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Full Length Research Paper

Histological and histochemical assessments on the effect of ethanol fruit extract of *Phoenix dactylifera* L. (Date Palm) on cerebral cortex of lead acetate treated wistar rats

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This study histologically and histochemically assess the effect of ethanol fruit extract of *Phoenix dactylifera* L. (EFPD) on the cerebral cortex of lead acetate exposed Wistar rats. Twenty rats were grouped into five groups (A to E, n=4). Group A (control) was administered distilled water (2 ml/kg), while groups B to E were treatment groups. Cerebral damage was induced in rats by the administration of lead acetate (120 mg/kg). Groups B, C, D and E were administered lead acetate (120 mg/kg) for a period of 3 weeks, after which groups C and D were administered EFPD (500 and 1000 mg/kg, respectively) and group E was administered dimercaptosuccinic acid (10 mg/kg) for 2 weeks. All administrations were via oral route, once daily. Microscopic examination of cerebral sections of lead acetate-treated rats revealed histo-architectural alteration; cortical degenerative changes, such as, necrosis, satellitosis, vacuolation and neuronal cytoplasmic shrinkage. However, administration of EFPD remarkably ameliorated lead acetate-induced cortical cerebral degenerative changes in the rats, in a dose dependently manner, as compared to the reference drug dimercaptosuccinic acid. Results suggest that EFPD is a potential therapeutic agent against lead acetate-induced cortical cerebral alterations in Wistar rats.

Key words: Cerebrum, lead acetate, *Phoenix dactylifera* L, Wistar rats.

INTRODUCTION

Human and animal populations interact with their environment via food, air and water on a daily basis, as such exposes them to toxic substances, such as chemicals and heavy metals, capable of causing harm or even death (Wade et al., 2002; Burger et al., 2013). Heavy metals are natural constituents of the earth crust, their biochemical balance are easily altered by human needs for improving quality of live and well-being (Das et
Once present in the environment even in trace amount can pose a serious problem for all organism and prolong exposure creating a deleterious health effect in humans, since they cannot be degraded or destroyed (Chen and Chen, 2001; Sedbrook, 2016). Lead is one of the common toxic heavy metals due to the ease in mining and refining. It is used in building construction, making of water pipes, lead-acid batteries, bullets and shot, weights, as part of solders, pewter’s fusible alloys, as well as radiation shield (Duah et al., 2012). The wide use of lead had turned lead poisoning into an ever present environmental and health challenge, hence the increased blood-lead level (Ahmed et al., 2013). Lead exposure affect both central and peripheral nervous system resulting to nerve cell degeneration and demyelination (Sanders et al., 2009; Abeer, 2012; Assi et al., 2016).

The cerebrum is rostral most part of the brain responsible for higher brain function, such as motor movements, perception of stimuli, emotions, problem solving and recognition (Singh, 2002; Owolabi et al., 2014). The cerebrum is vulnerable to damage from a variety of sources such as developmental defects, degenerative diseases, infectious processes, trauma and tumors (Klementiev et al., 2007). Heavy metals exposure which lead happen to be one, have been reported to be one of the leading cause of cerebral injuries (Korogi et al., 2011; Fonfria et al., 2005; Wagner et al., 2010; Owoe and Farombi, 2015). Pharmacotherapy and psychoactive drugs in the last two decades have gain recognitions, due to its efficacy in the management of neurological related disorders. However, several studies have revealed that such relieve are temporal with manifestation of various side effects (Handa, 1995; Mireille et al., 2017). Traditional medical practice has gained interest in the world over due to the wide spread usage of medicinal plants and its consumption, especially in developing countries (Ashafa and Olunu, 2011; Sujith et al., 2012).

Phoenix dactylifera L. (date palm) and its various parts are widely used in folk medicine for the treatment of various ailment and disorders, such as memory disturbance, fever, inflammation, paralysis, and even nervous disorders (Nadkarni, 1976; Elgindi et al., 2015; Alhaider et al., 2017). Several researchers have documented on the rich nutritional value, high dietary fibre and essential mineral of date palm, such as phosphorus, iron, potassium and a significant amount of calcium and vitamins (Mohamed and Al-Okbi, 2004; Usama et al., 2009; Yusuf et al., 2017). Several studies on extracts of date palm have indicated the presence of antioxidant properties (Mansouri et al., 2005; Al-Qarawi et al., 2008; Agbon et al., 2016); these antioxidant activities are attributed to a wide range of phenolic and flavonoid compounds and some Vitamin in date palm (Vayali, 2012; Bennedduort et al., 2013).

