

## Full Length Research Paper

# Laxative properties of aqueous and methanolic extracts of mature stem bark of *Neoboutonia velutina* Prain (Euphorbiaceae) in rats

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***Neoboutonia velutina***. Prain is a plant used in Cameroonian ethnomedicine for the treatment of digestive disorders such as constipation. This study was conducted to evaluate the laxative properties of aqueous and methanolic extracts of *N. velutina* in rats. The laxative properties as mentioned by the consulted traditional healer were firstly determined, through an evaluation of fecal emission outset, number of wet defecations, water content of faeces, ions secretion, gastrointestinal motility and intestinal fluid accumulation in an acute loperamide induced-constipation and secondly by evaluating the defecation outset, defecation frequency and water content of faeces in a pre-sub-chronic treatment. The results show that the two extracts after 08 h of observation, significantly reduced the fecal emission outset at 125 mg/kg and increased the number of wet defecations except 250 mg/kg of the aqueous extract. The methanolic extract significantly increased the fecal water content compared to the control groups. Both extracts stimulated the secretion of water and ions such as  $K^+$ ,  $Na^+$  and  $Cl^-$ , leading to intraluminal retention of water and an accumulation of intestinal fluid. Finally, the pre-sub-chronic treatment of constipated animals led to significant increase of fecal water content and defecations. These extracts could have laxative properties resulting from the presence of some phytoconstituents that could stimulate the increase in the fecal water content and the number of defecation. These results therefore support the traditional use of the plant in the treatment of constipation.

**Key words:** *Neoboutonia velutina*, mature stem bark, laxative properties, rats.

## INTRODUCTION

Constipation is an abnormal irregularity of defecation or a difficulty in evacuating fecal matters. In developed countries, its frequency is estimated at 6% in the general

population, 20 % in old subjects and even more than 50% in medium geriatric, with a female prevalence found in the majority of studies (Camara et al., 1999). In

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Cameroon, a study undertaken on a sample of 720 patients in Douala and Yaoundé towns showed that constipation accounts for 6.42% of ano-recto colic affections (Ndjitoyap et al., 1991). The causes of this pathology are multiple, such as an association of reduction in intestinal transit and faeces dehydration or from a disturbance in defecation mechanisms. It is also due to a diet low in liquid or fibers, the absorption of certain drugs and emotional shocks. Sometimes it is due to secondary causes like a psychiatric disorder. Added to quasi daily discomfort observed in patients (abdominal pains, nausea, anorexia and pelvic gravity), constipation complications can lead to a requirement of a medical intervention (Harari et al., 1994). As consequences, some related pathophysiology like fecaloma, fecal incontinence, anal lesions, hydro-electrolytic disorders, diverticulosis, diverticulitis and cardiovascular complications are observed.

The use of laxatives is very current for the treatment of constipation, but the abuse of these laxatives can be responsible for other symptoms such as intestinal mucosa irritation, causing hydro-electrolytic diarrhea (Guimbaud and Permemuter, 2001). So far, half of patients have not been satisfied with the effect of laxatives on improving the quality of life. Plants have long been a very important source of drugs against several diseases including constipation. *N. velutina* Prain also known as *N. melleri* Var is a plant belonging to the family Euphorbiaceae, and is largely distributed in Cameroon localities such as Bamenda, Ntem, Babadjou, Labane, Yakuba and Tukurua. The specie is found in marshy forests land, near river and stream side, with persistent leaves. *N. velutina* is well known in traditional medical practice, where the mature stem bark of the plant is extracted with water and taken orally for the treatment of constipation. No scientific report on the laxative activity of the mature stem bark of *N. velutina* plant has been done. The present study was carried out to evaluate the laxative properties of the aqueous and methanolic extracts of mature stem bark of *N. velutina*.

## MATERIALS AND METHODS

### Plant material collection and extraction

The mature stem bark of *N. velutina* (Euphorbiaceae) used for the study was harvested from a bush located in Mbouda (West region of Cameroon) in March 2013 and authenticated at the National Herbarium in Yaounde, Cameroon through a comparison with the voucher specimen No 50111/HNC. The collected fresh stem bark was scrapped, chopped, shade dried and coarsely powdered.

