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Research Article

Laxative Potential of Ethanol Leaf Extract of *Securinega virosa* in Loperamide Induced Constipated Wistar Rats

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ABSTRACT

Constipation is the inability of individuals to empty the bowel for a period of three times a week which causes discomfort and even sometimes lead to colorectal cancer. This work was aimed at estimating the laxative potential of ethanol leaf extract of *Securinega virosa* in constipated rats induced with loperamide. Experimental adult rats were induced with 3 mg/kg body weight of loperamide, while the normal control received normal saline solution. Upon confirmation of constipation, the rats were treated with 50, 100 and 200 mg/kg body weight of ethanol leaf extract of *Securinega virosa* for 7 days. Senokot was used as the reference drug. During the study period, faecal properties, feed intake, water intake, body weight gain and intestinal motility were recorded. The treatment of constipated rats with 50, 100 and 200 mg/kg body weight greatly improved the faecal properties, decreased body weight and elevated water intake statistically at $p \leq 0.05$ as well as the gastrointestinal motility when compared with the constipated untreated experimental group. These proved the laxative effect of the plant, with the best dose being 200 mg of the extract. Thus these results evidenced the ethnobotanical usage of *Securinega virosa* in the treatment of constipation.

Keywords: *Laxative, Constipation, Loperamide, Securinega virosa, Senokot.*

INTRODUCTION

The inability of individuals to have a free bowel movement as and when due is usually as a result of constipation. Constipation occurs from too much absorption of water from food by the colon, which leads to slow colon contraction. Constipation, also known as irritable bowel syndrome (IBS) is a common symptom-based functional gastrointestinal (GI) disorder, estimated to affect between 5 and 20% of the population (Heidelbaugh *et al*., 2015). The occurrence of constipation has been reported to be more prevalent in people of the lower socio-economic class (Higgins and Johnson, 2004). Although constipation can be mild in some cases, it could also become chronic (Sharma and Rao, 2016) as it can lead to colorectal cancer (Koloski *et al*., 2013). Chronic constipation can lead to blockage of the intestine, resulting in its closure which may require consideration of colectomy or cecostomy surgery (Sharma and Rao, 2016).

Loperamide is an opioid agonist anti-diarrhea drug that is widely used due to its ability to inhibit the secretion of intestinal water and peristalsis which lead to slower intestinal transit time and absorption of water and electrolytes (Griffith *et al*., 2022). