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Comparative Efficacy Of *Moringa Oleifera* Seed Extract Versus Enrofloxacin Against *Salmonella Pullorum* In Chickens

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A B S T R A C T

Poultry meat and eggs serves as major sources of protein and income worldwide. Poultry diseases and drug resistant disease organisms are major constraints to successful poultry production. This study aims to consider the effectiveness of *Moringa oleifera* aqueous seed extracts over Enrofloxacin drug on pullorum disease effects caused by *Salmonella Pullorum* in chicken. Sixty white Cockerel chicks purchased one day old was brooded for two weeks and vaccinated accordingly at Veterinary Teaching Hospital, Sabo-Oke, University of Ilorin. The chickens were randomly grouped into 4 groups (A, B, C and D). Birds in groups A, B and C were artificially infected orally with 0.5 ml of 10^8 CFU of *Salmonella Pullorum* isolates. Birds in group A and B each, were treated orally with 200 mg/kg of *Moringa oleifera* aqueous seed extract and enrofloxacin drug for 7 days, respectively. Birds in group C were infected but not treated as positive control. Birds in group D were uninfected and untreated as negative control. It was observed that *Moringa oleifera* aqueous seed extract showed a higher significant effect in the amelioration of the clinical signs (General weakness was observed in birds in groups A (10 %) and B (20 %) only, while birds in group C died before day 7 of treatment, birds in group D did not record any signs of weakness. Anorexia was 20% and 40% in groups A and B, respectively). At day 7 of treatment, chickens in groups A and B recorded mortality of 10% and 20%, respectively, while chickens in the uninfected untreated group D had no mortality. *Moringa oleifera* aqueous seed extract with lower percentage mortality as recorded in (Table 2 &3) in groups A 10%, B 20% and 10%, 16%, respectively, showed it has the potentials of reducing clinical signs effects as well as an efficient antimicrobial agent on infected and treated chickens than enrofloxacin drug.

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Introduction

Poultry meat and eggs serves as major sources of protein and income worldwide. Poultry diseases and drug resistant disease organisms are major constraints to successful poultry production. This study aims to consider the effectiveness of *Moringa oleifera* aqueous seed extracts over Enrofloxacin drug on pullorum disease effects caused by *Salmonella Pullorum* in chicken. Sixty white Cockerel chicks purchased one day old was brooded for two weeks and vaccinated accordingly at Veterinary Teaching Hospital, Sabo-Oke, University of Ilorin. The chickens were randomly grouped into 4 groups (A, B, C and D). Birds in groups A, B and C were artificially infected orally with 0.5 ml of 10^8 CFU of *Salmonella Pullorum* isolates. Birds in group A and B each, were treated orally with 200 mg/kg of *Moringa oleifera* aqueous seed extract and enrofloxacin drug for 7 days, respectively. Birds in group C were infected but not treated as positive control. Birds in group D were uninfected and untreated as negative control. It was observed that *Moringa oleifera* aqueous seed extract showed a higher significant effect in the amelioration of the clinical signs (General weakness was observed in birds in groups A (10 %) and B (20 %) only, while birds in group C died before day 7 of treatment, birds in group D did not record any signs of weakness. Anorexia was 20% and 40% in groups A and B, respectively). At day 7 of treatment, chickens in groups A and B recorded mortality of 10% and 20%, respectively, while chickens in the uninfected untreated group D had no mortality. *Moringa oleifera* aqueous seed extract with lower percentage mortality as recorded in (Table 2 &3) in groups A 10%, B 20% and 10%, 16%, respectively, showed it has the potentials of reducing clinical signs effects as well as an efficient antimicrobial agent on infected and treated chickens than enrofloxacin drug.

Materials and methods

Study area

This study was carried out in the Department of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria.

Materials

Dry seeds of *Moringa oleifera* were collected at Ilorin West L.G.A, Kwara State, Nigeria. The extraction technique was carried out according to [22]. Four hundred grams of the coarsely grounded seeds were loaded in a winster bottle and soaked with one litre of distilled water for 72 hrs with intermittent shaking. The extract was filtered and dried in an incubator at 56^0C. The weight of the dried extract was determined using a digital weighing balance by subtracting the weight of the beaker from the weight of the beaker with the extract and then kept at 4^0C until needed.

