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Original article

Influence of Sex on Food Intake and Body Weight in Wistar Rats in a Repeated Administration of *Bridelia ferruginea* Benth. Aqueous Extract

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Abstract

Background and objectives: *Bridelia ferruginea* Benth. , a medicinal plant widely used in traditional medicine in Ivory Coast to treat many pathologies such as diabetes mellitus, is usually taken during a short or long period and its bark used as vegetables contains anti-nutritional factors.. The aim of the present study was to assess the effects of the plant extract on food consumption and body weight in Wistar female and male rats. **Methods:** The aqueous extract was prepared using the powder (100 g) of the stem bark of *Bridelia ferruginea* macerated in distilled water (1 Liter) during 24 hours. For the present study, thirty-two rats were randomly divided into four groups of 8 animals each (8; 4/sex). The experiment was performed in 90 days, according the protocol described by the Organization for Economic Cooperation and Development guideline 408 for testing chemicals in sub chronic toxicity study in rodents. Food consumption and animal body weight were measured each day during the experiment period. **Results:** The food consumption decreased in female group compared to the male rats receiving the same treatment ($p<0.05$). During three months of measurement, decrease of food consumption was observed in all female rat groups (control and treated, $p<0.001$) compared to male groups having the same treatment. Weekly measures of body weight showed decrease in female rats compared to male groups receiving the same dose. **Conclusion:** *Bridelia ferruginea* caused lower body weight and food consumption in female rats compared to the male that suggested female Wistar rats were more sensitive than male.

Keywords: aqueous extract; body weight; *Bridelia ferruginea*; food consumption; Wistar rat

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Introduction

Bridelia ferruginea Benth. (Euphorbiaceae) is a woody shrub that grows in the Savannah or rain forests of Africa and has traditionally been used to treat many diseases such as diabetes and arthritis [1-3]. It is known that the use of medicinal plants is not without danger to health and poses problems like toxicity, overdose and side effects [4]. However, it is thought that herbs are considered 'Natural' and thus are free from risk [5]. In literature surveys, *Bridelia ferruginea* is known traditionally in Ivory Coast as a purgative, diuretic, antidiabetic, aphrodisiac and

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anti-gonococcal agent [6,7]. Previous studies have demonstrated that the aqueous extract of *Bridelia ferruginea* possesses diuretic and vasorelaxant activities [8,9] and do not disrupt the markers of renal function (urea and creatinine) in rats after one day treatment [10]. The phytochemical screening of *Bridelia ferruginea* aqueous extract showed the presence of quinones, gallic and catechic tannins, alkaloids, sterols, polyterpenes, polyphenols, reducing compounds (sugars), flavonoids and saponosides [11]. In a sub chronic study previously carried out in rats from February 2016 to May 2016, where the comparison was made in each group, the findings showed that the aqueous stem-bark extract of *Bridelia ferruginea* used in the doses of 100, 200 and 400 mg/kg, did not cause mortality in both sex of Wistar rats [12]. According to these authors, the plant didn't disturb parameters like lipid profile, liver and kidney functions in male or female rats after the period of the experiment. Some authors also shown that *Bridelia ferruginea* possessed antinutrients substances [13]. In view of the non-toxicity of the aqueous extract of *Bridelia ferruginea* in rats during the sub chronic toxicity study and the presence of antinutrients in this plant, the aim of the present work was to know the effects of this extract on food consumption and body weight, when comparing male to female.

Material and Methods

Ethical considerations

The animal experiments were carried out from February 2016 to May 2016, following the guideline of animal use of the Department of Pharmacology of JSS college of Pharmacy Ooty (Tamil Nadu, India), approved by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethics Committee (IAEC) Chennai, Government of India, under the number JSSCP/IAEC/PH.COLOGY/PH.D/01/2015-16.

Plant material

Bridelia ferruginea stem-bark was obtained in Abidjan (Yopougon), Ivory Coast. These stem barks were identified and authenticated by a botanist, Mr. Assi Jean, of the National Floristic Center of Félix Houphouët-Boigny University (Ivory Coast). Voucher specimen (herbarium No. 17148, 2015) has been deposited in this center.