### Preparation of aqueous extract

Powdered *N. velutina* stem barks (180 g) were macerated in 3 L of distilled water for 72 h. The macerated mixture was filtered through Whatman N°3 filter paper and evaporated to dryness in an air oven at 40°C to give 19.7 g of aqueous extract corresponding with an extraction yield of 10.9 %. The extract was stored in a refrigerator (20°C) until use.

### Extraction of the methanol plant material

150 g of the stem bark powder of *N. velutina* was macerated in 4 L of methanol for 72 h. The filtrate was concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 65°C to give 12.6 g of methanol extract (08.4% yield).

### Animals

Albino Wistar rats (*Rattus norvegicus*) of 1 month and weighing on average 80 g were used for sub-chronic treatment whereas those of 2 to 2.5 months and weighing on average 140 g were used on acute loperamide induced-constipation. They were bred in the Animal House of the Department of Animal Biology, University of Dschang, Cameroon. The animals were kept in standard cages with good ventilation (Temperature:  $22 \pm 1^\circ\text{C}$ , photoperiod: 12 h natural light and 12h dark, humidity: 45-50 %) and received food and water *ad libitum*. Prior to experimental protocol, the rats were acclimatized for 48 h to laboratory conditions for minimizing any non specific stress. Experimental protocols used in this study were approved by the laboratory committee (Laboratory of Animal Physiology and Phytopharmacology, Department of Animal Biology, Faculty of Science, University of Dschang, Cameroon) according to the standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

### Drugs and chemicals

Loperamide hydrochloride (Imodium, Janssen-Cilag, France), sodium chloride (NaCl, BDH Chemicals, England), deactivated charcoal (Carbophos, Tradiphar, France) and castor oil (Geochim, Cameroon) were purchased in a certified drugstore.

### Induction of constipation and experimental design

Constipation was induced in the animals by oral administration of loperamide (3 mg/kg b.w for laxative and gastrointestinal transit test and 5 mg/kg b.w for water and electrolyte secretion test). The elimination of reduced, hard and dry fecal pellets indicated constipation in the rats. The rats were grouped into 08 groups of six rats each. The animals in group 1 (negative control) were treated with distilled water (1 ml/100 g b.w), group 2 (positive control) received castor oil (2 ml), groups 3, 4 and 5 comprised constipated rats respectively treated with doses 125, 250 and 500 mg/kg b.w of aqueous extract of *N. velutina*, while groups 6, 7 and 8 comprised constipated rats receiving 125, 250 and 500 mg/kg b.w of methanolic extract of *N. velutina* respectively. All the substances were administered through oral route and the animals were fasted for 18 hours, prior to the beginning of each experiment.

### Laxative activity on loperamide induced constipation in acute treatment

This study was carried out, as earlier described by Saito et al. (2002), with slight modification; an inductive dose of 3 mg/kg of loperamide was used, other than 5 mg/kg. Rats were placed individually in cages lined with clean paper, and allowed to fast for 18 h. All the animals received loperamide (3 mg/kg b.w). 1 h later, animals received *per os*, aqueous and methanolic extracts and castor oil for the positive control. The fecal emission outset, defecation frequency and water content of feces in all eight groups were monitored for 8 h.

### Water and electrolyte secretion

The method of Robert et al. (1976), with slight modifications was used. Distilled water other than saline solution given to negative control group and the delay time (01 hour other than 02 hours) before animals sacrifice, was used. Rats were divided as earlier mentioned. 01 hour after oral administration of aqueous and methanolic extract and castor oil, rats were sacrificed and the small intestine from the pylorus to caecum was extracted. The intestinal content was collected in graduated tubes by milking and the volume was measured. It was therefore centrifuged (3900 rpm for 15 min) and the supernatant served as fluid sample for the determination of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{Ca}^{2+}$  concentrations, using flame photometry assay.

### Gastrointestinal transit (GIT)

Gastrointestinal transit was measured according to the method of Nagakura et al. (1996). All animals firstly received loperamide, followed by the oral administration of the different substances (extracts, castor oil and distilled water) to different groups one hour later. After 30 min, the animals were given 1 ml of freshly prepared deactivated charcoal meal. 1 h later, animals were sacrificed by an overdose of anesthesia and the abdomen was immediately cut open, to excise the whole small intestine (pylorus region to caecum). The distance across which the charcoal had covered and the total length of the small intestine were measured. The GIT propulsion was expressed as percentage of distance covered by the charcoal relative to the total length of the small intestine.