Salmonella Pullorum isolates (CD 20) were obtained from National Veterinary Research Institute, Vom, Plateau State, Nigeria for the study. Enrofloxacin 20% was purchased from a reputable Veterinary pharmaceutical company in Ilorin, Kwara State.

Experimental Birds

Sixty (60) day old white leghorn cockerel chicks were acquired from a reputable hatchery also at Ilorin, Kwara State. The birds cloacal swap using a sterile swab sticks were collected and screened against *Salmonella Pullorum* infections in Microbiology Laboratory of the Veterinary Teaching Hospital before they were brooded for two weeks. The chickens were fed with vital® feeds and clean drinking water was provided ad libitum. The birds were vaccinated with Lasota vaccine from National Veterinary Research Institute Vom, Nigeria (NVRI) against Newcastle disease at day (1) and Infectious bursa disease Gumboro vaccine at day (14).

Experimental design

The birds were divided into four experimental groups (A, B, C and D) consisting of 15 birds per group. Chicks from groups A, B and C were infected through the oral route with *Salmonella Pullorum* at a dose of 0.5 ml of 10^8/ ml CFU (Mac Farland Standard Solution), while chicks in uninfected untreated group D, served as negative control, and were kept separate from the infected groups. Aqueous seed extract of *Moringa oleifera* 0.5 g was dissolved in 1 milliliter of water to give 500 mg/ml. A dose of 200 mg/kg of the aqueous seed extract of *Moringa oleifera* was used for the experimental treatment of birds in group A, while a dose of 200 mg/kg of enrofloxacin® was used in the treatment of birds in group B. Chickens in group C were infected but not treated (Positive control), while birds in group D were uninfected untreated (Negative control). Treatment of experimental groups commenced at the onset of clinical signs 48 hours post infection with aqueous seed extract of *Moringa oleifera* at 200 mg/kg orally for 7 days. Chickens in group B were treated with enrofloxacin® at 200 mg/kg orally for 7 days as well. Chickens in group C were left untreated, while chickens in group D were uninfected untreated control group. The study was for 10 days and the parameters that were monitored in all the groups include clinical signs, morbidity and mortality

Clinical signs, morbidity and mortality

Clinical signs, morbidity and mortality across the groups were monitored and recorded for 10 days from the experimental groups throughout the period of study.

Data analysis

Clinical signs and mortality were expressed using percentage descriptive statistics and presented in Tables.

Results

Effect of aqueous seed extract of *Moringa oleifera* on Clinical signs and Morbidity rate

The clinical signs observed in chickens experimentally infected with *Salmonella Pullorum* 48 hours post infection and before treatment was instituted is presented in (Table 1). General weakness in groups A, B and C were 14%, 20% and 20%, respectively. Birds in groups A, B and C had 7%, 20% and 20% anorexia, respectively. Whitish diarrhea observed were 14%, 13% and 30% in groups A, B and C, respectively. Somnolence was observed in birds in groups A (7%), B (7%) and C (10 %). The percentage of birds that showed droopy wings in group A only was 7%. Peeping sound was only recorded in chickens in group B (7%), while 7% gasping was observed in chickens in group A only.

The clinical observation on the effect of aqueous seed extract of *Moringa oleifera* (ASEMO) and enrofloxacin on 7 days' treatment of chicks experimentally infected with *Salmonella Pullorum* were presented in Table 2: General weakness was observed in birds in groups A (10 %) and B (20 %) only while birds in group C died before day 7 of treatment, birds in group D did not record any signs of weakness. Anorexia was 20% and 40% in groups A and B, respectively. In all the groups, only birds in group A showed 10% seclusion at day 7. All other clinical signs were absent by day 7 of treatment.

Effect of plant extract on survival of the chicks

Percentage (%) mortality recorded in the different groups of birds treated with aqueous seed extract of *Moringa oleifera* and enrofloxacin drug during the 10 days' experimental infection and treatment are presented in (Table 3). Mortality was recorded 72 hrs in group C (10%) birds; also, on day 4, chickens in groups B and C recorded 20% and 50% mortality, respectively. On day 5 mortality recorded in groups A, B and C were 25%, 50% and 75 %, respectively; while on day 6, chickens in groups A and B had 8% and 14% mortality, respectively, while (100%) mortality was recorded in birds in the infected untreated group C (positive control). At day 7 of treatment, chickens in groups A and B recorded mortality 10% and 16%, respectively except chickens in uninfected untreated group D (negative control). At day 8 of the experiment, and at the termination of the experiment, mortality was not recorded against all the experimental groups and chickens in group D (negative control) that were apparently healthy throughout the period of study.