Preparation of extract

The extract was prepared in Swiss Center of scientific Research (Abidjan, Ivory coast). Stem barks were dried in the shade at room temperature around 25°C and powdered with a micro-crusher. Hundred (100) g of powder was macerated for 24 h in a 1 L of distilled water using a magnetic stirrer. The supernatant was filtered with Whatman No 1 filter paper and it was evaporated using a rotating evaporator (Buchi, France) water to reduce solvent before lyophilization process. The concentrate was frozen at -30°C before and lyophilized using a lyophilisator (Telstar, Spain). A brown colored powder was obtained.

Animals

Thirty-two Wistar rats (male or female) weighing 150-250 g, from the animal house of JSS College of Pharmacy, Udhagamandalam, Tamil Nadu (India) were used in the study. The animal house was well ventilated with room temperature around 25 ± 1°C and animals had 12 h day and night schedule. The animals were fed with standard feed for rat and received water ad libitum.

Sub-chronic toxicity study

The sub-chronic toxicity was carried out according to the Organization for Economic Cooperation and Development guidelines (OECD) Guideline-408, adopted on 2st September 1998. This study is part of findings of sub chronic toxicity conducted by Nene Bi et al. [12] at the department of pharmacology, JSS College of Pharmacy Ooty (Tamil Nadu, India) from February 2016 to May 2016. The animals were randomly divided into four groups of 8 animals each (8; 4/sex). Thus, to evaluate the effects of the aqueous extract of *Bridelia ferruginea*, rats of both sexes were distributed as follows:

Group I: Male (M1) and female (F1) control, received only distilled water orally (10 mL/kg of body weight);

Group II: Treated male (M2) and female (F2) rats received 100 mg/kg of *Bridelia ferruginea* aqueous extract;

Group III: Treated male (M3) and female (F3) rats received 200 mg/kg of *Bridelia ferruginea* aqueous extract;

Group IV: Treated male (M4) and female (F4) rats received 400 mg/kg of *Bridelia ferruginea* aqueous extract.

The control and all treated rats received the appropriate test solution daily. The body weight and feed intake were measured weekly throughout the study period (90 days).

Statistical analysis

For statistical analysis, we used the R Core Team [14], a language and environment for statistical computing of R Foundation for Statistical Computing, Vienna, Austria. The experimental results have been expressed as the mean ± SD. Significant differences were determined using Tukey's one-way multiple comparison tests and differences were considered significant at $p< 0.05$.

Results and Discussion

It is known that taking chemicals such as herbal remedies or modern medicines often poses side effects [4]. The present study was carried out to evaluate the effects of an aqueous extract of *Bridelia ferruginea* on food consumption and body weight in Wistar rats (female and male).

In this study, food intake was measured every day during 13 weeks (figure 1). Food consumption in female rats' groups was lower than male groups. After the first week of treatment (figure 1A), a significant difference was observed in food intake in female rats treated respectively, with 200 (F1-F3, $p = 0.0319$) and 400 mg/kg (F1-F4, $p = 0.0348$) of the aqueous extract of *Bridelia ferruginea* (Euphorbiaceae), compared to the female control group. Decrease in food consumption was observed in female rats treated with 200 (12.02 ± 0.92 g/rat) and 400 mg/kg (12.09 ± 2.11 g/rat) compared to female control group (19.29 ± 8.48 g/rat). At the same time, there was no significant difference ($p>0.05$) in food intake between female and male rats.