### Laxative activity test on constipation in a pre-sub-chronic treatment

This study was carried out using Xu et al. (2012) method with some modifications. Rats grouped as earlier mentioned, were treated orally once a day for 14 consecutive days. On day 15, all animals received 5 mg/kg body weight of loperamide followed by the oral administration of 1 ml of charcoal meal 10 min after. They were therefore placed in individual cages dressed with clean white filter paper. Fecal emission outset, frequency of defecation and water content of feces in all eight groups were monitored for 8 h.

### Statistical analysis

Results were expressed as mean  $\pm$  standard deviation (s.d). For statistical analysis, data were normally distributed and multiple comparisons were carried out using one-way analysis of variance (ANOVA) followed by a Tukey test for post-hoc analysis. Statistical significance was acceptable at a level of  $p < 0.05$ . Data analysis was achieved using the software program GraphPad InStat.

## RESULTS

### Laxative activity on loperamide induced constipation in acute treatment

#### Fecal emission outset

The results of laxative effect of aqueous and methanolic extracts of mature stem bark of *N. velutina* on fecal emission outset in constipated rats during 8 h of

observation are reported in Table 1. The methanolic extract was more active due to the fact that it significantly ( $p < 0.05$ ) reduced the fecal emission outset compared to untreated animals group that received distilled water. The more important effect was obtained with dose 125 mg/kg of the same extract ( $58.67 \pm 12.32$  min) compared to  $480 \pm 00.00$  min as noted in negative control group. Similar results were obtained when compared with castor oil used as standard laxative. The aqueous extract at the only dose of 125 mg/kg provoked a significant ( $p < 0.05$ ) reduction in the fecal emission outset.

### Wet defecation frequency

The effects of aqueous and methanolic extracts of mature stem bark of *N. velutina* on the number of wet fecal output in constipated rats are presented in Table 2. Except the dose 250 mg/kg of aqueous extract, all the other doses of the two extracts significantly ( $p < 0.05$ ) increased the number of wet fecal outputs compared to untreated animal groups which received distilled water. The dose 125 mg/kg of methanolic extract was the most effective ( $03.50 \pm 00.22$  compared to  $00.00 \pm 00.00$  animals of negative control group). Only the doses 125 and 250 mg/kg of the methanolic extract significantly ( $p < 0.05$ ) increased the number of wet fecal outputs when compared to animals which received castor oil.

### Water content (%)

Table 3 shows the effects of aqueous and methanolic extracts of mature stem bark of *N. velutina* on water secretion on the intestinal fecal matter in constipated rats after 8 h of observation. A significant ( $p < 0.001$ ) increase in water content was obtained with the methanolic extract at all doses ( $75.82 \pm 06.44$  ;  $92.78 \pm 00.44$  and  $83.99 \pm 03.56$  %) respectively for 125, 250, and 500 mg/kg compared to constipated rats which received distilled water ( $00.00 \pm 00.00$  %). A similar effect was obtained with 125 and 500 mg/kg of aqueous extract ( $59.55 \pm 05.41$  and  $60.26 \pm 05.29$  % respectively). A non-significant ( $p > 0.05$ ) increase of water content was obtained in animals receiving 250 and 500 mg/kg of methanolic extract compared to animals which received castor oil used as standard laxative.

### Intestinal electrolyte secretion

#### $\text{Ca}^{2+}$ ions

The results of  $\text{Ca}^{2+}$  ions secretion are shown in Table 4. A non-significant ( $p > 0.05$ ) secretion of this ion was observed with all doses of aqueous and methanolic extracts of mature stem bark of *N. velutina* compared to

**Table 1.** Laxative effects of aqueous and methanolic extracts of mature stem bark of *N. velutina* evaluated through fecal emission outset in loperamide induced constipation.

| Treatment                                | Dose (mg/kg)  | Fecal emission outset (min)   | % of reduction |
|------------------------------------------|---------------|-------------------------------|----------------|
| Distilled water                          | 1ml/100 g b.w | 480.00 ± 00.00                | 00.00          |
| Castor oil                               | 2 ml          | 252.00 ± 19.93                | 47.50          |
|                                          | 125           | 142.80 ± 58.54 <sup>a</sup>   | 70.24          |
|                                          | 250           | 480.00 ± 00.00                | 00.00          |
| Aqueous extract of <i>N. Velutina</i>    | 500           | 413.20 ± 12.32                | 13.92          |
|                                          | 125           | 58.67 ± 12.32 <sup>c***</sup> | 87.78          |
| Methanolic extract of <i>N. velutina</i> | 250           | 88.50 ± 11.40 <sup>c**</sup>  | 81.56          |
|                                          | 500           | 119.20 ± 30.85 <sup>c*</sup>  | 75.17          |

<sup>a</sup>p<0.05; <sup>c</sup>p<0.001: significant differences compared to negative control receiving distilled water.\*p<0.05;\*\*p<0.01 and \*\*\*p<0.001: significant differences compared to the positive control (castor oil treated group). Values of each column represent the mean ± SD (n= 6).

**Table 2.** Laxative effects of aqueous and methanolic extracts of mature stem bark of *N. velutina* evaluated on wet defecation frequency.

| Treatment                                | Dose (mg/kg)  | Wet defecation frequency      |
|------------------------------------------|---------------|-------------------------------|
| Distilled water                          | 1ml/100 g b.w | 00.00 ± 00.00                 |
| Castor oil                               | 2 ml          | 01.83 ± 00.16 <sup>c</sup>    |
|                                          | 125           | 01.50 ± 00.34 <sup>c</sup>    |
| Aqueous extract of <i>N. velutina</i>    | 250           | 00.00 ± 00.00                 |
|                                          | 500           | 01.16 ± 00.16 <sup>a</sup>    |
|                                          | 125           | 03.50 ± 00.22 <sup>c***</sup> |
| Methanolic extract of <i>N. velutina</i> | 250           | 02.88 ± 00.31 <sup>c*</sup>   |
|                                          | 500           | 02.00 ± 00.26 <sup>c</sup>    |

<sup>a</sup>p<0.05; <sup>c</sup>p<0.001: significant differences compared to negative control receiving distilled water.\*p<0.05; \*\*\*p<0.001: significant differences compared to the positive control (castor oil treated group). Values of each column represent the mean ± SD (n= 6).

negative control which received distilled water. Meanwhile, at all tested doses of the aqueous extract, a significant (p<0.001) inhibition of Ca<sup>2+</sup> secretion was recorded, compared to castor oil administered as standard drug.

### K<sup>+</sup> ions

The effect of the aqueous and methanolic extracts of mature stem bark of *N. velutina* on K<sup>+</sup> ions secretion is presented in Table 4. Both extracts at all tested doses, significantly (p<0.001) stimulated K<sup>+</sup> secretion. This can be seen by an increased K<sup>+</sup> concentration in intestinal fluid, compared to negative control group, treated with distilled water.

### Cl<sup>-</sup> ions

Laxative effects of aqueous and methanolic extracts of mature stem bark of *N. velutina* on Cl<sup>-</sup> secretion are presented in Table 4. Both extracts at all doses except the dose 250 mg/kg provoked an increase in Cl<sup>-</sup> secretion. The most significant (p<0.001) effect was obtained with dose 500 mg/kg of the aqueous extract and 125 and 250 mg/kg of methanolic extract, compared to negative control group; and better than the standard dose of 500 mg/kg of the aqueous extract.

### Na<sup>+</sup> ions

The results of laxative effects of aqueous and methanolic

**Table 3.** Laxative properties of aqueous and methanolic extracts of mature stem bark of *N. velutina* evaluated on water content.

| Treatment                                | Doses (mg/kg) | Water content (%)           |
|------------------------------------------|---------------|-----------------------------|
| Distilled water                          | 1ml/100 g b.w | 00.00 ± 00.00               |
| Castor oil                               | 2 ml          | 77.85 ± 05.66 <sup>c</sup>  |
|                                          | 125           | 59.55 ± 05.41 <sup>c</sup>  |
| Aqueous extract of <i>N. velutina</i>    | 250           | 00.00 ± 00.00 <sup>**</sup> |
|                                          | 500           | 60.26 ± 05.29 <sup>c</sup>  |
|                                          | 125           | 75.82 ± 06.44 <sup>c</sup>  |
| Methanolic extract of <i>N. velutina</i> | 250           | 92.78 ± 00.44 <sup>c</sup>  |
|                                          | 500           | 83.99 ± 03.56 <sup>c</sup>  |

<sup>c</sup>p<0.001: significant differences compared to negative control receiving distilled water. <sup>\*\*</sup>p<0.01: significant differences compared to the positive control (castor oil treated group). Values of each column represent the mean ± SD (n= 6).

**Table 4.** Laxative activities of aqueous and methanolic extracts of mature stem bark of *N. velutina* measured on electrolyte secretion in constipated rats.

| Treatment                                | Doses (mg/kg) | Ca <sup>2+</sup> (meq %)      | K <sup>+</sup> (meq %)     | Cl <sup>-</sup> (meq %)       | Na <sup>+</sup> (meq %)       |
|------------------------------------------|---------------|-------------------------------|----------------------------|-------------------------------|-------------------------------|
| Distilled water                          | 1ml/100 g b.w | 03.33 ± 00.40                 | 00.00 ± 00.00              | 02.88 ± 00.02                 | 00.31 ± 00.00                 |
| Castor oil                               | 2 ml          | 03.78 ± 00.12                 | 00.05 ± 00.00              | 03.38 ± 00.01                 | 00.78 ± 00.11                 |
| Aqueous extract of <i>N. velutina</i>    | 125           | 01.73 ± 00.02 <sup>c***</sup> | 00.04 ± 00.00 <sup>c</sup> | 03.79 ± 00.12 <sup>a</sup>    | 01.25 ± 00.00 <sup>c***</sup> |
|                                          | 250           | 02.61 ± 00.09 <sup>**</sup>   | 00.04 ± 00.00 <sup>c</sup> | 03.39 ± 00.13                 | 00.63 ± 00.00 <sup>a</sup>    |
|                                          | 500           | 02.48 ± 00.10 <sup>**</sup>   | 00.05 ± 00.00 <sup>c</sup> | 05.35 ± 00.07 <sup>c***</sup> | 00.62 ± 00.00 <sup>a</sup>    |
| Methanolic extract of <i>N. velutina</i> | 125           | 04.21 ± 00.41                 | 00.06 ± 00.00 <sup>c</sup> | 04.09 ± 00.35 <sup>c</sup>    | 01.25 ± 00.00 <sup>c***</sup> |
|                                          | 250           | 03.92 ± 00.22                 | 00.05 ± 00.00 <sup>c</sup> | 04.33 ± 00.27 <sup>c*</sup>   | 01.09 ± 00.16 <sup>c*</sup>   |
|                                          | 500           | 02.93 ± 00.16                 | 00.04 ± 00.00 <sup>c</sup> | 04.06 ± 00.19 <sup>b</sup>    | 00.93 ± 00.00 <sup>c</sup>    |

<sup>a</sup>p<0.05; <sup>b</sup>p<0.01; <sup>c</sup>p<0.001: significant differences compared to negative control receiving distilled water. <sup>\*</sup>p<0.05; <sup>\*\*</sup>p<0.01; <sup>\*\*\*</sup>p<0.001: significant differences compared to the positive control (castor oil treated group). Values of each column represent the mean ± SD (n= 6).

extracts of mature stem bark of *N. velutina* on Na<sup>+</sup> secretion are recorded in Table 4. It was observed that both extracts significantly (p<0.05) stimulated Na<sup>+</sup> secretion, compared to the negative control. The most important secretion was obtained with both extracts at the same dose of 125 mg/kg compared to animals treated with distilled water and those receiving castor oil (01.25 ± 00.00 %).

#### Effect of the aqueous and methanolic extracts of mature stem bark of *N. velutina* on gastrointestinal transit (intestinal motility test)

##### Effect on the distance travelled by deactivated charcoal meal

Table 5 shows the value obtained from the distance

travelled by deactivated charcoal after administration of both extracts to constipated rats. All extracts doses tested, significantly (p<0.001) stimulated an increase in the distance travelled by charcoal meal compared to the negative control animals. The greatest propulsion was obtained with the methanolic extract at dose 125 mg/kg (89.22 %) and better than the standard laxative drug (88.34 %).

