2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 328-337.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj39
PII: S232245682400039-14

The Effects of Acute Oral Toxicity of *Jatropha multifida* and *Hyptis suaveolens* on Zootechnical Parameters in Local Chickens

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ORIGINAL ARTICLE
Received: June 25, 2024
Revised: July 30, 2024
Accepted: August 19, 2024
Published: September 25, 2024

ABSTRACT

The substantial use of medicinal plants in traditional poultry farming is a well-established practice. The present study aimed to determine the median lethal dose (LD_{50}) of ethanolic extracts of *Jatropha multifida* and *Hyptis suaveolens* in local chickens and to assess the effects of these extracts on feed intake, water intake, and average body weight. The methodology followed OECD Directive 223, which involves administering the highest dose of the extract to the chickens and assessing any mortality. Three homogeneous groups of five chickens each were formed for the limit dose test. The control group received distilled water, while batches 1 and 2 received 700 mg of ethanolic extract of *J. multifida* and *H. suaveolens*, respectively. The average body weight of the chickens was 350g ± 20, and the extracts were administered via gavage at a suspension of 2000 mg/kg.bwt of the extract dissolved in water. The results of the phytochemical tests indicated the presence of several chemical compounds known for their therapeutic effects. The productivity of the extract was 2.75 ± 0.19 for *J. multifida* and 3.3 ± 0.27 for *H. suaveolens*. After administration of the suspensions, observation for 14 days revealed no mortality. This finding indicated that the LD_{50} of the utilized ethanolic extracts exceeds the limit dose (2000 mg/kg.bwt). However, feed intake (49 ± 3 > 46±4) and average body weight (436 ± 31 > 388 ± 37) in batch 2 were significantly higher than those in batch 1. Future research should explore the subacute toxicity of *J. multifida* and *H. suaveolens* across various chicken breeds.

Keywords: Body weight, Lethal Dose, Local chicken, Medicinal plant, Mortality, Toxicity

INTRODUCTION

Jatropha multifida of the Euphorbiaceae family and *Hyptis suaveolens* of the Lamiaceae family are medicinal plants in Benin's traditional pharmacopoeia, which are widely used by traditional therapists (Agban et al., 2012; Koudokpon et al., 2015) as traditional recipes in the treatment of several animal diseases. In southern Benin, Sèdégan et al. (2023a) reported that traditional poultry farmers use the leaves and stems of *J. multifida* and *H. suaveolens* in the treatment of fowl pox with inconclusive results, although they have been cited as one of the plants with proven veterinary uses (Adjanohoun et al., 1989; Dassou et al., 2015). The use of the phytotherapeutic heritage cannot, therefore, remain static and be limited solely to the collection of traditional recipes (Fokunang et al., 2011). Enhancing the value of medicinal flora requires phytochemical, pharmacological, and clinical studies (Kouchadé et al., 2017), as well as pharmaco-toxicological studies to rationalise administration doses (Sèdégan et al., 2023b; 2024), with a view to developing improved traditional medicines (Agban et al., 2020). Previous studies have demonstrated the biological effects and pharmacological activities of these medicinal plants in *in vitro* tests on germs responsible for common diseases. For instance, ethanolic and hydroethanolic extracts of *J. multifida* are thought to have very strong inhibitory activity against *C. albicans* (Agban et al., 2012), which justifies the use of the plant in the management of candidiasis in certain African countries such as Nigeria (Adesola and Adetunji, 2007). The bark and leaves are used to treat itchy skin and eczema (Hamza et al., 2006), while the sap has been shown to have strong healing properties (Philippe et al., 2012; Klotoe et al., 2014). However, the fruits can cause severe diarrhoea, dehydration and liver failure (Levin et al., 2000). The sap and aqueous extract of *J. multifida* organs have been studied for their toxicity in Wistar rats (Dougnon et al., 2012; Falodun et al., 2014; Senou et al., 2022). In contrast, no toxicity has been reported in humans or animals from the use of *H. suaveolens* organs (Santos et al., 2007; Sèdégan et al., 2024), whose efficiency in the treatment of candidiasis is proven with its leaves used in food and feed (Kouchadé et al., 2017).