Figure 1B shows the effects of the aqueous extract in rats, after 2 weeks of daily administration. There was a significant decrease (M3-F3, $p<0.001$) in food consumption in females compared to male rats that received 200 mg/kg of the aqueous extract. After 3 weeks (figure 1C), there was a decrease of food consumption in female treated respectively, with 200 and 400 mg/kg compared to the male rats at the same doses. This diminution observed in the two doses was significant (M3-F3, $p=0.0326$), (M4-F4, $p=0.0161$). In 4 weeks of daily treatment (figure 1D), there was a difference in food consumption between female rats and males treated respectively with 100 and 200 mg/kg of the extract. At the dose of 100 mg/kg, a significant decrease (M2-F2, $p = 0.01828$) in food intake was observed in females compared to the male which received the same dose. The dose of 200 mg/kg caused decrease (M3-F3, $p<0.001$) in food consumption in females compared to male rats treated with the same dose. Figure 1F shows the effects of the extract in rats after six weeks of daily treatment. The findings obtained in the control group of female and male rat, showed significant decrease (M1-F1, $p=0.02962$) of food consumption in females (13.85±2.55 g/rat) compared to the males (16.33±1.87 g/rat). At the dose of 100 mg/kg, food intake was significantly reduced in the female group compared to males (M2-F2, $p<0.001$). After 7 weeks of daily administration of *Bridelia ferruginea* aqueous extract, food consumption was significantly reduced (M1-F1, M2-F2, M3-F3, M4-F4, $p<0.001$) in all female groups compared to male rats having received the same treatments (figure 1G). In figure 1H (8 weeks), the food consumed was decreased ($p < 0.001$) in control and treated female groups compared to control and treated male groups with the same doses. After 9 weeks of daily administration of the aqueous extract of *Bridelia ferruginea*, food consumption in control and treated female groups significantly reduced ($p< 0.001$) compared to male rats receiving the same treatments (figure 1I). Figure 1J shows the effects of the extract after 10 weeks of daily treatment in rats. These results showed the decrease ($p<0.001$) of food consumption in female control rats' and those treated with 100 and 400 mg/kg compared to male groups having the same treatment. In 11 weeks, the extract caused a significant decrease in food consumption in the female control (M1-F1, $p< 0.001$) and those which were treated at the doses of 100 (M2-F2, $p < 0.001$), 200 (M3-F3, $p< 0.001$) and 400 (M4-F4, $p < 0.001$) mg/kg bw compared to male rats receiving the same treatments (figure 1K). Figure 1L showed, after twelve (12) weeks daily treatment in rats of the effects of the aqueous extract of *Bridelia ferruginea*. In female control (M1-F1, $p < 0.001$) and those treated 100 (M2-F2, $p < 0.001$), 200 (M3-F3, $p < 0.01$), 400 (M4-F4, $p < 0.001$) mg/kg bw, the findings indicated the decrease in food consumption compared to male rats having the same treatment. After thirteen (13) weeks of treatment, a significant decrease ($p< 0.001$) was observed in food consumption in all female rats' groups (control and treated) compared to male

groups receiving the same treatment (figure 1M). Figure 2 shows the effects of *B. ferruginea* aqueous extract on food intake in rats after three months treatment. In all female rat groups (control and treated), food consumption significantly decreased ($p<0.001$) compared to male groups having the same treatment.