The aim of this study was to histologically and histochemically assess the therapeutic effect of ethanol fruit extract of P. dactylifera (EFPD) against lead acetate-induced cerebral alterations in Wistar rats.

MATERIALS AND METHODS

Plant collection and identification

Dried P. dactylifera (date palm) fruits were obtained at a local market (Samaru) in Zaria, Nigeria and was authenticated and given a Voucher Specimen Number of 7130, at the Herbarium Unit of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Extract preparation and phytochemistry

Extraction of P. dactylifera fruit and phytochemical screening were conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The method of maceration as reported by Agbon et al. (2013) for the preparation of ethanol fruit extract of P. dactylifera was adopted. The method of Trease and Evans (2002) as reported by Oni et al. (2015) was adopted for phytochemical screening.

Experimental animals

Twenty Wistar rats (male and female; 100 to 180 g) were obtained from Animal House of the Department of Human Anatomy, Faculty Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University, Zaria and housed in new wired cages in the same animal house were rats acclimatized for two weeks prior to the commencement of the experiment. The rats were separated into five groups; one control and four treatment groups. The rats were housed under standard laboratory condition, light and dark cycles of 12 h and were provided with standard rodent pellet diet and water ad libitum. The treatment groups were administered, in addition to feed and water, lead acetate/EFPD/DMSA for a period of five weeks. The rats were weighed before and after the experiment and weight changes were computed and analysed.

Drug

Lead acetate (Analytical) manufactured by British Drug Houses (BDH) Laboratory Chemicals Division, Poole, England, was obtained and used as neurotoxin for the experiment. Dimercaptosuccinic acid (DMSA, Analytical) manufactured by Best of Chemical (BOC) Sciences, New York, USA was obtained and used for the experiment as standard chelating drug.

Experimental procedure

Twenty Wistar rats were grouped into five groups (A to E) of four rats each. Group A (control) was administered distilled water (2 ml/kg), while groups B to E were treatment groups. Cerebral damage was induced in rats by the administration of lead acetate (120 mg/kg; 20% LD50) (Sujatha et al., 2011) as reported by Yusuf et al. (2017). Groups B, C, D and E were administered lead acetate (120 mg/kg) for a period of 3 weeks, after which groups C and D were administered ethanol fruit extract of P. dactylifera (500 and 1,000 mg/kg, respectively, that is, 10 and 20% of LD50 oral in rats (Agbon et al., 2017) and group E was administered DMSA (10
mg/kg) (Chen et al., 1999) for an additional period of 2 weeks. All administrations were via oral route, once daily.

Histological and histochemical studies

At the end of the experiment, rats were euthanized and brain organs were harvested. Harvested brain organs were fixed in Bouin’s fluid and tissues processed using routine histological techniques, stained with Haematoxylin and Eosin (H&E) and histochemical stains (Cresly Fast Violet [CFV] for Nissl substance and Bielschowsky for neurites/nerve fibres) for light microscopic examination.

Data analysis

Results obtained were analysed using the statistical software, Statistical Package for Social Sciences (IBM SPSS version 20.0) and results were expressed as mean ± standard error of mean (SEM). Presence of significant difference among mean of the groups was determined using one-way analysis of variance (ANOVA) with Tukey post hoc test. Paired sample t-test was employed for the comparison of means as appropriate. Values were considered significant when p < 0.05.

RESULTS

Phytochemical analysis

Phytochemical analysis of ethanol fruit extract of P. dactylifera (EFPD) produced positive reaction for secondary metabolite and negative for some shown in Table 1.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Athraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Positive (present); - = Negative (absent).

The weight of the rats in all groups were observed to have increased (p>0.05), except in the lead acetate-treated group (p<0.05) when initial and final weights were compared. However, there was no significant difference (p>0.05) in weight change (difference in initial and final weights) when treated groups were compared with the control (Table 2).

Histological and histochemical examination

Histological examination of sections of cerebral cortex of rats stained with routine (H&E) histological and histochemical (CFV and Bielschowsky) stains revealed the following.

The cerebral sections of rats in the control group revealed normal histoarchitecture of the cerebral cortex with distinctive appearance of cortical neurones arranged in six layers (I to VI). Morphology of the ganglionic layer (Layer V) revealed dense distribution of large pyramidal cells; ganglion or Betz cells and glial cells. Histochemical (CFV) staining for Nissl substance revealed normal appearance of distinct intensely stained pyramidal cells and Bielschowsky revealed normal neuronal fibres (Plate 1A).

The cerebral sections of lead acetate-treated rats revealed histo-architectural degeneration of neurons such as satellitosis, perineuronal vacuulations, necrosis and neuronal cytoplasmic shrinkage. CFV staining revealed chromatolysis, cytoplasmic shrinkage and indistinct staining intensity and Bielschowsky stain revealed pyramidal neuronal degeneration as loss of neuronal fibres when compared with the control (Plates 1B, 2B and 3B).

Examination of the cerebral sections of ethanol fruit extract of P. dactylifera (500 and 1,000 mg/kg)-treated rats, revealed mild cerebral cortex histo-architectural distortions such as perineuronal/cytoplasmic vacuolation and pyknosis; CFV staining showed reduced staining intensity and Bielschowsky staining revealed loss of neuronal fibres when compared with the severe distortions of the lead acetate-treated group (Plates 1C, 1D, 2C and 2D).

The histological features of the DMSA-treated group revealed relatively similar histo-architecture, with mild distortion, when compared with control (Plates 1E, 2E and 3E).

DISCUSSION

In this study, phytochemical analysis of ethanol fruit extract of P. dactylifera revealed the presence of secondary metabolites such as, flavonoids, saponins, tannins and alkaloids which have been reported to have neuroprotective activities (Chan et al., 2012; Wan Ismail and MohdRadzi, 2013; Hwang et al., 2015). This finding
Table 2. Effect of *P. dactylifera* on body weight of lead acetate exposed Wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>IW (g)</th>
<th>FW (g)</th>
<th>t-value</th>
<th>p-value</th>
<th>FW-IW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>114.28±26.37</td>
<td>131.30±24.13</td>
<td>3.02</td>
<td>0.03</td>
<td>17.02±2.24</td>
</tr>
<tr>
<td>PbA</td>
<td>153.05±20.20</td>
<td>146.93±19.39</td>
<td>12.53</td>
<td>0.00</td>
<td>-6.12±0.81</td>
</tr>
<tr>
<td>PbA+PDLo</td>
<td>155.37±18.67</td>
<td>169.63±12.79</td>
<td>3.56</td>
<td>0.07</td>
<td>34.25±5.88</td>
</tr>
<tr>
<td>PbA+PDHi</td>
<td>143.83±17.06</td>
<td>163.15±10.95</td>
<td>2.65</td>
<td>0.08</td>
<td>19.32±6.11</td>
</tr>
<tr>
<td>PbA+DMSA</td>
<td>148.28±18.68</td>
<td>165.70±26.64</td>
<td>1.78</td>
<td>0.17</td>
<td>17.42±7.96</td>
</tr>
</tbody>
</table>

n = 4; mean ± SEM; Paired sample t-test; One way ANOVA LSD post hoc test; p>0.05 when compared with the control. Control (2 ml/kg distilled water); PbA: Lead acetate (120 mg/kg); PDLo: ethanol fruit extract of *Phoenix dactylifera* (500 mg/kg); PDHi: ethanol fruit extract of *Phoenix dactylifera* (1000 mg/kg); DMSA: dimercaptosuccinic acid; IW: initial weight; FW: final weight.

Plate 1. Micrograph of cerebral cortex (Layer V) section of Wistar rat. H and E stain (Mag. x250). (A) Section of cerebral cortex of control (untreated) group showing normal histology of the cerebral cortex. Granule cell (G); Pyramidal cell (P); Stellate cell (S). (B) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) showing distortion in the histology of the cerebral cortex. Pyramidal cell (P); Stellate cell (S); Neuronal degeneration (Na); perineuronal vacuolations (Nc), cytoplasmic shrinkage, satellitosis and necrosis. (C) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) followed by EFPD (500 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Astrocyte (A); Neuronal degeneration (N); perineuronal vacuolations/pyknosis and satellitosis. (D) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) followed by EFPD (1000 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Astrocyte (A); Stellate cell (S); Purkinje cell (P); Neuronal degeneration (N); perineuronal vacuolations and cytolysis. (E) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) followed by DMSA (10 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Astrocyte (A); Neuronal degeneration (N); pyknosis and clumping of cells.

is in agreement with the reported phytochemical constituents in fruit extract of *P. dactylifera* L. (Faqir et al., 2012; Abiola et al., 2017).