To cite this paper: Sèdégan EBF, Akpo Y, Boko KC, Azalou M, Adoligbé C, Iwaka Ch, Attakpa E, and Alkoiret TI (2024). The Effects of Acute Oral Toxicity of *Jatropha multifida* and *Hyptis suaveolens* on Zootechnical Parameters in Local Chickens. *World Vet. J.,* 14(3): 328-337. DOI: https://dx.doi.org/10.54203/scil.2024.wvj39

In southern Benin, 29.1% of traditional poultry farmers use medicinal plants to treat diseases in their flocks (Sèdégan et al., 2023a), often without guidance on safe doses of administration and the potential toxicity risks induced by the used plants. Furthermore, the toxicity of medicinal plants *(J. multifida* and *H. suaveolens*) in both traditional and modern poultry farming is poorly documented despite their growing use in traditional pharmacopoeia in general, and in traditional poultry farmers in particular (Sèdégan et al., 2023b). As a result, this study aimed to address this gap by determining the median lethal dose (LD_{50}) of ethanolic extracts from *J. multifida* and *H. suaveolens* in local chickens. Specifically, it evaluated the impact of these extracts on feed and water consumption as well as the body weight of the chickens.

MATERIALS AND METHODS

Ethical approval

All authors declare that the experiments were examined and approved by the Ethics Committee of the Communicable Diseases Research Unit at the Applied Biology Research Laboratory of the University of Abomey-Calavi under approval number 002 2023/EPAC/LARBA/URMAT/CE/R. The experiments were carried out in accordance with the ethical standards defined in the 1964 Declaration of Helsinki.

Study setting

The study was conducted at Lumière Agropastorale, a farm breeding site in the locality of Massi, commune of Zogbodomey, department of Zou, Republic of Benin. Figure 1 showed the geolocation of the clinical trial sites and the collection sites for the used plants.

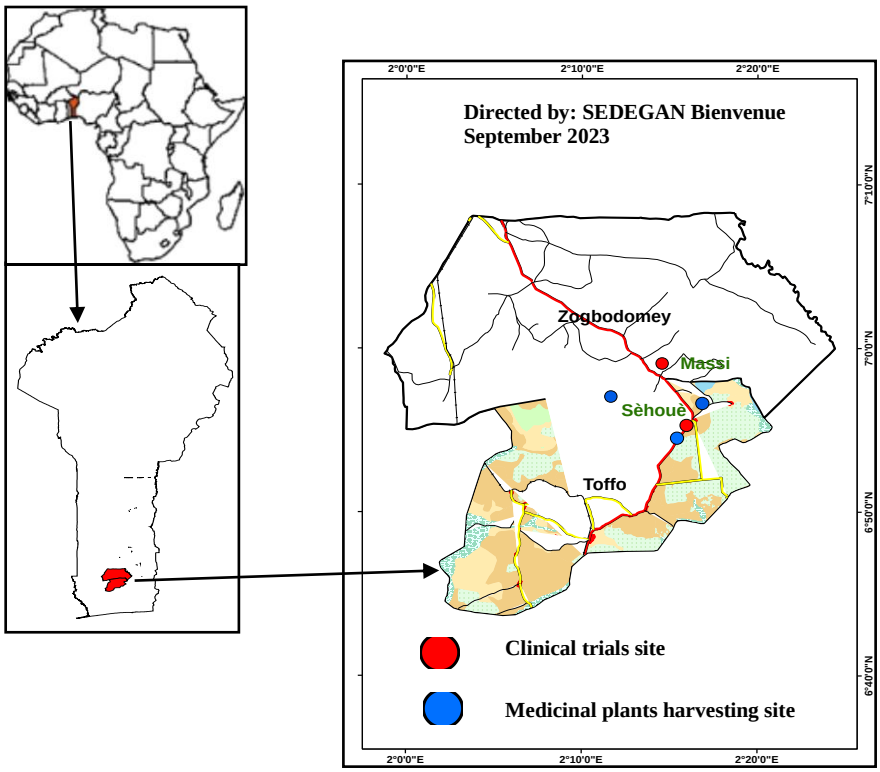

Figure 1. Clinical trial and medicinal plant harvesting sites for evaluating acute oral toxicity of *Jatropha multifida* and *Hyptis suaveolens* in Benin local chickens

Material

Plant materials consisted of the leaves and stems of *J. multifida* and *H. suaveolens*, collected in April 2022 in the Toffo region following an ethnobotanical survey of local traditional practitioners. The plants were identified at the national herbarium of the University of Abomey-Calavi under the identification number YH 775/HNB for *J. multifida* and YH 774/HNB for *H. suaveolens*.