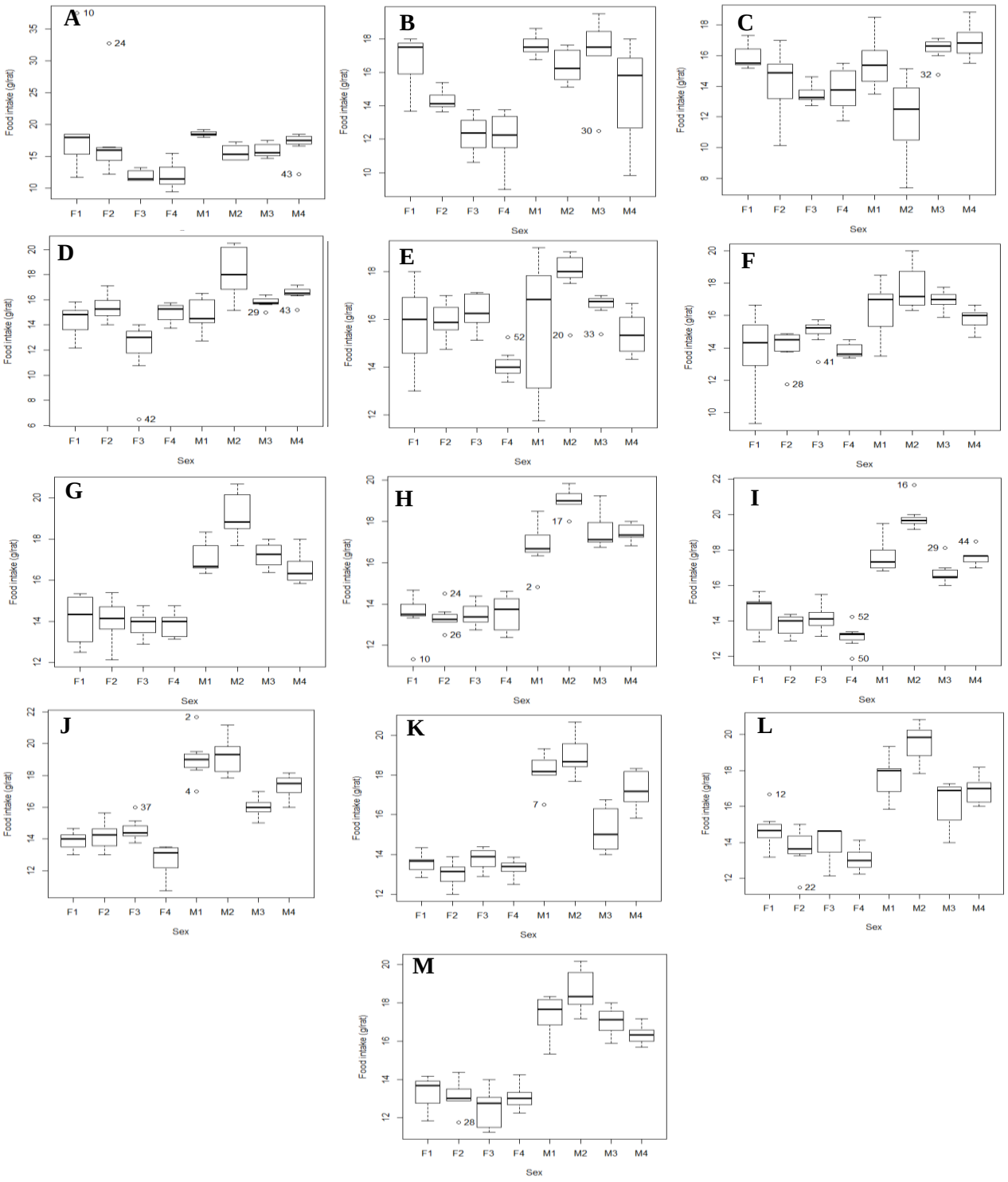

Figure 1. Effects of *Bridelia ferruginea* aqueous extract on food intake in male and female rats in weekly measurement after daily oral administration; A: Week1; B: Week2; C: Week3; D: Week4; E: Week5; F: Week6; G: Week7; H: Week8; I: Week9; J: Week10; K: Week11; L: Week12; M: Week13. F1: Female control; F2: 100mg/kg; F3: 200mg/kg; F4: 400mg/kg; M1: Male control; M2: 100 mg/kg; M3: 200 mg/kg; M4: 400 mg/kg. Values are expressed as mean ± SD; M: Male (n= 4); F: Female (n=4).

When the female rats were compared each other, a significant decreased ($p<0.001$) of food consumption in those that received 200 and 400 mg/kg were observed. This could be due to the sensitivity to chemicals in female rats treated with the aqueous extract of *Bridelia ferruginea* Benth., by reducing food intake.The gender difference in dietary toxicokinetic was demonstrated by some authors who showed that the clearance of 2,4-Dichlorophenoxyacetic acid, mediated by organic anion transporter 1 was depending on sex [15,16]. The decrease of food consumption in female groups could also be linked to the chemicals found in the extract of *B. ferruginea*. Similar findings were obtained by Maronpot et al. [17], using myricitrin in Sprague-Dawley rats. The variation of body weight and food consumption in male and female Wistar rats groups, can be linked to a basic difference in physiology and body composition in both sexes, according to the authors who demonstrated that the absorption of some drugs may be affected by gastric acid secretion and gastric emptying which are lower in women [18,19]. Some authors showed that estrogen reduces food consumption in female rats [20].

Regarding food consumption, an assessment of the effects on the body weight of *B. ferruginea* aqueous extract was carried out on female and male Wistar rats. Animal body weight was not statistically different between groups before the beginning of the experiments at week 0 (W0) (table 1).