Table 1. Clinical observation (Percentage) of chickens treated with aqueous seed extract of *Moringa oleifera* and enrofloxacin 3 days' post experimental infection with *Salmonella Pullorum*

Treatment Groups	Mortality	Weakness	Anorexia	Whitish diarrhea	Somnolence	Droopy wings	Seclusion	Peeping sound	Gasping
A	0	14	7	14	14	7	7	0	7
B	0	20	20	13	0	7	0	7	0
C	10	20	20	30	0	10	0	0	0
D	0	0	0	0	0	0	0	0	0

Key: Treatment groups; A=ASEMO (200 mg/kg), B= Enrofloxacin (200 mg/kg), C= Infected untreated group (positive control), D= Uninfected untreated group (negative control): -Percentages were computed to the nearest whole number

Table 2. Clinical observations (%) of chickens treated with aqueous seed extract of *Moringa oleifera* and enrofloxacin 7 days' post experimental infection with *Salmonella Pullorum*

Treatment Groups	Mortality	Weakness	Anorexia	Whitish diarrhea	Somnolence	Droopy wings	Seclusion	Peeping sound	Gasping
A	10	10	20	0	0	0	10	0	0
B	20	20	40	0	0	0	0	0	0
C	-	-	-	-	-	-	-	-	-
D	0	0	0	0	0	0	0	0	0

Key: Treatment groups; A=ASEMO (200 mg/kg), B= Enrofloxacin (200 mg/kg), C= Infected untreated group (positive control), D= Uninfected untreated group (negative control): Percentages were computed to the nearest whole number. Negative (-) all birds in this group died.

Table 3. Summary of mortality (%) pattern in chickens experimentally infected with *Salmonella Pullorum* and treated with aqueous seed extract of *Moringa oleifera* and enrofloxacin for 7 days

Days post treatment	Mortality (%) among experimental groups			
	A	B	C	D
1	0	0	0	0
2	0	0	0	0
3	0	0	10	0
4	0	20	50	0
5	22	25	75	0
6	8	14	100	0
7	10	16	0	0

Key: Treatment groups; A =ASEMO (200 mg/kg), B= Enrofloxacin (200 mg/kg), C= Infected untreated group (positive control), D= Uninfected untreated group (negative control): -Percentages were computed to the nearest whole number. Negative (-) all birds in this group died.

Discussion

Acute manifestation of clinical signs effects observed in the experimentally infected untreated group of chicks within (48 hours) in this study is synonymous with the acute manifestation of clinical signs of pullorum disease caused by *Salmonella pullorum* in chickens [5]. This also supports the assertion that, *Salmonella pullorum* field isolates obtained from clinical disease are more virulent. The observed clinical signs of

whitish diarrhea, anorexia and weakness observed in this study were typical of pullorum disease as an enteric disease of chickens [17]. However, chicks treated with aqueous seed extract of *Moringa oleifera* showed a lower clinical signs manifestation effects compared to chicks treated with the enrofloxacin conventional drug. This is possible because *Salmonella pullorum* field isolates are known to be more resistant to most conventional poultry drugs presently in use [5], it could also be due to probably to wrong drug constitution, dilution and administration. Excessive use of the same drug over time or even the use of expired drugs by ignorant farmers going into self medication those are desperate to treat their birds due to the absence of Veterinary Doctors in the field [1].