Preparation of extracts and identification of the main chemical groups

Fresh leaves and stems were weighed, washed, disinfected with VIRUNET$^{@}$, rinsed with running water and then air-dried in a dark room at ambient temperature (25°C on average) (Agban et al., 2020). The dried plants were ground using

a RETSCH mill (type SK 100, Verder Scientific, Germany). The two powders obtained were used to prepare the extracts. The dry matter content was determined by weighing the powders using an electronic balance, applying the the following formula.

$$T(MS) = \frac{\text{Masse de feuille sèche}}{\text{Masse de feuille fraîche}} \times 100 \qquad \text{(Formula 1)}$$

To identify the main chemical groups, present in the plants, five grams of dry powder from each plant was boiled in fifty milliliters of water for 15 minutes. The resulting decoctate was filtered to obtain an aqueous extract. Qualitative phytochemical screening was conducted on aqueous extracts using the standard method based on colorimetric and precipitation reactions by different reagents (Adjatin et al., 2013). The phytochemical analyses were carried out at the Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA) of the Ecole Polytechnique d'Abomey-Calavi (EPAC/UAC). The presence or absence of these secondary metabolites was indicated by the presence of legend (+) or its absence (-). To prepare the ethanolic extract, 200 grams of each powder was placed in 500 milliliters of 95% ethanol supplied by the Biochemistry Laboratory of the Faculty of Health Sciences (FSS) at the University of Abomey-Calavi (UAC). The mixture was stirred for 15 to 20 minutes and then allowed to stand for 24 hours to facilitate the extraction the chlorophyll and its derivatives (Agban et al., 2012). The mixture was filtered and the extraction process was repeated on the residue for three more consecutive times at 24-hour intervals. Each obtained extract was evaporated using a rotavapor at the Pharmacology and Essential Oils Laboratory of the Institute of Advanced Bio-Medical Sciences (ISBA-FAST/UAC) in order to obtain dry extracts. The productivity of each extract was determined by the following formula.

$$R = \frac{\text{Dry extract mass}}{\text{Dry sheet mass}} \times 100 \qquad \text{(Formula 2)}$$

Experimental design

Young local breed chickens (*Gallus gallus domesticus*), aged between 8 and 10 weeks and weighing 350±70 g, were used. The chickens were obtained after the natural hatching of chicks reared in a building specially fitted out for the purpose and complying with chick hygiene standards. They were fed *ad libitum*, with a commercial feed, produced and certified in Benin by Groupe Veto Services (GVS). The feed consisted of 2830 Kcal/kg metabolizable energy, 4.9% crude fat, and 19% crude protein. The chickens were given distilled water to drink. Prevention against New Castle disease was carried out by subcutaneous injection with the inactivated ITA NEW ND$^{@}$ vaccine in oily emulsion, manufactured and marketed by LAPROVET, under Marketing Authorization N°UEMOA/V/00021/2014/04/30. For the acute toxicity test, three batches of five chickens were formed and housed in three different cages, each with a surface area of 1m^2 laced. The groups were treated as follows:

- Control Group: consisting of five chickens given distilled water
- Treatment 1: consisting of five chickens, each administered 700 mg of ethanolic extract of *J. multifida*
- Treatment 2: consisting of five chickens, each given 700 mg of ethanolic extract of *H. suaveolens*

The trial which lasted 14 days was conducted at room temperature (24°C to 26°C) with sufficient ventilation allowing at least ten air changes per hour. The chickens were exposed to 12-hour light cycles daily.

Acute oral toxicity test (Lethal Dose 50) and limit dose test

The acute oral toxicity test was conducted in accordance with OECD (2016), Guideline 223, for the testing of chemicals. This *in vivo* test evaluated the toxicity of ethanolic extracts from the leaves and stems of *J. multifida* and *H. suaveolens* in chickens. The extracts were weighed, suspended, and administered via gavage. The chickens were fasted, with no access to feed or water, for 12 to 15 hours during overnight prior to the administration of the suspensions (Zann, 1996). Feed and water were reintroduced two hours post-administration. The trial involved administering a single, high dose of ethanolic extract (2000 mg/kg.bwt) to each chicken in the respective treatment groups of *J. multifida* and *H. suaveolens*. The administered volume remained constant in relation to the body weight of the chickens, not exceeding 10 ml/kg body weight (Zann, 1996). The control group received 2 ml of distilled water. The test was conducted at ambient temperature and humidity, with sufficient ventilation to allow at least ten air changes per hour. Administration of medications was avoided for 14 days before and after the test (OECD 223, 2016). Average weight of the chickens and average feed consumption were calculated over a period of 14 days as indices for assessing the acute toxicity of the extracts (Pissang et al., 2018).

Observation of test chicks and measurement of study parameters

Chickens were observed continuously for the first two hours following the administration of the extracts. Observations focused on signs such as aggression, mobility, vigilance, droppings, regurgitation, abnormal behavior, and time to death. Three more observations were made at intervals during the daylight hours on the first day. From the second day onwards, two observations during the day were required. The body weight of the chickens was recorded

using a precision electronic balance before the administration of each extract on days 3, 7, and 14 to track weight variations. Daily feed and water consumptions were also measured throughout the 14-day trial period.

Statistical analysis

Data were compiled into an Excel database, and statistical analyses were performed using R software version 3.6.2, 2019. Means were calculated using the Summary procedure, and relative frequencies were assessed using the t-test procedure. Confidence intervals (CI) for the percentages were calculated using the following formula.

$$CI = 1{,}96\sqrt{\frac{[p(1-p)]}{N}} \qquad \text{(Formula 3)}$$

where p was the observed percentage and N was the total number of participants. The results from the experimental groups were compared with the control group treated with distilled water, as well as between each experimental group. In order to determine the presence of significant differences among the groups, p values less than 0.05 were considered significant.

RESULTS

Ethanolic extraction yield and phytochemical compositions

The obtained dry matter content (TMS) was 16±1.3% for *J. multifida* and 20±2.3% for *H. suaveolens*. The productivity of ethanolic extract was 2.75 ± 0.19 % for *J. multifida* and 3.3 ± 0.27 % for *H. suaveolens*. Table 1 presented; the results of phytochemical screening carried out on the aqueous extracts of these plants. As was seen, the harvested leaves and stems were rich in secondary metabolites, including alkaloids, catechic tannins, flavonoids, anthocyanins, mucilages, and coumarin quinones. Only *J. multifida* contained leuco-anthocyanins. Neither plant contained gall tannins, reducing compounds, saponosides, cyanogenic derivatives, and anthraquinones.

Table1. Effects of phytochemical test results of *Jatropha multifida* and *Hyptis suaveolens* on zootechnical parameters of local chickens

Chemical groups	***J. multifida***	***H. suaveolens***
Alkaloids	+	+
Gall tannins	-	-
Catechin tannins	+	+
Flavonoids	+	+
Anthocyanins	+	+
Leuco-anthocyanins	+	-
Reducing compounds	-	-
Mucilages	+	+
Saponosides	-	-
Cyanogenic derivatives	-	-
Anthraquinones	-	-
Coumarin quinones	+	+

+ Present; - Absent

Toxicological study

Administering a single limit dose of 2000 mg/kg.bwt of the ethanolic extract from *J. multifida* and *H. suaveolens* did not cause any mortality in the treated chickens (treatment group 1 and treatment group 2) during the fourteen-day observation period. Except for some signs of distress observed in chickens from batch 1 on the first day, no signs of aggression, regurgitation, or abnormal behavior were observed in either treatment group during the experiment.