#### Intestinal fluid volume

The results recorded in Table 6 illustrate the effect of aqueous and methanolic extracts on intestinal fluid accumulation. The doses 250 and 500 mg/kg of aqueous extract and all tested doses of methanolic extracts significantly (p<0.001) increased the intestinal fluid volume, compared to negative control group. The

**Table 5.** Laxative effects of aqueous and methanolic extracts of mature stem bark of *N. velutina* measured on distance travelled by deactivated charcoal.

| Treatment                                | Doses (mg/kg)  | Distance travelled by deactivated charcoal (cm) | Total length of intestine (cm) | % of propulsion |
|------------------------------------------|----------------|-------------------------------------------------|--------------------------------|-----------------|
| Distilled water                          | 1 ml/100 g b.w | 08.00 ± 00.0                                    | 77.12 ± 00.19                  | -               |
| Castor oil                               | 2 ml           | 68.61 ± 00.38                                   | 77.20 ± 00.24                  | 88.34           |
| Aqueous extract of <i>N. velutina</i>    | 125            | 82.21 ± 04.95 <sup>c</sup>                      | 67.59 ± 04.23                  | 85.62           |
|                                          | 250            | 55.64 ± 00.74 <sup>c</sup>                      | 77.03 ± 00.24                  | 88.99           |
|                                          | 500            | 72.68 ± 00.79 <sup>c</sup>                      | 77.03 ± 00.34                  | 89.19           |
| Methanolic extract of <i>N. velutina</i> | 125            | 74.18 ± 00.52 <sup>c</sup>                      | 77.16 ± 00.28                  | 89.22           |
|                                          | 250            | 72.79 ± 00.95 <sup>c</sup>                      | 75.97 ± 00.95                  | 89.01           |
|                                          | 500            | 49.28 ± 00.52 <sup>c ***</sup>                  | 76.98 ± 00.26                  | 83.77           |

<sup>c</sup>p<0.001: significant differences compared to negative control receiving distilled water. \*\*\*p<0.001: significant differences compared to the positive control (castor oil treated group). Values of each column represent the mean ± SD (n= 6).

**Table 6.** Laxative effects of aqueous and methanolic extracts of mature stem bark of *N. velutina* measured through the intestinal fluid volume.

| Treatment                                | Doses (mg/kg) | Volume of intestinal fluid (ml) | % of increase |
|------------------------------------------|---------------|---------------------------------|---------------|
| Distilled water                          | 1ml/100 g b.w | 00.42 ± 00.09                   | -             |
| Castor oil                               | 2 ml          | 01.16 ± 00.08 <sup>c</sup>      | 64.29         |
| Aqueous extract of <i>N. velutina</i>    | 125           | 00.60 ± 00.06 <sup>*</sup>      | 30.55         |
|                                          | 250           | 01.35 ± 00.15 <sup>c</sup>      | 69.14         |
|                                          | 500           | 01.55 ± 00.11 <sup>c</sup>      | 78.26         |
| Methanolic extract of <i>N. velutina</i> | 125           | 03.05 ± 00.19 <sup>c***</sup>   | 86.34         |
|                                          | 250           | 01.67 ± 00.11 <sup>c</sup>      | 75.00         |
|                                          | 500           | 02.37 ± 00.07 <sup>c***</sup>   | 82.39         |

<sup>c</sup>p<0.001: significant differences compared to negative control receiving distilled water. \*p<0.05; \*\*\*p<0.001: significant differences compared to the positive control (castor oil treated group). Values of each column represent the mean ± SD (n= 6).

methanolic extract at doses 125 and 500 mg/kg was the most active since it showed a significant (p<0.001) increase in intestinal fluid accumulation more than the standard treated group receiving Castor oil.

