parasitol144:172-175.

Johnson J, Reid WM (1970) Anticoccidial drugs lesion scoring techniques in battery and floor pen experiments with chickens. Exp Parasitol 28:30-36.

Juman M, Nordin ML, Woldegiorgis EA, Zulkiple SA, Shaari R (2020) *In vitro* study of antifungal activity of *Averrhoa bilimbi* extracts against *Candida* species. Int J Vet Sci 9: 104-110.

Ijaz MU, Khan MA, Yousaf S, Nasir S, Naz H, Anwar H, Younis T, Samad A (2020). Methanolic extract of *Fraxinus xanthoxyloides* attenuates cisplatin-induced reproductive toxicity in male albino rats. Pak Vet J 40: 489-493.

Khan MN, Sajid MS, Rizwan HM, Qudoos A, Abbas RZ, Riaz M, Khan MK (2017) Comparative efficacy of six anthelmintic treatments against natural infection of fasciola species in sheep. Pak Vet J 37: 65 - 68.

Khater HF, Ziam H, Abbas A, Abbas RZ, Raza MA, Hussain K, Younis EZ, Radwan IT, Selim A (2020) Avian Coccidiosis: Recent Advances in Alternative Control Strategies and Vaccine Development. Agrobiol Records 1: 11-25.

Khaskheli AA, Khaskheli MI, Khaskheli AJ, Khaskheli AA (2020) Dietary influence of *Yucca schidigera* on broilers and layers: a review. Int J Vet Sci 9: 458-461.

Masood A, Qureshi AS, Shahid RU, Jamil H (2020). Effects of oral administration of essential oil (Mix Oil®) on growth performance and intestinal morphometry of Japanese quails (Coturnix coturnix *japonica)*. Pak Vet J 40: 385-389.

Mikail HG, Abubakar IA, Mohammed BR, Yusuf M, Hussain G (2019) Effects of Methanolic Leaf Extract of Lannea schimperi on Some Organs Histopathology in Experimentally Induced Coccidiosis in Broiler Chickens. J Vet Sci Ani Husb 6: 602

Molan AL, Liu Z, Shampa DE (2009) Effect of pine bark (*Pinusradiata*) extracts on sporulation of coccidian oocysts. Fol Parasitol 56: 1-5.

Nghonjuyi NW, Tiambo CK, KimbiHK, Manka N, Juliano F, Lisita RS (2015) Efficacy of ethanolic extract of *Carica papaya* leaves as a substitute of sulphanomide for the control of coccidiosis in KABIR chickens in cameroon. J Anim Health Prod 3: 1-21.

Nweze NE, Obiwulu IS (2009) Anticoccidial effects of *Ageratum conyzoides*. J Ethnopharmacol 122:6-9.

Qamar H, Li A, Zeng Z, Wang Y, Mehmood K, Waqas M, Iqbal M, Zhang J, Li J (2020). Effect of grape seed extract on tibial dyschondroplasia incidence, liver weight, and tibial angiogenesis in chickens. Pak Vet J 40 : 187-194.

Raheel IAR, Orabi A, El Masry A (2019) Natural herbs CLEANACTIV®; immune-modulator, health activator and growth promoter in broiler chickens. Int J Vet Sci 8: 67-270.

Ragab E, Badr H, AbuElkheir A, Enbaawy MI (2020) The effectiveness of methanolic extracts of five plants on different *Salmonella* isolates. Int J Vet Sci 9: 379-384.

Ryley JF, Meade R, Burst JH, Robinson TE (1976) Methods in coccidiosis research Separation of oocysts from faeces. J Parasitol 73: 311-326.

SAS (2004) SAS Statistical Software Version 9.1. SAS Institute Inc. Cary NC USA.

Shahid SRA, Shah MA, Riaz A, Malik AM, Hasan MU, Xiangrui L, Babar W and Shahid SKA, (2020) Identification and molecular characterization of *Eimeria tenella* based on EtMic5 gene in Pakistan. Pak Vet J 40: 443-448.

Wang D,Zhou L, Li W, Zhou H, Hou G (2016) Anticoccidial effect of *Piper sarmentosum* extracts in experimental coccidiosis in broiler chickens. Trop Anim Health Prod 5: 1071-1078.

YangWC, Tien YJ, Chung CY, Chen C,Chiou WH, Hsu SY, Liu HY, Liang CL, Chang CLT (2015) Effect of *Bidens pilosa* on infection and drug resistance of Eimeria in chickens. Res Vet Sci 98: 74-81.

Yi Y, Yao M, Sun P, Cheng J, Khan A, Guo J, Li H, Sun N (2020). Effect of ethanol extract and fractions of physalis calyx seu fructus on inflammation and *Mycoplasma Gallisepticum*. Pak Vet J 40: 283-288.

Youn HJ, Kang YB, Jang DH (1993) Effects of g-irradiation from cobalt-60 on pathogenicity of *Eimeria tenella*. Korean J Vet Res 33:649-655.

Zhang K, Li X, Na C, Abbas A, Abbas RZ, Zaman MA (2020) Anticoccidial effects of *Camellia sinensis* (green tea) extract and its effect on Blood and Serum chemistry of broiler chickens. PakVet J 40: 77-80.