Effect of ethanolic extract of *Jatropha multifida* on feed consumption, water consumption, and body weight

Figure 2 illustrated the variations in feed consumption, water consumption, and average body weight of chickens fed with *J. multifida* ethanolic extract. The average feed (54 ± 6.43 g) and water (88.5 ± 9.69 ml) consumption in the control treatment was higher than the average feed (46.3 ± 4.04 g) and water (76.7 ± 8.12 ml) consumption in Treatment 1. There was a remarkable drop in feed and water consumption in treatment 1 over the 14 days of observation. The average body weight of the control group (422 ± 55.5 g) was higher than that of Treatment 1 (388 ± 37 g) from the third day of observation onwards. In addition, the mean difference in feed consumption (FC) between the control group and Treatment 1 was 7.78 g with a 95% confidence interval of [4.52 g; 11 g]. Also, the t-test confirmed a significant difference ($p = 0.001$) in feed consumption between the control group and Treatment 1. Similarly, the mean difference in water consumption between the control group and Treatment 1 was 11.7 ml with a 95% confidence interval of [3.89 ml;

19.6 ml], indicating a significant difference in water consumption of the two compared groups (p = 0.001). While the average body weights of the chickens increased during the observation period, the mean difference in body weight between the control group and Treatment 1 was 33.7 g with a 95% confidence interval of [-20.7 g ; 87.7 g]. However, no significant difference in average weight (p = 0.14) was found in treatment 1 compared to the control group on the fourteenth day.

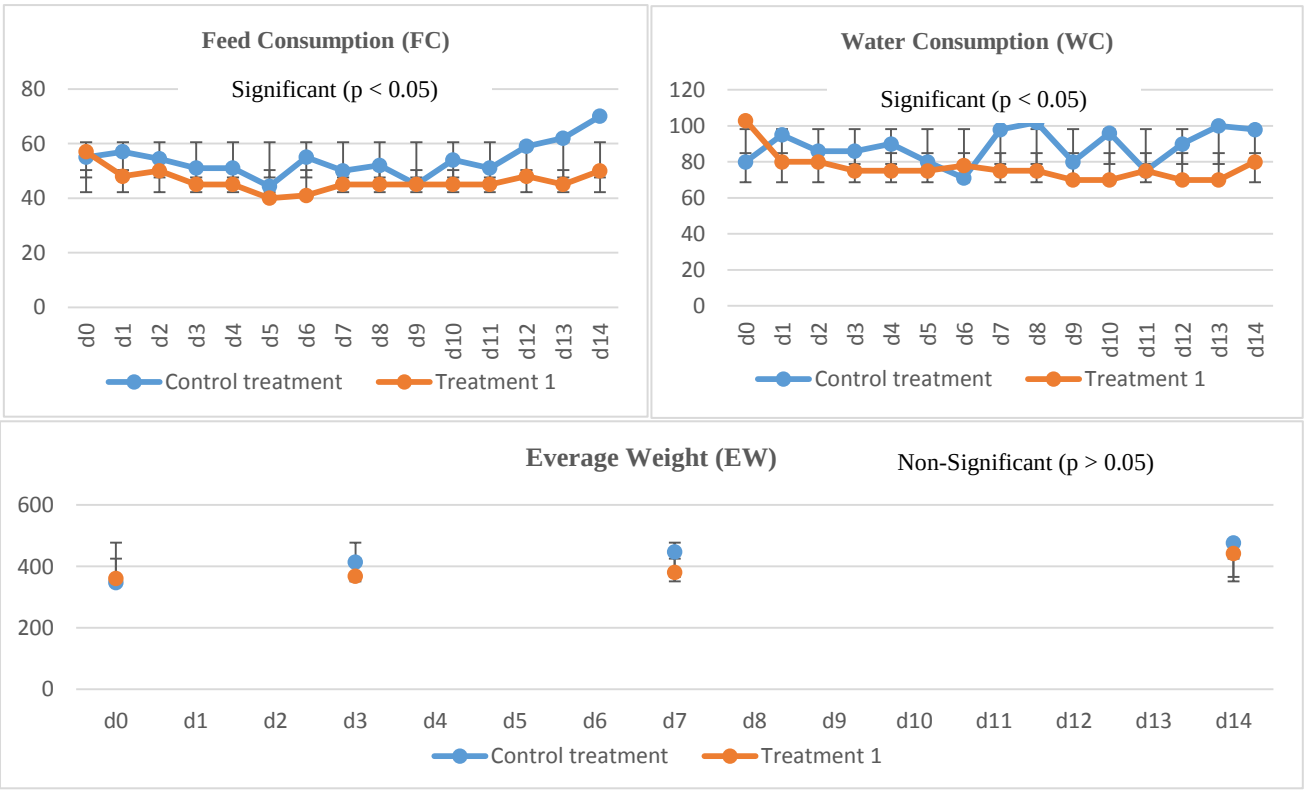

Figure 2. Effects of ethanolic extract of *J.multifida* on feed and water consumption and average weight in treatment 1 of local chicken

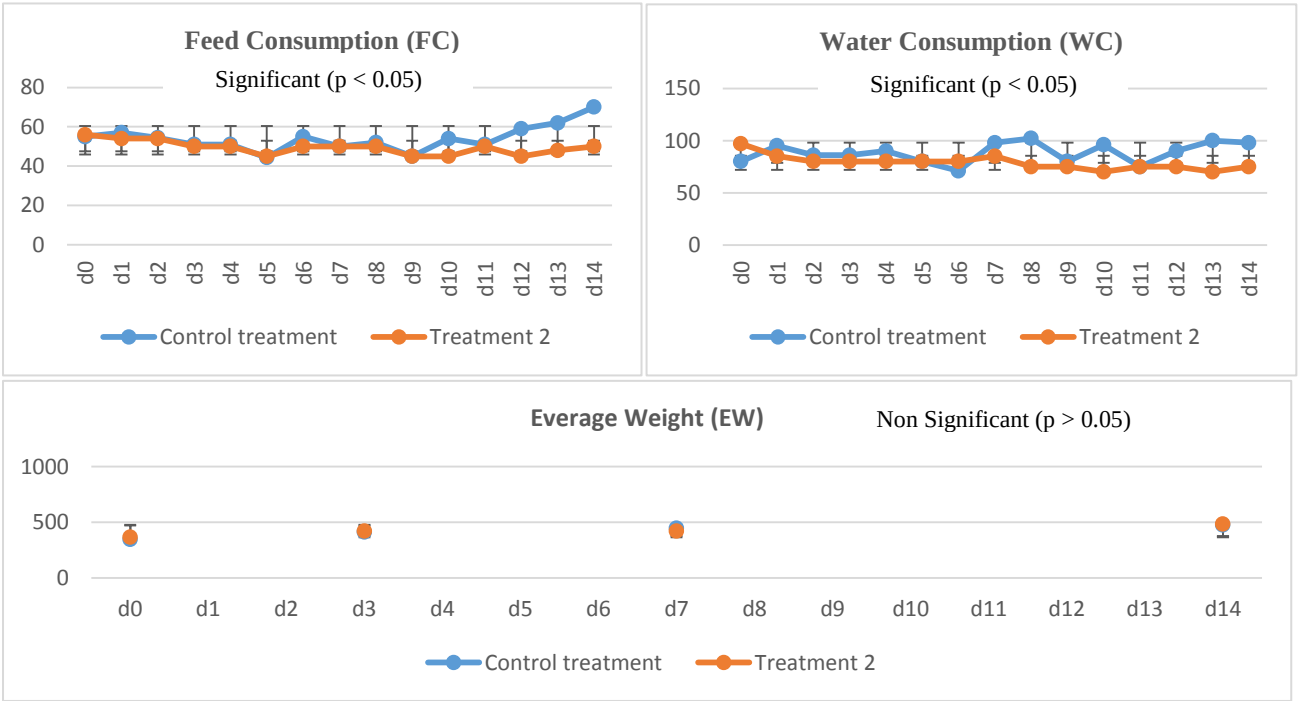

Figure 3. Effects of ethanolic extract of *H. suaveolens* on feed and water consumption and average weight in treatment 2 of local chicken

Effect of ethanolic extract of *Hyptis suaveolens* on feed consumption, water consumption and body weight

Figure 3 illustrated the variations in feed consumption, water consumption and average body weight of the chickens administered the ethanolic extract of *H. suaveolens*. The control group exhibited higher average feed consumption (54 ± 6.43 g) and water intake (88.5 ± 9.7 ml) compared to Treatment 2, which recorded 49.5 ± 3.5 g for feed and 78.8 ± 6.8 g for water consumption of. In Treatment 2, there was a drop in feed consumption from the tenth day, while a slight decrease in water consumption began in this group from the seventh day. The mean difference in feed consumption between Treatment 2 and the control group was 4.6 g with a 95% confidence interval of [0.9 g; 8.2 g]. Thus, the t-test established a significant difference (p = 0,002) in feed consumption between the two groups. Similarly, the mean difference in water consumption was 9.7 ml, with a 95% confidence interval of [2.3 ml; 17.1 ml], also showing a significant difference (p = 0.001) between Treatment 2 and the control group on the fourteenth day. Whereas the average weights on the third and seventh days were approximately equal, the average weight of Treatment 2 (436 ± 31.6 g) surpassed that of the control group (422 ± 55.5 g) on the fourteenth day.

The average weight difference between Treatment 2 and the control group was -14.6 g with a 95% confidence interval of [-78.9 g; 49.7 g]. Despite these variations, no significant difference in average weight was detected between the treatment groups (p = 0.012).

Comparison of parameters studied between experimental batches

Table 2 presented the average values for feed and water consumption, average weights, confidence intervals, and p-values comparing treatment groups 1 and 2. The data indicated that chickens in Treatment 2 performed better than those in Treatment 1 in terms of feed consumption, water consumption, and average weight. However, a significant difference was observed in feed consumption and average weight between Treatment groups 1 and 2, suggesting that the ethanolic extract of *J. multifida* may have reduced the appetite of chickens in Treatment 1, leading to decreased feed intake and, consequently, lower body weight. In contrast, the ethanolic extract of *H. suaveolens* may have enhanced the appetite of chickens in Treatment 2, resulting in increased feed and water consumption and subsequent weight gain.

Table 2. Effects of *Jatropha multifida* and *Hyptis suaveolens* on feeding and body weight of local chickens aged 14 days

	Treatment 1	Treatment 2	Dif moy	CI1	CI2	p-value	Décision
FC (g)	46.3 ± 4.04	49.5 ± 3.46	-3.2	-5	-1.39	0.001	S
WC (ml)	76.7 ± 8.12	78.8 ± 6.77	-2.06	-4.41	0.27	0.079	NS
AW(g)	388 ± 37.1	436 ± 31.6	-48.3	-61.7	-34.8	0.001	S

FC: Feed consumption; WC: Water consumption; AW: Average weight; Dif moy: Average difference between treatment 1 and treatment 2; CI: Confidence interval; S: Significant; NS: Not significant. Treatment 1 : 700 mg of ethanolic extract of *J. multifida*, Treatment 2 : 700 mg of ethanolic extract of *H. suaveolens*

DISCUSSION

This study represented a pioneering evaluation of the acute oral toxicity of the ethanolic extracts from *Jatropha multifida* and *Hyptis suaveolens* in local breed chickens. The plant materials selected for the present study were chosen due to their common traditional use in treating infectious diseases such as candidiasis and enteritis (Dougnon et al., 2012; Klotoe et al., 2014; Kouchadé et al., 2017; Agban et al., 2020), Additionally, the two plants were the most commonly mentioned plants by traditional poultry farmers for treating fowl pox (Sèdégan et al., 2023a). Given the increasing use of medicinal plants for managing certain infectious diseases (candidiases and enteritis) and parasitic diseases (Intestinal parasitosis and ectoparasitosis) (Iwaka et al., 2022a; 2022b; Sèdégan et al., 2023b), ongoing evaluation of therapeutic doses and potential side effects induced by these plants remains crucial.

Phytochemical screening of the powdered leaves and stems of *J. multifida* and *H. suaveolens* revealed the presence of several secondary metabolites known for their therapeutic and toxic effects. For *J. multifida*, the results corroborated those of Aiyelaagbe et al. (2008), Biswanath et al. (2008), Bruneton (2009), Agban et al. (2012; 2020), and Senou et al. (2022), which confirmed the presence of alkaloids, tannins, and flavonoids in *J. multifida* leaves and stems. In contrast to present findings, Senou et al. (2022) confirmed the presence of gall tannins and saponosides in *J. multifida* leaves, but rejected the presence of quinone derivatives (coumarin quinones), which were toxic compounds (Kollin and Uziel 2006). For *H. suaveolens*, the results were similar to those of Koné (2009), Traoré (2016), and corroborated those reported by Kouchadé et al. (2017) and Soumahoro et al. (2020), who confirmed the presence of chemical compounds such as gall tannins, leuco-anthocyanins, and anthraquinones in *H suaveolens*. In addition, these secondary metabolites contribute to a range of beneficial properties, including antiplasmodic, antioxidant, antibacterial, antifungal, antidiabetic,