Reduced physical activity observed among the lead acetate-treated rats, reflects treatment-related toxicity. This agrees with the findings on drug-related toxicity and physical activity as reported by Agbon et al. (2014) and Yusuf et al. (2017). Body weight changes are strong pointers of general health status and drug related toxicity in animals (Mukinda, 2007; Salawu et al., 2009).

Significant weight decrease observed in lead acetate-treated rats is indicative of heavy metal related toxicity. Heavy metals intoxication, have been implicated for poor appetite and malabsorption of nutrients in animal models
Plate 2. Micrograph of cerebral cortex (Layer V) section of Wistar rat. CFV stain (Mag. ×250). (A) Section of cerebral cortex of control (untreated) group showing normal histology of the cerebral cortex. Granule cell (G); Pyramidal cell (P); Stellate cell (S). (B) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) showing distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal degeneration (N); perineuronal vacuolations, chromatolysis, cytoplasmic shrinkage, and poor staining intensity. (C) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) followed by EFPD (500 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Astrocyte (A); Neuronal degeneration (N); perineuronal vacuolations and indistinct staining intensity. (D) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) followed by EFPD (1000 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Astrocyte (A); Stellate cell (S); Purkinje cell (P); Neuronal degeneration (N); chromatolysis cytoplasmic vacuolations and cytolysis reduced staining intensity. (E) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) followed by DMSA (10 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Astrocyte (A); Neuronal degeneration (N); cytolysis and reduced staining intensity.

(Jadhav et al., 2007; Wadaan, 2009). Finding is in accordance with the report of Yusuf et al. (2017) which observed remarkable decrease in weight in lead-exposed rats. Body weight gain observed in EFPD-treated rats could be attributed to the plant’s wide range of essential nutrients with high caloric value, particularly carbohydrates and lipids (Shaba et al., 2015; Punia, 2016; Megbo et al., 2017). This finding is in agreement with the report of Wahab et al. (2010) and Haouas et al. (2014).

Neuronal degeneration have been identified as one of the major causes of neuronal cell apoptosis (cell death), which could be as a result of disease (pathological) or natural (aging) condition (Mazanetz and Fischer, 2007; Kumar and Khanum, 2012) exerting extrinsic insults or traumatic stresses on the cells (Kumar et al., 2009; Sivanandam and thakur, 2012). Characteristic neuronal histo- and cyto-architectural distortions, such as neuronal shrinkage, perineuronal vacuolation, satellitosis, and indistinct staining intensity, loss of pyramidal neuron processes, necrosis and pynkosis observed in this study are indicators of neurodegenerative changes. Satellitosis observed in this study is indicative of treatment related toxicity, a condition marked by an accumulation of neuroglia cells around damaged or necrosed neurones of the central nervous system, often as a prelude to neuronophagia (Manickam et al., 2009). Findings are in consistence with the reports on neurodegenerative changes following heavy metals (lead, mercury, cadmium) exposure. These metals have capacity to induce nervous tissue insult (Amal and Mona, 2009; Fakunle et al., 2013; Wani et al., 2015; Butt et al., 2018) and disrupt the release mechanism of calcium-dependent neurotransmitter and DNA repairs (Hartwig, 1994; Sharma et al., 2014).

In light microscopy, rough endoplasmic reticulum and free ribosomes appear as basophilic granular areas (Nissl bodies) with Cresy Fast Violet staining. Neuronal degeneration has been related to reduction in Nissl substances (Akassoglou et al., 2004) involving degradation of β-tubulin, an important component of the neuronal cytoskeleton, and these effects are evoked by
Plate 3. Micrograph of cerebral cortex (Layer V) section of Wistar rat. Bielschowsky stain (Mag. ×250). (A) Section of cerebral cortex of control (untreated) group showing normal histology of the cerebral cortex. Granule cell (G); Pyramidal cell (P); Stellate cell (S); Glia cell (G); Dendrite (D). (B) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) showing distortion in the histology of the cerebral cortex. Pyramidal cell (P); Stellate cell (S); Severed dendrite (D); Neuronal process degeneration (N); loss of neuronal fibres. (C) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) followed by EFPD (500 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Stellate cell (S); Neuronal degeneration (N); reduced nerve fibres. (D) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) followed by EFPD (1000 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Neuronal process (D); Basket cell (B); (mild neuronal loss). (E) Section of cerebral cortex of the group administered lead acetate (120 mg/kg) followed by DMSA (10 mg/kg) showing mild distortion in the histology of the cerebral cortex. Pyramidal cell (P); Glia cell (G); Neuronal degeneration (N); mild neuronal fibre loss.