The mean absolute response at each dose level of body weight, is shown in figure 3. The body weight was measured every day. Daily administration of the extract did not cause any changes ($p>0.05$) of body weight in rats between male and female compared each to other after 7 weeks (figure 3A-H). From 8^{th} to 12^{th} weeks, significant changes of body weight were observed in male rats and female treated with *B. ferruginea* aqueous extract at the dose of 200 mg/kg ($p<0.05$, figure 3I-M). In figure 3I (8 weeks), the body weight in female and male rats was statistically different (M3-F3, p=0.0421) at the dose of 200 mg/kg. At this dose, the body weight of females (215.75 ± 7.41 g) were lower than that of males (278.75 ± 15.20 g). Figure 3J (9 weeks) at the dose of 200 mg/kg of *Bridelia ferruginea* showed that the body weight in male rats (279.5 ± 14.08 g) increased compared to those of female (219 ± 5.42 g). This difference was significant (M3-F3, p=0.0408). After 10 weeks of treatment with the aqueous extract of *B. ferruginea* in rats, (figure 3K) the changes in body weight between male rats (281.25 ± 9.36 g) and female (218.75 ± 6.95 g) were significant (M3-F3, p=0.0390).

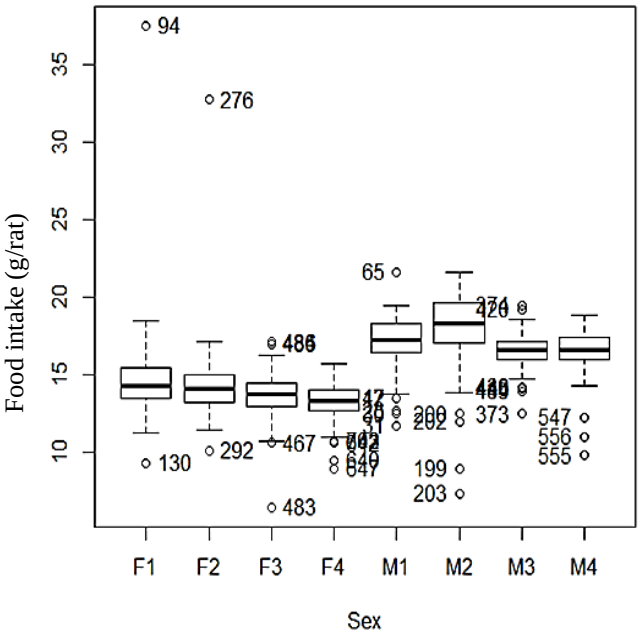

Figure 2. Effects of *Bridelia ferruginea* aqueous extract on average food intake in male and female rats after 13 weeks of daily oral treatment. F1: Female control; F2: 100 mg/kg; F3: 200 mg/kg; F4: 400 mg/kg; M1: Male control; M2: 100 mg/kg; M3: 200 mg/kg; M4: 400 mg/kg. Values are expressed as mean ± SD; M: Male (n= 4); F: Female (n= 4)

In figure 3L, the variations in body weight in the dose of 200 mg/kg bw in male (282.75 ± 12.84 g) and female (220.25 ± 6.95 g) were statistically different (M3-F3, p = 0.0454). In 12 weeks (Figure 3M), there was the significant difference (M3-F3, p = 0.0324) between body weight in male and female rats after daily administration of the aqueous extract of Bridelia ferruginea at 200 mg/kg bw, with the increase of body weight in male (288.5±16.58 g) rats compared to the female (220.25 ± 9.11 g). After 13 weeks (figure 3N), body weight changed significantly in the control group (M1-F1, p = 0.03758) and in the of rats treated with 200 mg/kg bw (M3-F3, p = 0.00459) of *Bridelia ferruginea*. In both cases, the body weight of female rats was lower than those of males. Figure 4 shows the effects of the extract in the body weight of female and male Wistar rats treated daily during 3 months.

Table 1. Animal body weight before the beginning of the experiments at week 0 (W0)

Groups	Body weight (g)		p value
	Male	Female	
Group I (control)	188 ± 31.97	199 ± 27.86	> 0.05
Group II (100 mg/kg bw)	188.50 ± 11.73	199 ± 18.81	
Group III (200 mg/kg bw)	186 ± 20.26	215.75 ± 8.26	
Group IV (400 mg/kg bw)	238 ± 65.85	208.75 ± 28.30	

Data are presented as mean ± SD; M: Male (n= 4); F: Female (n= 4). There was no significant difference between the body weight in male and female rats ($p > 0.05$).

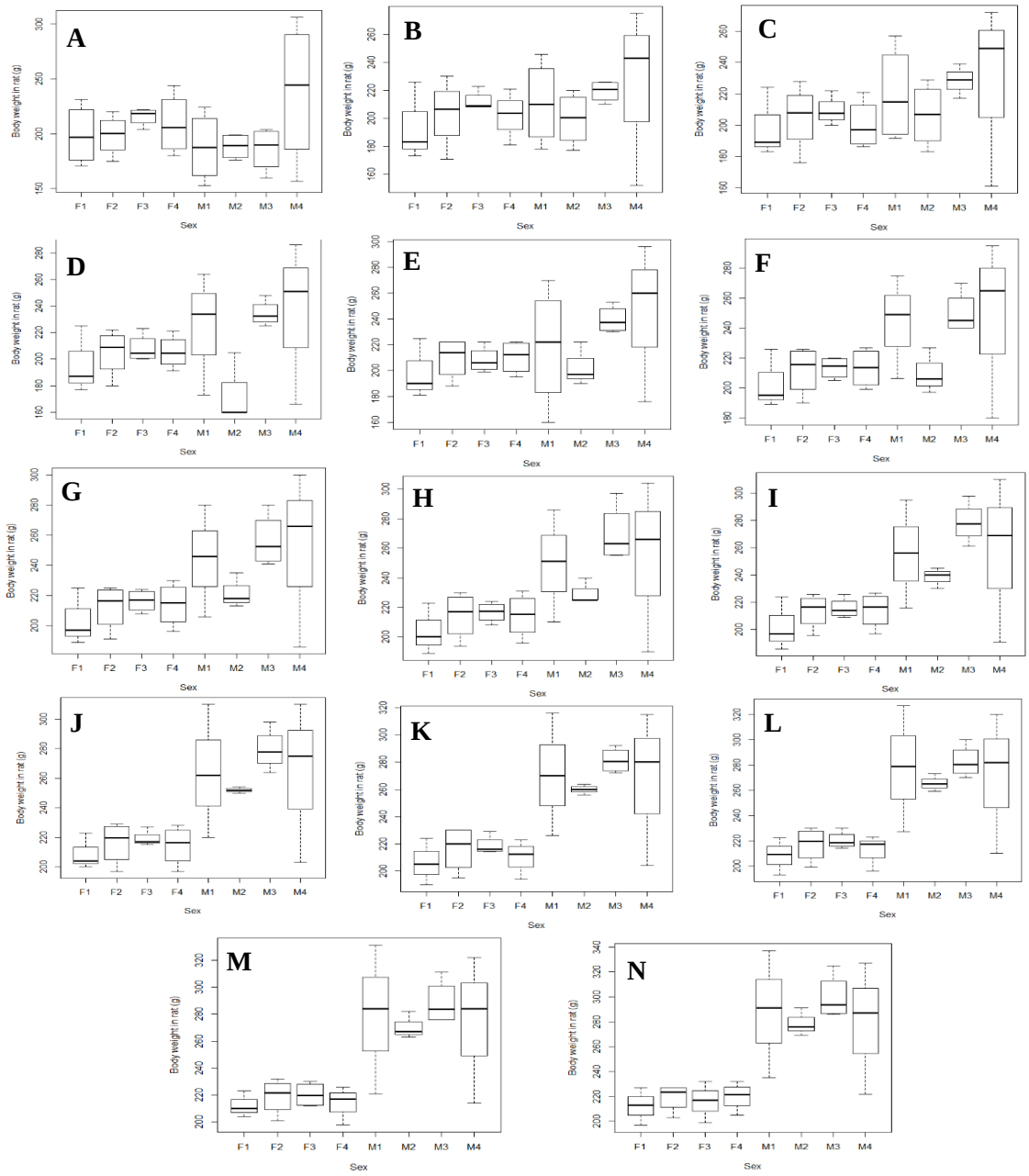

Figure 3: Body weight changes in male and female rats during 13 weeks treatment with *Bridelia ferruginea*. A: Before experiment, B: Week1, C: Week2, D: Week3, E: Week4, F: Week5, G: Week6, H: Week7, I: Week8, J: Week9, K: Week10, L: Week11, M: Week12, N: Week13. F1: Female control, F2: 100mg/kg, F3: 200mg/kg, F4: 400mg/kg, M1: Male control, M2: 100mg/kg, M3: 200mg/kg, M4: 400mg/kg. Data are presented as mean as the mean ± SD, M: Male (n= 4), F: Female (n= 4).

After this time, the significant changes of the body weight were observed in the control group (M1-F1, $p < 0.001$) and those treated with 200 (M3-F3, $p < 0.001$) and 400 mg/kg bw (M4-F4, p

< 0.001) with a decrease of body weight in female's compared to male. The findings in the present study showed that *B. ferruginea* aqueous extract caused a gradual dose-dependent non-significant increase of body weight in male and female rats during the experimental period, compared to control groups. The similar results were obtained with some authors studying the acute and sub-acute toxicity of the ethanol leaf extract of *Aegialitis rotundifolia* Rox in Wistar rats [21]. These results are comparable to those of Venkatesan et al. [22], whom in sub-acute toxicity studies of acetaminophen, an analgesic substance, in Sprague Dawley Rats, showed no changes in body weight in both sexes. At the same time, body mass was lower in female rat groups treated with 200 mg/kg compared to male groups at the same dose. This effects of *Bridelia ferruginea* on body weight would be due to its analgesic activities as observed by Akuodor et al. [23] in mice. It is known, according the guideline of the OECD 423, that female rats are used in a toxicity study because they are slightly more sensitive than males [24,25].

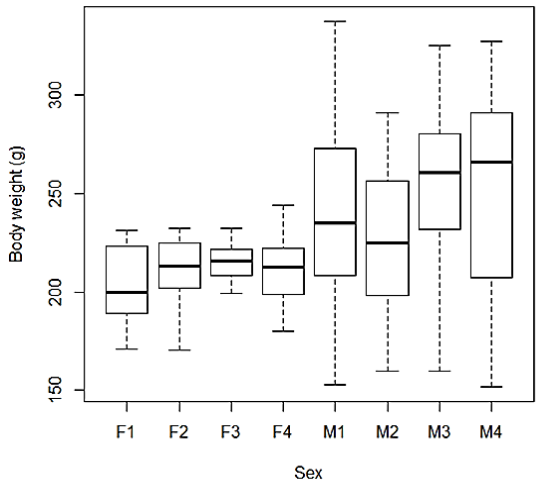

Figure 4. Effects of *Bridelia ferruginea* aqueous extract on the body weight changes in male and female rats after 13 weeks of daily oral treatment. F1: Female control, F2: 100 mg/kg, F3: 200 mg/kg, F4: 400 mg/kg, M1: Male control, M2: 100 mg/kg, M3: 200 mg/kg, M4: 400 mg/kg. Data are presented as mean as the mean ± SD, M: Male (n= 4), F: Female (n= 4)

In accordance with Tarttelin, Gorski [26], body weight and food intake in female rats decreased because of the effects of estrogen and progesterone. Since estrogen was higher in female rats, food consumption decreased, resulting in decreasing body weight [27]. For Fukushima et al. [28], who demonstrated the effects of gonadectomy in these species, these results appear to be due the effects of gonadal steroid hormones in rodents. As demonstrated by Clegg et al. [29] in their study, the brains of female rats are more sensitive to catabolic actions of leptin, hormone controlling the feeling of fullness. The administration of this hormone in female rats, reduced food intake and body weight. According to the findings of the present study, the body weight and food intake were lower in female rats' compared to male groups which could be linked to the sensitivity of female rats to chemicals found in the extract of *Bridelia ferruginea* (Euphorbiaceae). It could be due to the effects of estrogen in female rats.

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Author contributions

Semi Anthelme Nene-Bi prepared the extract and conducted toxicity study; Ramachandran Vadivelan supervised the work and participated in the literature survey; Palanisamy Dhanabal provided the infrastructure to carry out the experiments; Ouga Stanislas Zahoui analyzed the data; Semi Anthelme Nene-Bi drafted the manuscript; Flavien Traore and Semi Anthelme Nene-Bi corrected the final paper.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

OECD: Organization for Economic Cooperation and Development