antirheumatic, antisuporific, antiinflammatory, and antiseptic effects helping in burns and multiple skin complications (Grassi et al., 2006). However, the absence or non-existence of secondary metabolites in a plant could be due to factors such as the place and season of harvest, the handling conditions (quality of reagents used) and the professionalism of the operator, the variation in genetic make-up, the weather conditions, the part of the plant studied, its good preservation (water content in the dry state), its purity (ash content), and the employed extraction method (Koné 2009; Akhtar and Ihsan-ul-Haq, 2018).

The absence of mortality after the administration of ethanolic extracts suggests that the Lethal Dose 50 (LD_{50}) for ethanolic extracts of *J. multifida* and *H. suaveolens* exceeds the limit dose of 2,000 mg/Kg.bwt. This finding was in line with those of earlier studies on *J. multifida* extract (Agban et al., 2020; Senou et al., 2022) and *H. suaveolens* extract (Attawish et al., 2005; Santos et al., 2007). However, *Jatropha multifida* extract could be considered toxic in that it caused a reduction in feed consumption and consequently in average weight in chickens, in contrast to an increase in feed consumption associated with a loss of average weight (Agban et al., 2020). These results indicated that *J. multifida* extract was a cause of the drop in body weight of chickens in Treatment 1. This supported the claim that *J. multifida* intoxication was similar to organophosphate intoxication, which caused muscle paralysis in animal species (Kollin and Uziel, 2006). However, the use of *H. suaveolens* as an aperitif in traditional medicine (Moreira et al., 2010) justified the significant increase in feed consumption and average weight of chickens in Treatment 2 compared with chickens in Treatment 1. It was therefore certain that the toxicity of *H. suaveolens* extract has never been proved (Attawish et al., 2005; Santos et al., 2007; Traoré, 2009; Ansm, 2021; Sèdégan et al., 2024). Overall, this study indicated that, at a dose of 2,000 mg/Kg.bwt, the ethanolic extract of *H. suaveolens* was preferable to that of *J. multifida* for *in vivo* use in local chickens for all purposes.

CONCLUSION

The results of this study demonstrated the use of ethanolic extracts of *Jatropha multifida* and *Hyptis suaveolens* at a dose limit of 2,000 mg/kg.bwt would not result in mortality among chickens. This indicated that the Lethal Dose 50 (LD_{50}) for both of these plant extracts taken from Benin's traditional pharmacopoeia was higher than the limited dose. Notwithstanding, whereas the *J. multifida* extract had a negative impact on feed consumption and the average body weight of the chickens, thereby reflecting its toxicity, the *H. suaveolens* extract improved feed consumption and the average body weight of the chickens. These findings highlight the need for further research to explore the subacute toxicity as well as potential hematological and biochemical disturbances induced by these plant extracts in both local and commercial breeds of chickens.

DECLARATIONS

Funding

This study received no financial support.

Availability of data and materials

All data from the current study are available in this article.

Authors' contributions

Enagnon Bienvenue Florent Sèdégan, Yao Akpo, and Kadoéito Cyrille Boko designed and planned the study, supervised data collection, and analyzed the data. Enagnon Bienvenue Florent Sèdégan, Maximilien Azalou, and Christophe Iwaka collected data and drafted the first version of the manuscript. Camus Adoligbé, Christophe Iwaka, and Enagnon Bienvenue Florent Sèdégan wrote the final version of the document and carried out the critical review. Eloi Attakpa, Ibrahim Alkoiret Traoré, Yao Akpo, and Kadoéito Cyrille Boko revised the document. All authors read and approved the final version of the article.

Competing interests

The authors declare that they have no conflict of interest.

Ethical considerations

The authors took ethical concerns and farmers' consent into account prior to the surveys. This article was originally written without copying from other articles.

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