N-methyl D-aspartate receptor (NMDAR) function (Xu et al., 2012). Findings in these studies are in accordance with the reports of Ajibade et al. (2012) and Agbon et al. (2014) with loss of Nissl substance in cerebellar neurones and shrinkage of the nucleus following chemically induced toxicity. The brain among other tissues and organ with high lipid content is the most sensitive target of heavy metal intoxication. Lead neurotoxic effect results from its ability to cross the blood brain barrier readily and influence alteration of certain membrane bound enzymes responsible for protecting the biological systems against reactive oxygen species (ROS) and oxidative stress (Xu et al., 2008; Yun et al., 2011; Fakunle et al., 2013; Ibegbu et al., 2013). Naqi (2015) and Owolabi et al. (2017) reported cortical cerebellar histo-architectural distortions on exposure to lead in rats. Bielschowsky staining technique demonstrates neuronal processes of the central nervous system. Loss of neuronal processes observed in the lead-treated rats is indicative of treatment related toxicity. Heavy metals have been reported to disrupt microskeletal like structures in the brain cells (Leong et al., 2001).

Neuronal injury may result in reversible or irreversible cell damage or cell death (Kumar et al., 2006). Mild cortical cerebral histo-architectural distortion in rats treated with DMSA and EFPD (500 and 1000 mg/kg) after exposure to lead acetate, was observed when compared with the severe histo-architectural distortions observed in the lead acetate-treated group.

In this study, histo-architectural features of DMSA (succimer)-treated group were comparable with the control. Thus, indicates the therapeutic activity of succimer. Succimer is an established metal ion chelating agent recommended for the treatment of heavy metal toxicity, such as lead (Kalia and Flora, 2005; Lowry, 2010).

Natural agents with antioxidant properties are beneficial in attenuating drug-induced oxidative stress in biological systems (Musa et al., 2012; Bauchi et al 2016). *P. dactylifera* antioxidant activities are attributed to a wide range of phenolic phytochemical constituents (Vayali,
2012; Benmeddour et al., 2013). Flavonoid, a polyphenolic compound, found in *P. dactylifera* fruits have been reported to have strong ROS scavenging and metal ions chelating activities; an antioxidant that shields neurones from lethal damage and has ability to suppress neuroinflammation (Pujari, 2011; Rice, 2001; Komaki et al., 2015). Findings are in line with the reported ameliorative potentials of *P. dactylifera* fruit extract; Agbon et al. (2017) reported ameliorative activity of ethanol fruit extract against inorganic mercury induced cerebral and cerebellar alterations in Wistar rats and Yusuf et al. (2017) reported neuroprotective activity on lead acetate-induced toxic effects in cerebellum. Thus, indicating the therapeutic potentials of EFPD on heavy metals induced cortical cerebral alterations.

**Conclusion**

In light of the observed results of the present study, it could be concluded that ethanol fruit extract of *P. dactylifera* possess therapeutic potentials against lead acetate-induced cortical cerebral alterations in Wistar rats. The therapeutic property of the extract, comparable to the standard drug, DMSA, could be attributed to the antioxidant properties of its constituent phytochemicals, such as flavonoid.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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Full Length Research Paper

Determination of egg production and weight in layers experimentally infected with *Salmonella gallinarum*

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The present study was conducted to determine egg production and weight in layers experimentally infected with *Salmonella gallinarum*. Twenty layers were used for the research. The layers were purchased at the age of 18 weeks from certified commercial poultry farm in Kujama Farm, Kaduna State, Nigeria and housed in the Animal Research Unit of the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The birds were examined to certify that they were disease free by collecting samples from the cloacal. The birds were assigned to two groups (infected and control) of ten layers each. The infected group was challenged with *Salmonella gallinarum* orally at the dose of 0.5 ml of 9 × 10⁸ CFU/ml. All the birds in the control group were orally given 0.5 ml of normal saline. After the infection, all the infected layers were closely observed for clinical signs of fowl typhoid. Percentage of egg production and body weight were measured from each group at days zero (Day 0), 2, 4, 7, 14, 21, 28, 35 and 42, post-infection (pi). By day seven post infection, all birds in the infected group showed clinical signs typical of fowl typhoid, namely, ruffled feathers, weakness, somnolence, greenish-yellow diarrhea, huddling together, decrease in feed and water consumption, and five of the layers died. There were, however, significant drop in egg production and loss of body weight in the *S. gallinarum* infected group.

Key words: Fowl typhoid, *Salmonella*, inoculum, layers, egg production, body weight.

INTRODUCTION

*Salmonella* species belong to the Family, Enterobacteriaceae. They are Gram negative, non-spore forming rods (Popoff et al., 2003). Fowl typhoid caused by *Salmonella enterica serovar gallinarum* in birds, is a

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severe systemic disease that affect both young and adult birds with macroscopic and microscopic lesions leading to massive economic losses due to high morbidity and mortality (Parmer and Davies, 2007).

Fowl typhoid (FT) has been discovered in many African countries which include Tanzania, Uganda (Okoj, 1993), Senegal (Arbelot et al., 1997), Nigeria (Sa‘idu et al., 1994) and Morocco (Bouzoubaa et al., 1987). FT is a septicemic infection affecting chicken and turkey mostly, but some natural infections in many other avian species has been studied (Wray et al., 1996; Shivaprasad, 1997). The outbreak of FT in young chicks may be due to vaccination against FT practiced by many farmers which result in vertical transmission of the infection (Jordan and Pattison, 1992; Roa, 2000). The control of FT through hygienic measures, together with some serological testing and slaughter of positive reactors, have resulted in the elimination of Salmonella gallinarum in many countries (Barrow, 1999). However, FT remains a leading disease of the poultry industry in many areas of the world (Okwori et al., 2013). Respiratory distress and depression is seen in acute FT and the clinical signs include greenish-yellow diarrhea, there may be enlargement and congestion of the liver, spleen and kidney. The liver may have pale multiple foci of 2 to 4 mm in diameter (Beyaz et al., 2010). In acute to subacute cases, there is multiple necrosis of the liver parenchyma with accumulation of fibrin and infiltration of heterophils mixed with a few lymphocytes and plasma cells can be seen in the liver (Kokosharov et al., 1997; Hossain et al., 2006). In sub-acute outbreaks, sporadic mortality over a long period is experienced while in chronic cases, especially in cases where there are large nodules in the heart, the liver will have congestion with interstitial fibrosis. The spleen may have severe congestion or fibrin deposits and severe hyperplasia (Chishti et al., 1985). The transmission of S. gallinarum can be through faecal droppings of infected birds, bird carcasses and laid eggs. The infection could be introduced by importation of live infected chickens and hatched eggs. Mechanical spread may be by humans, wild birds, mammals, flies, ticks, feed sacks, etc (Steigh and Duguid, 1989).

Poultry production in Nigeria has witnessed a rapid growth to a well-established commercial enterprise. This increase in the production activity is greatly pronounced and has resulted in new challenges (Hassan et al., 2006). Poultry production is the most efficient and cost-effective way of increasing the availability of high-protein food, as eggs are known to provide the most perfectly balanced food containing all the essential amino acids, minerals and vitamins (FAO, 1987; Branckaert et al., 2000). Salmonellosis in poultry causes egg shell abnormalities including shell-less and infertile eggs with early embryonic mortality (Welish et al., 1997; Coufal et al., 2003). This study evaluated the determination of egg production and weight in layers experimentally infected with Salmonella gallinarum in Zaria, Kaduna State, Nigeria.

MATERIALS AND METHODS

Study area

This study was carried out in Zaria, Kaduna State, which is located within the Northern Guinea Savannah Zone of Nigeria, between latitude 7° and 11°N, and longitude 7° and 44°E; the average rainfall of this zone ranges from 1,000 to 1,250 mm, and the average temperature ranges from 17 to 33°C (Sa‘idu et al., 1994).

Experimental chickens

Twenty eighteen-week-old hens were purchased from a commercial farm in Kuja, Kaduna State, Nigeria. These birds were vaccinated against other diseases but with the exception of fowl typhoid. On arrival, at the venue of the research, the birds were housed in the animal research unit of the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The birds were kept for a period of four weeks to get used to the handling conditions they would be subjected to during the research. During this period, they were on layer mash (Hybrid®).

Experimental design

Allocation of chickens to experimental groups

At 22 weeks old, the hens were randomly allocated to two groups (infected and control) of 10 layers each. The control group of chickens was then moved to the research pen of Department of Veterinary Pathology as a precautionary measure against transmission of the experimental fowl typhoid disease to the control group. At this point, both groups were fed commercial layer mash (Hybrid feeds®) until termination of the experiment. Water was provided to the layers ad libitum, throughout the experimental period that lasted for 42 days.

Source of bacterial organism

S. gallinarum was obtained from the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Bacteriological analysis

Cloacal swabs were collected from both infected and control groups of layers and dipped into a buffered peptone water for recovery of the S. gallinarum and subcultures were then made from each broth onto MacConkey agar. The agar plates were incubated aerobically at 37°C for 24 h using methods described by Wigley et al. (2001) and Parmer and Davies (2007).

Challenge bacteria

The challenge bacteria were collected from the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The bacteria from the slant were re-plated on MacConkey agar (MCA). The subcultured plates were then examined for their characteristic features, such as color, morphology using Gram’s stain (Gram negative). Some colonies
were picked from the cultured plate and placed in sterile test tube with normal saline of 20 ml of 0.5% and turbidity equivalent to 9 × 10⁸ CFU/ML was obtained. Challenge of the layers was done orally using sterile syringes. The infected group was given a dose of 0.5 ml of 9 × 10⁸ CFU/ML of the bacterium, but the control group were not infected with the organism, but received distilled water only.

Clinical observation

After challenge of the infected birds with the bacterial organism, the infected group was daily observed for typical signs of FT and findings were recorded.

Determination of body weight and egg production

Beginning from the day of infection (day 0) and throughout the experimental period, that lasted for 42 days, the live weights and egg production of the birds were recorded.

Bacteriological isolation

At necropsy, tissue samples of the liver, kidney, ovary and spleen were aseptically taken for isolation of S. gallinarum using standard laboratory methods (Wigley et al., 2001; Parmer and Davies, 2007).

Statistical analysis

Data obtained were expressed as ± SEM. Values were subjected to student T-test and values of P<0.05 were considered to be significant.

RESULTS

Clinical signs of fowl typhoid in the infected birds

All the birds in the control group appeared healthy throughout the experiment. Following challenge with S. gallinarum, the birds appeared clinically normal until day 7 post challenge when the birds started passing greenish-yellow diarrhea, having depression and huddling, rough feathers, somnolence, reduction in feed and water consumption, decreased egg production and sudden death.

Bacterial recovery from infected birds

S. gallinarum organisms were isolated in some of the samples collected which include liver, kidney, spleen and ovary of the challenged birds. Biochemical test revealed indole negative, urea negative, catalase and citrate positive and it produces hydrogen sulphide (H₂S) in triple sugar iron agar TSI.

Effect of S. enterica serovar gallinarum infection on egg production and body weight in the layers

Mean weekly percentage egg production

The mean weekly percentage egg production in the S. enterica serovar gallinarum experimentally infected and control groups is presented in Figure 1. The mean weekly percentage egg production in the infected birds on week 0 pi (87.12 ± 3.26%) was not significantly different (P>0.05) from that of the control group (84.13 ± 4.31%). But by week 1 pi, a significant decrease (P< 0.05) in mean weekly percentage egg production was observed in the infected group (31.03 ± 1.49%) when compared with that of the control (89.05 ± 3.26%) with the infected group reaching its lowest value on week 4 pi (20.11 ± 3.37%).
Table 1. Mean weekly percentage egg production in *Salmonella gallinarum* infected and control layers.

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>84.13 ± 4.31</td>
<td>87.12 ± 3.26</td>
</tr>
<tr>
<td>1</td>
<td>79.05 ± 3.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.03 ± 1.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>73.21 ± 2.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.01 ± 2.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>77.5 ± 3.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.13 ± 2.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>77.45 ± 4.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20.11 ± 3.37&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>80.30 ± 3.22&lt;sup&gt;e&lt;/sup&gt;</td>
<td>34.03 ± 2.30&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>83.08 ± 2.29&lt;sup&gt;f&lt;/sup&gt;</td>
<td>52.06 ± 3.20&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with the same superscript alphabets are significantly different with p<0.05.

