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## Original Research Article

# Effect of *Murdannia edulis* Faden roots on serum prolactin level and mammary glands of lactating female albino rats

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## ABSTRACT

**Background:** *Murdannia edulis* (Stokes) Faden (Myit-Cho in Myanmar) is a herb which has widely been used to induce or increase lactation by ethnic groups living in Tamu township of Sagaing division in Myanmar. This study aimed to explore the effect of *Murdannia edulis* on serum prolactin level and mammary glands of the lactating female rats.

**Methods:** The type of the study was laboratory based experimental study. Thirty-six lactating dams were divided into six groups of 6 lactating rats in each group (n=6), namely group I (normal saline) as control, group II (5 mg/kg metoclopramide) as standard, groups III and IV (1 gm/kg and 2 gm/kg watery extract of *Murdannia edulis*), groups V and VI (1 gm/kg and 2 gm/kg ethanolic extract of *Murdannia edulis*), respectively. The extract and the drugs were orally administered from day 4 to 15 of lactation. At 16 days of lactation, the heart blood of lactating dams was collected under chloroform anaesthesia and serum prolactin level of dams was measured by using ELISA kit. The histological changes of mammary glands of the rats were also assessed.

**Results:** The significant higher serum prolactin level were seen in ethanolic extract 2 gm/kg body weight treated group when compared to control. Histology of mammary glands in ethanolic extract treated rats showed stimulation of lobuloalveolar cell development.

**Conclusions:** The lactogenic activity of the roots of *Murdannia edulis* may be due to promoting prolactin secretion and lobuloalveolar cell development of mammary glands.

**Keywords:** Lactating rats, Mammary glands, *Murdannia edulis*, Serum prolactin

## INTRODUCTION

Traditionally, postpartum Myanmar women employed a combination of herbs and dietary measures to sustain or augment lactation following childbirth. Concerns frequently arise among mothers regarding the adequacy of breast milk quantity. Throughout the lactational period, women often endeavor to enhance breast milk production by incorporating herbs and foods identified as "galactogogues".<sup>1</sup> A diverse array of common galactogogues includes almonds, anise, asparagus, borage, caraway, chaste tree fruit, chicken soup, cilantro, coconut, coriander, cumin, dandelion, dill, fennel, fenugreek, garlic, ginger, hops, lettuce, marshmallow root, millet,

mushrooms, nettle, oat straw, papaya, pumpkin, red clover, red raspberry, rice, sage, seaweed soup, sesame seeds, sunflower seeds, thistles, vervain, and numerous others.<sup>2</sup>

Lactating mothers in the Tamu district of the Sagaing region in Myanmar specifically employ the roots of *Murdannia edulis* (Stokes) Faden (Myit-Cho in Myanmar) (Figure 1) to induce or enhance milk production postpartum. This plant naturally grows in the local forest. Residents of that area harvest the roots of *Murdannia edulis*, boiling them in hot water to create a tea with purported lactogenic effects. The efficacy of this herbal practice in promoting lactation is well-established within

the community. However, the use of *Murdannia edulis*, as a traditional medicine for treatment of other conditions is rare. The lactogenic activity of *Murdannia edulis* was scientifically evaluated in the study of Htoon et al.<sup>3</sup> The significance increase in milk production of lactating female rats and average weight gain of the pups was seen in ethanolic and watery extracts of *Murdannia edulis* treated group when compared to control group.<sup>3</sup>



**Figure 1: Plants and roots of *Murdannia edulis* Stokes (Faden).**

*Murdannia edulis* is a rarely investigated herb. A researcher from Kaohsiung medical university of Taiwan studied the anti-inflammatory activity of *Murdannia edulis* and it was described that three new coumarins (7,8-dimethoxy-4-hydroxymethyl coumarin, 7-methoxy-8-hydroxy-4-hydroxymethyl coumarin, 8-hydroxy-4-hydroxymethyl coumarin) together with seven known organic compounds (isovitexin, vitexin, epi-loliolide, (E)-N-feruloyltyramine, vanillic acid, syringic acid and bracteanolide A) could be isolated from the whole plant extract of *Murdannia edulis*.<sup>4</sup>

The purpose of this study is to evaluate the possible mechanism of action of *Murdannia edulis* by studying its effect on serum prolactin level and the morphology of mammary glands in lactating female albino rats.

## METHODS

This study is a laboratory based experimental animal study. The research work relating to the animals was conducted at the animal house of the Department of Medical Research, Yangon, Myanmar, and the measurement of serum prolactin level and the histological examination of rat mammary glands were done at department of clinical pathology of Defense Services Liver Hospital, Yangon, Myanmar.

## Plant material collection

Plants of *Murdannia edulis* including flowers were collected from Tamu district, Sagaing Division, Myanmar, and subsequently identified at the Department of Botany, University of Yangon, Myanmar. The roots were sectioned, air-dried in shade, and then stored in glass bottles for subsequent analyses.

## Plant extract preparation

Solid-liquid extraction was performed on *Murdannia edulis* roots using ethanol and water. Approximately 400 g of air-dried roots were placed into two 5-liter conical flasks, with each flask receiving 2.5 L of the respective solvent. The extraction process involved heating the flasks in a boiling water bath at the appropriate boiling points for the solvents (60 °C for ethanol and 100 °C for water). Following a 6-hour extraction period, the mixture was cooled to room temperature, filtered using Whatman filter paper, and the residues were discarded. The filtrate was then evaporated to dryness in a Petri dish on a boiling water bath. The resulting dried extract was stored in a desiccator and dissolved in distilled water to achieve the desired concentration prior to testing.

## Acute toxicity testing

The acute toxicity of ethanolic and aqueous extracts of *Murdannia edulis* was evaluated in mice using the OECD test guideline 425: up-and-down procedure (Main test).

## Animal model

Thirty-six female albino rats weighing between 180-240 g were procured from the animal house of the department of medical research, Yangon. These rats were housed and mated with males in stainless steel metal cages under standard laboratory conditions with a 12-hour dark/light cycle. The rats were provided with commercial feeds and tap water ad libitum, with the first day of birth designated as the first day of lactation.

## Serum prolactin level determination

Thirty-six lactating dams at onset of lactation were divided into 6 groups, each comprising six lactating rats (n=6). The groups included group I (normal saline) as a control, group II (5 mg/kg metoclopramide) as a standard, groups III and IV (1 gm/kg and 2 g/kg watery extract of *Murdannia edulis*), and groups V and VI (1 gm/kg and 2 gm/kg ethanolic extract of *Murdannia edulis*).

Both extracts and drugs were orally administered, and all animals received daily treatment starting from the evening of the third day of lactation up to 15 days of lactation. On the 16th day of lactation, 0.5 mL of heart blood was collected from all rats, and serum prolactin levels were analyzed using a rat prolactin ELISA kit (Cloud-Clone CEA846Ra).

### Histological examination of rat mammary glands

Two female rats from the control, metoclopramide, and ethanolic extract-treated groups (at doses of 1 gm/kg and 2 gm/kg body weight) were sacrificed under chloroform anesthesia. Inguinal mammary glands were resected for subsequent histological examination under H and E stain to compare the differences of mammary gland histology in different groups.

Only two of the rats from each group were selected and sacrificed, the others to be reproducible as a surrogate mother for the remaining pups because the pups had still depended on their mother to some extent although they ate some solid food independently

### Statistical analysis

Data obtained were expressed as mean  $\pm$  SEM. Student t test was used for comparisons between the groups. SPSS software was used for analysis. Values of  $p < 0.05$  were considered significant.