#### Laxative activity of extracts on constipated rats submitted to a pre-sub-chronic treatment

##### Effects on the fecal emission outset

The results of laxative effects of the aqueous and methanolic extracts of mature stem bark of *N. velutina* on fecal emission outset are reported in Table 7. The aqueous extract at doses 500 mg/kg (63.17 ± 05.12 min), 125 mg/kg (58.50 ± 04.54 min) and 250 mg/kg (41.17 ± 03.11 min) of methanolic extract significantly (p<0.001)

reduced the fecal emission outset in pre-treated constipated rats, compared to the negative control group (380.33 ± 13.89 min). When this activity is compared to that of castor oil (198.50 ± 09.83 min), it is realized that at the same doses of the extracts, there was a significant (p<0.001) reduction of the time of fecal output than the standard laxative.

##### Effects on defecation frequency

Table 8 presents the effect of the aqueous and methanolic extracts of mature stem bark of *N. velutina* on defecation frequency. It was observed that none of the doses of both extracts caused a significant (p>0.05) variation in defecation frequency after 14 days of pre-treatment compared to the negative control group.

**Table 7.** Laxative activities of aqueous and methanolic mature stem bark extracts of *N. velutina* evaluated through fecal emission outset.

| Treatment                                | Doses (mg/kg)  | Fecal emission outset (min)   | % of inhibition |
|------------------------------------------|----------------|-------------------------------|-----------------|
| Distilled water                          | 1 ml/100 g b.w | 380.33 ± 13.89                | -               |
| Castor oil                               | 2 ml           | 198.50 ± 09.83 <sup>c</sup>   | 47.81           |
| Aqueous extract of <i>N. velutina</i>    | 125            | 480.00 ± 00.00                | -26.21          |
|                                          | 250            | 342.83 ± 11.36                | 09.86           |
|                                          | 500            | 63.17 ± 05.12 <sup>c***</sup> | 83.39           |
| Methanolic extract of <i>N. velutina</i> | 125            | 58.50 ± 04.54 <sup>c***</sup> | 84.62           |
|                                          | 250            | 41.17 ± 03.11 <sup>c***</sup> | 89.18           |
|                                          | 500            | 344.33 ± 10.97                | 09.47           |

<sup>c</sup>p<0.001: significant differences compared to negative control receiving distilled water. \*\*\*p<0.001: significant differences compared to the positive control (castor oil treated group). Values of each column represent the mean ± SD (n= 6).

**Table 8.** Laxative effects of aqueous and methanolic extracts of mature stem bark of *N. velutina* evaluated by the number of defecation.

| Treatment                                | Doses (mg/kg) | Defecation frequency       | % of increase |
|------------------------------------------|---------------|----------------------------|---------------|
| Distilled water                          | 1ml/100 g b.w | 01.00 ± 00.00              | -             |
| Castor oil                               | 2 ml          | 02.33 ± 01.03 <sup>c</sup> | 57.14         |
| Aqueous extract of <i>N. velutina</i>    | 125           | 00.00 ± 00.00              | 00.00         |
|                                          | 250           | 01.17 ± 00.41              | 14.29         |
|                                          | 500           | 01.17 ± 00.41              | 14.29         |
| Methanolic extract of <i>N. velutina</i> | 125           | 01.50 ± 00.55              | 33.33         |
|                                          | 250           | 01.33 ± 00.52              | 25.00         |
|                                          | 500           | 01.00 ± 00.00              | 00.00         |

<sup>c</sup>p<0.001: significant differences compared to negative control receiving distilled water. Values of each column represent the mean ± SD (n= 6).

### Effects on water content of faeces

The water content of faeces measured after 8 h of observation in pre-treated rats is recorded in Table 9. Only dose 500 mg/kg of aqueous extract significantly (p<0.001) increased the water content of faeces (88.53 ± 02.08 %) after a pre-sub-chronic treatment compared both to negative control group (45.28 ± 02.18 %) and positive control group (56.79 ± 20.89 %).