Table 2. Mean weekly body weight (g) of *Salmonella gallinarum* infected and control layers.

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1733 ± 6.00</td>
<td>1733 ± 5.14</td>
</tr>
<tr>
<td>1</td>
<td>1756 ± 4.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1644 ± 1.90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>1769 ± 6.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1603 ± 6.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1784 ± 2.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1557 ± 5.90&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>1834 ± 4.71&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1511 ± 1.91&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>1877 ± 2.13&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1526 ± 3.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>1891 ± 3.90&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1543 ± 6.95&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with the same superscript alphabets are significantly different with p<0.05.

Thereafter, a gradual rise was observed in the infected group on week 5 pi (34.03 ± 2.30%) until the end of the experiment on week 6 pi (52.06 ± 3.20%) Table 1.

**Mean weekly body weight**

The mean body weights of the *S. enterica* serovar *gallinarum* experimentally infected and control groups are presented in Figure 2. The mean weekly body weight (g) of the infected birds on week 0 pi (1733 ± 5.14 g) and control (1733 ± 6.00 g) showed no significant difference (P>0.05). A significant decrease (P< 0.05) in mean weekly body weight was also observed on week 1 pi in the infected group (1644 ± 1.90 g) when compared with that of the control (1756 ± 4.40 g), with the infected group reaching its lowest value on week 4 (1511 ± 1.91g) post-infection. Following this, a gradual rise from its week 4 value was observed in the infected birds on week 5 pi (1526 ± 3.00g) till the termination of the experiment on week 6 pi (1543 ± 6.95 g) Table 2.

**DISCUSSION**

The clinical signs observed in the *S. gallinarum*-infected group on week 5 pi (34.03 ± 2.30%) until the end of the experiment on week 6 pi (52.06 ± 3.20%) Table 1.
layers in this study, which included depression, ruffled feathers, huddling, loss of body weight, drop in egg production, somnolence and greenish-yellow diarrhoea were consistent with findings in previous reports (Shivaprasad, 2000; Freitas Neto et al., 2007; Ezema et al., 2009; Garcia et al., 2010). The 50% mortality in the layers recorded in this study was in the range (10 to 100%) reported previously (Shivaprasad, 1996; Uzzau et al., 2000; Oliveira et al., 2005; Paiva et al., 2009) in chickens. A significant (P<0.05) progressive drop in egg production was observed in the infected layers from 1st week pi and reaching its maximum drop on the 4th week pi. The significant drop in egg production in the S. gallinarum infected group recorded in this study was in the range of 50 to 70% reported previously by Shivaprasad (1997) and Ezema et al. (2009) in laying birds. The drop in egg production, which was recorded by week 4 pi showed that the disease progressed with increased severity. The drop in egg production observed in this study could be due to a number of factors. The factors known to cause drop in egg production in S. enterica serovar gallinarum-infected layers include decrease in feed and water consumption with consequent nutritional imbalances and possible impairment of renal and intestinal calcium absorption due to the infection-induced lesions in these systems (Ezema et al., 2009). The loss of body weight observed in the S. enterica serovar gallinarum infected birds was similarly reported by Ezema et al. (2009) in commercial layers afflicted by fowl typhoid and may be due to decrease in feed consumption which was supported by results of measurement of their feed consumption and intestinal disturbances evidenced by diarrhoea, which could have interfered with nutrients absorption as had been reported by Shah et al. (2013) in S. enterica serovar gallinarum infected broiler chickens.

CONCLUSION AND RECOMMENDATION

This study has shown that experimental infection of layers with S. enterica serovar gallinarum can cause significant reduction in egg production and weight loss. Therefore, those keeping layers should adhere to strict biosecurity measures as means of prevention and control of fowl typhoid in poultry farms, as this disease could lead to decrease in egg production, weight loss and other eggshell abnormalities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Related Journals:

- Clinical Reviews and Opinions
- Journal of Medicinal Plant Research
- African Journal of Pharmacy and Pharmacology
- Journal of Dentistry and Oral Hygiene
- Journal of Parasitology and Vector Biology
- Journal of Pharmacognosy and Phytotherapy
- Journal of Medical Laboratory and Diagnosis
- Journal of Diabetes and Endocrinology
- Medical Practice and Reviews

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