However, treatment with aqueous seed extract of *Moringa oleifera* for seven days showed a significant reduced clinical signs manifestation on birds as from days 5-7 better than enrofloxacin drug used in this study. This tends to show that the aqueous seed extract of *Moringa oleifera* had an ameliorative effect in pullorum disease infected birds. These reduced manifested clinical signs effects by the orally administered extract in experimental birds *in vivo* further confirms the efficacy of the aqueous and ethanol seed extracts of *Moringa oleifera* as having an *in vitro* antibacterial activity against Gram positive and Gram-negative bacteria [14]. Also, [12] reported that aqueous root extract of *Moringa oleifera* had antibacterial activity against some Gram-positive and Gram-negative bacteria *in vitro*. It could therefore be said that ASEMO has both in vitro and in vivo antibacterial activities, and is likely to have some active pharmacological constituents' or therapeutic molecules that might be responsible for reduction or amelioration of severe clinical signs and reduced mortality in birds suffering from pullorum disease better than enrofloxacin drug. However, *Salmonella pullorum* was incapable of resisting the ameliorative and therapeutic effects of aqueous seed extract of *Moringa oleifera* in the experimentally infected and treated chickens.

These positive effects of the aqueous seed extract of *Moringa oleifera* in this study suggests that the extract has some antimicrobial pharmacological molecules that cannot be resisted by Salmonella organisms. The aqueous seed extract molecules could be said to be highly effective in the treatment of pullorum disease in experimental chickens. *Moringa oleifera* have also been known to have active pharmacochemical constituent and large protein molecules that binds to many polypeptides' moieties [18] which could be said to be responsible for its therapeutic and antimicrobial abilities. Also, *Moringa oleifera* seed extract was found to improve growth and weight gain in broilers [20]. Some major reasons for the improved growth and weight gain may be attributed to the richness of *Moringa oleifera* seeds in proteins rich in sulfur amino acids and their high oil content and beneficial unsaturated fatty acids [21].

The effect on mortality from this study was observed in birds of more than two weeks of age as against less than one week as reported by [19], who reported that Pullorum disease causes substantial mortality in chicks, but less frequently in birds of more than a week old [19]**.** In this study, mortality was observed in the infected untreated groups and in the enrofloxacin treatment group within 2-3 days' post infection, suggesting the virulent pathogenic potentials of Salmonella organisms that caused weakness, anorexia and even death in birds.

The difference in duration of experimental mortality recorded from this study could be associated with birds' inability to eat to maintain a healthy life and sustain immune status which could be derived from dietary intake. Also, the effect of manifested clinical signs of diarrhea, anorexia, and weakness lead to exhaustion, weakness, depression and death [16].

Although mortality from the treatment groups was not significantly different within the treated groups, the aqueous seed extract was able to cause a reduced mortality rate in chickens treated with the extract better than enrofloxacin treated chickens. This is in agreement with report from [19] that the frequently found *Salmonella field strains* are sensitive to many commonly available antibiotics, but strains from free ranging birds have varying degrees of resistance.

This further re-enforces the potentials of both the seed extract of *Moringa oleifera* and enrofloxacin drug in treating pullorum disease in chicken through increased production of heterophils immune response cells. This immune response capability of the aqueous seed extract of *Moringa oleifera* might be responsible for the inability of salmonella organisms to resist the effects of the extracts on experimental birds. This also confirms that some medicinal plants parts such as *Moringa oleifera* aqueous seed extracts is safe in chickens and has the potential of increasing the immune status of chicken when administered through the oral route [8], [14] in the phase of disease outbreak.

It can therefore be concluded that, *Moringa oleifera* aqueous seed extract is capable of ameliorating the clinical signs effects of pullorum disease in chickens, reduce mortality and, can be considered a potential antimicrobial agent in the treatment of pullorum disease in chickens.

Conclusion

Absence of overt clinical signs and reduced mortality and in extract treated group showed the effectiveness and therapeutic benefits derivable from *Moringa oleifera* aqueous seed extract. It can therefore be concluded that, the extract had a high significant therapeutic effect on pullorum disease infected chicken than enrofloxacin drug.

References

Declaration of Interests*:* The authors declare that they have no competing interests.

Author Contributions*:* Conceptualization – N. A. F and A. G. A.; Methodology − N. A. F., A.G.A., and H.A.; Formal Analysis − N.A.F., A.G.A., and A.C.J.; Investigation − N.A.F. and H.A.; Resources − N.A.F.; Data Curation − N.A.F. and H.A.; Writing Original Draft Preparation − N.A.F.; Writing, Review, and Editing − A.G.A., H.A., and A.C.J.; Supervision − A.G.A. and A.C.J.; Project Administration − N.A.F., A.G.A., and A.C.J.; Funding Acquisition − N.A.F. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data that support the findings of this study are available upon request from the corresponding author.

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