### DISCUSSION

The present study was undertaken in order to evaluate the laxative properties of the aqueous and methanolic extracts of mature stem bark of *N. velutina* on loperamide-induced constipation models. The activities

of these extracts would be related to their permissive role on loperamide mechanisms of action, since they shorten the latent time of loperamide in inducing constipation (Reza et al., 2010). As previously mentioned, the observed increase in number of wet defecation could be due to an inhibiting action made by those extracts, since it has been shown that identified phenols, sterols and flavonoids have potential laxative properties (Zhao et al., 1997). The increase in water content of faeces once more confirms the laxative properties of the mature stem bark extracts of *N. velutina* which could be explained by a cathartic effect of extracts due to the accumulation of water, similar to the action of castor oil (Sharma et al., 2011). The colon absorbs Na<sup>+</sup> and Cl<sup>-</sup> ions but secretes K<sup>+</sup> ions which lead to a retention of water and a concentration of faeces. According to the work completed by Deachapunya et al. (2005), the majority of natural

**Table 9.** Laxative properties of aqueous and methanolic of mature stem bark of *N. velutina* measured by water content.

| Treatment                                | Doses (mg/kg) | Water content (%)             |
|------------------------------------------|---------------|-------------------------------|
| Distilled water                          | 1ml/100 g b.w | 45.28 ± 02.18                 |
| Castor oil                               | 2 ml          | 56.79 ± 20.89                 |
| Aqueous extract of <i>N. velutina</i>    | 125           | 00.00 ± 00.00                 |
|                                          | 250           | 20.90 ± 18.75                 |
|                                          | 500           | 88.53 ± 02.08 <sup>c***</sup> |
| Methanolic extract of <i>N. velutina</i> | 125           | 40.41 ± 16.13                 |
|                                          | 250           | 26.46 ± 03.28                 |
|                                          | 500           | 33.01 ± 04.04                 |

<sup>c</sup>p<0.001: significant differences compared to negative control receiving distilled water. \*\*\*p<0.001: significant differences compared to the positive control (castor oil treated group). Values of each column represent the mean ± SD (n= 6).

laxatives exert their effects on the colonic epithelium by stimulating secretion of Cl<sup>-</sup> ions and/or by inhibiting absorption of Na<sup>+</sup> ions, leading to an accumulation of the intestinal fluid as well as an increase of colon motility (Méité et al., 2010). Both extracts increased the distance covered by charcoal, which can be assimilated as an increased intestinal peristalsis in rats (Wintola et al., 2010).

The increase in intestinal fluid volume as observed after treatment of constipated animals with all doses of the various extracts could attest the effects of these extracts on the various secretory mechanisms (water and electrolytes secretions). However, the intestinal contents of the animals treated with the extracts were more viscous than that of those treated with the castor oil which is known to allow electrolytes transport and the contractility of intestinal smooth muscles. Its cathartic action is due to an accumulation of water in the intestinal tract (Méité et al., 2010).

Besides, the administration of castor oil used as standard substance generated the release of ricinoleic acid which provoked an accumulation of mucous fluid and hypersecretion of electrolytes (Na<sup>+</sup> and Cl<sup>-</sup>), thus softening the faeces (Adeyemi et al., 2011). The results also show that both extracts increase the intestinal propulsion. This could be probably due to an increase in the peristaltic movement of the gastro-intestinal tract of rats in response to a stimulation of the cholinergic receptors (Sharma et al., 2011) by identified phyto-chemical components of *N. velutina* extracts as sterols, flavonoids and glycosides which are recognized as potent laxative agents. Previous studies have shown that an increase in smooth muscles layers contractile activity is generally linked to mediators and neurotransmitters such as acetylcholine, known as particular enteric excitatory neurotransmitters, thus mediating intestinal propulsion

(Méité et al., 2010).

The sub-chronic treatment was undertaken with the aim of checking and researching the peristaltic-potentialisation role of *N. velutina* mature stem bark extracts. It was observed that *N. velutina* extracts have the capacity to reduce fecal emission outset and increase the water content of faeces in pre-treated constipated rats. This could be due to an increase in intestinal smooth muscles contraction, leading to an increase of the small intestine transit. In relation with the studies undertaken by Xu et al. (2012), the mature stem bark extracts of *N. velutina* could act in a similar way of insoluble fibers which lengthily remain not fermented in the colon and retain water, thus facilitating the softening of the faeces.

## Conclusion

This study has shown that mature stem bark of *N. velutina* has laxative properties in loperamide-induced constipation in rats. This suggests the beneficial effects of the plant in improving intestinal motility add to the various physiological effect of the plant. Thus, further studies are required to isolate the active components in the crude extract.

## Conflict of interests

The authors have not declared any conflict of interest.

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