## RESULTS

### Yield percentage

The ethanolic extraction of *Murdannia edulis* roots resulted in a yield of 14.7 gm of dried extract per 200 gm (7.35%) of dried crude powders. The aqueous extraction yielded 33.7 gm of dried extract per 200 gm (12.4%) of dried crude root powder.

### Acute toxicity

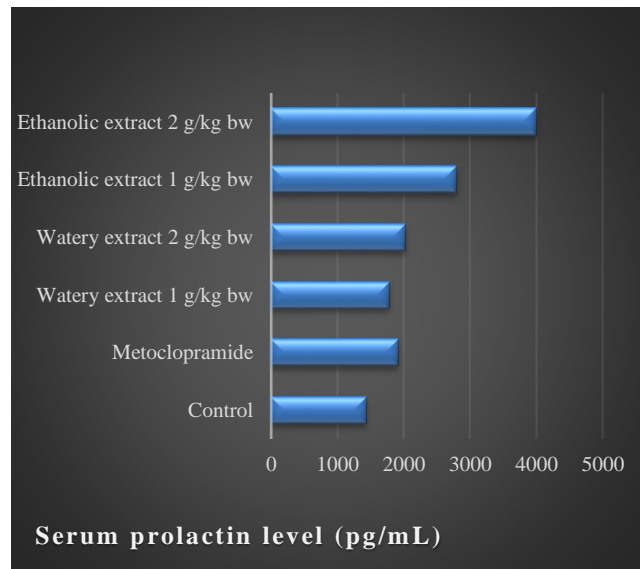
No lethality was observed in mice for up to 14 days, even at the maximum administered dose (5 gm/kg bw) of both ethanolic and aqueous extracts. Consequently, the extracts were determined to be devoid of acute toxic or harmful effects, with an estimated LD<sub>50</sub> exceeding 5 gm/kg.

### Rat serum prolactin level changes

The result of average rat serum prolactin level analysis were described in Table 1 and Figure 2. Serum prolactin level of lactating female rats in metoclopramide treated group was significantly higher than the control group ( $p < 0.05$ ). Among the tested groups, the rat serum prolactin level of ethanolic extract 2 gm/kg bw treated group was significantly higher ( $p = 0.002$ ) compared to control group and those of other tested groups were not different statistically.

In regard to serum prolactin level of metoclopramide treated group, that of ethanolic extract 2 gm/kg bw treated group was also significantly higher ( $p = 0.007$ ).

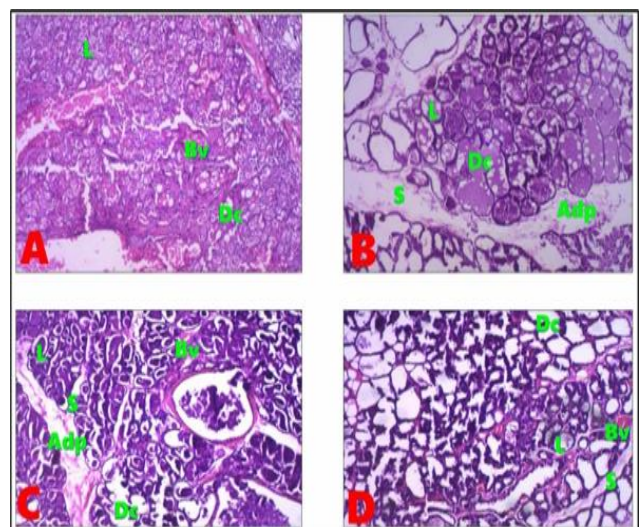
Therefore, this result showed that the efficacy of ethanolic extract of *Murdannia edulis* 2 gm/kg bw dose is higher than the standard drug metoclopramide.



**Figure 2: Rat serum prolactin level of lactating female rats in control, metoclopramide and extract treated groups.**

### Mammary gland histology changes of lactating rats

In ethanolic extract treated groups, lobuloalveolar development of mammary glands were seen. The membranes of mammary ducts and lobules were dilated though cell linings are still intact. However, they became very large ducts and lobules. Nuclei were regular. Blood vessels were congested, dilated and filled with red blood cells. Intervening stroma were scanty. These effects were dose-dependent and more prominent in 2 gm/kg dose ethanolic extract treated group as in Figure 3.



**Figure 3 (A-D): Histological features of rat mammary gland in normal saline group, in metoclopramide group, in ethanolic extract 1 gm/kg group and in ethanolic extract 2 gm/kg group and (Magnification: A $\times$ 40, B-D $\times$ 100).**

Adp-Adipose tissue; Bv-Blood vessel, Dc-Ductus; L-lobule; S-Connective tissue stroma.

**Table 1: Rat serum prolactin level of in control, metoclopramide and extract-treated groups.**

Groups	Rat serum prolactin level (pg/mL)	Level of significance vs control (p<0.05)
<b>Control</b>	1430.24±394.10	
<b>Metoclopramide</b>	1918.56±262.26	S, p=0.03
<b>Watery extract 1 gm/kg bw</b>	1786.04±733.58	NS, p=0.32
<b>Watery extract 2 gm/kg bw</b>	2030.16±922.66	NS, p=0.174
<b>Ethanollic extract 1 gm/kg bw</b>	2797.33±1864.48	NS, p=0.109
<b>Ethanollic extract 2 gm/kg bw</b>	4006.83±1357.59	S, p=0.002

S=statistically significant, NS=statistically not significant.

## DISCUSSION

Lactation, being an intricate neurohormonal process, involves the coordinated action of various hormones, including estrogen, progesterone, prolactin (PRL), and placental lactogen, which are complemented by the influence of insulin, cortisol, and several growth factors.<sup>5</sup> Prolactin, a principal lactogenic hormone secreted by the anterior pituitary, plays a crucial role in establishing lactation, determining milk macronutrient content, and regulating milk production.<sup>6</sup>

While some herbal medicinal plants have demonstrated positive effects on milk production in experimental animals, their effects on the serum prolactin levels of lactating female rats have been variable. For instance, the aqueous extract of *Acacia nilotica* ssp *adansonii*, *Hibiscus sabdariffa* L. seed extract, *Launaea taraxacifolia* leaf extracts, *Erythrina indica* Lam. leaves and the ethanolic extract of *Pluchea indica* Less leaf have been reported to stimulate the synthesis and release of prolactin hormone in rats.<sup>6-9</sup> In contrast, a study by Simelane et al showed that female lactating rats administered the extract of *Gunnera perpensa* produced more milk than controls but did not significantly influence serum prolactin levels.<sup>10</sup> In another study, the ethyl acetate fraction of *Coleus amboinicus* L. leaves (EA) induced milk in lactating dams, but it did not significantly stimulate the synthesis of serum prolactin.<sup>11</sup>

In this study, the impact of two extracts derived from the roots of *Murdannia edulis* on the serum prolactin levels in lactating female rats was investigated. The rat serum prolactin level of the ethanolic extract at a dose of 2 gm/kg body weight was significantly higher compared to the control group and the metoclopramide-treated group. Therefore, the efficacy of the ethanolic extract of *Murdannia edulis* at a dose of 2 gm/kg body weight resulted in a higher serum prolactin level than the standard drug metoclopramide. The lactogenic activity of *Murdannia edulis* roots may be attributed to its promotion of prolactin secretion from anterior pituitary gland.

Another potential mechanism contributing to increased milk production in lactating rats may involve the enhancement of cell proliferation in their mammary glands following extract administration. Some galactagogue herbs have been reported to profoundly affect proliferation of mammary secretory cells, which serves as an indicator of activity of these cells in milk secretion.<sup>6-7</sup> In this study,

the effect of different doses of ethanolic extract from the roots of *Murdannia edulis* on the rat inguinal mammary glands was investigated and the ethanolic extract appeared to stimulate the development of lobuloalveolar cells, and the observed morphological changes, as illustrated in Figures 3, indicated a dose-dependent effect.

## Limitations

In this study, only serum prolactin level and rat mammary gland histology was evaluated to search for the underlying pharmacological mechanism of the roots of *Murdannia edulis* and the other hormonal changes which may influence lactation such as cortisol and oxytocin were not studied due to time and financial limitations.

## CONCLUSION

The study on the effects of the roots of *Murdannia edulis* on rat serum prolactin levels and mammary gland histology suggests that the ethanolic extract may exert a lactogenic effect by promoting prolactin secretion from the anterior pituitary gland and enhancing the development of lobuloalveolar cells in mammary glands.

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*Conflict of interest:* None declared

*Ethical approval:* The study was approved by the Institutional Ethics Committee

## REFERENCES

- Nice FJ. Common herbs and foods used as galactagogues. ICAN: Infant. Child Adolescent Nutrit. 2011;3(3):129-32.
- Marasco L. Increasing your milk supply with galactagogues. J Hum Lact. 2008;24(4):455-6.

3. Htoon KZ, Kyaing KO, Oo AW, Htay TM, Phyu KP. Effect of ethanolic and watery extracts of *Murdannia edulis* (Stokes) Faden roots on milk production of lactating female albino rats. J Myan Mil Med. 2021;11(1):57-63.
4. Shia TH. Studies on the Chemical Constituents of *Murdannia edulis* and Anti-Liver Cancer Activities of *Gynura divaricata*. Kaohsiung Medical University Electronic thesis and dissertation services; 2016. Available at: <https://www.airitilibrary.com/Article/Detail/U0011-2308201619282000>. Accessed on 25 September 2023.
5. Rhoades RA, Bell DR, editors. Medical Physiology: Principles for Clinical Medicine. Lippincott Williams and Wilkins. 2013;724-6.
6. Gaya IB, Mohammad OMA, Suleiman AM, Maje MI, Adekunle AB. Toxicological and lactogenic studies on the seeds of *Hibiscus sabdariffa* Linn (*Malvaceae*) extract on serum prolactin levels of albino Wistar rats. Internet J Endocrinol. 2009;5(2):1-6.
7. Lompo-Ouedraogo Z, Van Der Heide D, Van Der Beek EM, Swarts HJ, Mattheij JA, Sawadogo L. Effect of aqueous extract of *Acacia nilotica* ssp *adansonii* on milk production and prolactin release in the rat. J Endocrinol. 2004;182(2):257-66.
8. Sani NI, Kawu MU, Bako IG. Effects of *Launaea taraxacifolia* and resveratrol on milk yield and serum prolactin and oxytocin levels: a lactogenic study. Int J Vet Sci Med. 2019;7(1):71-7.
9. Chithra S, Priya S, Raiby PP. Evaluation of the Lactogenic activity of *Erythrina indica* Lam. leaves. Indian J Tradit Knowl. 2022;21(3):552-7.
10. Syarif RA, Anggorowati N, Yuniyanti MM, Murti DNMA, Adyaksa, Wahyuningsih MSH. Lactogenic activity of ethanolic extract of *Pluchea indica* Less leaf in lactating rats. J Herbmед Pharmacol. 2023;12(3):380-87.
11. Simelane MBC, Lawal OA, Djarova TG, Musabayane CT, Singh M, Opoku AR. Lactogenic activity of rats stimulated by *Gunnera perpensa* L. (*Gunneraceae*) from South Africa. Afr J Tradit Complement Altern Med. 2012;9(4):561-73.
12. Damanik RM, Kustiyah L, Hanafi M, Iwansyah AC. Evaluation of Lactogenic Activity of Ethyl Acetate Fraction of Torbangun (*Coleus amboinicus* L.) Leaves. IOP Conf Ser Earth Environ Sci. 2017;101:012007.

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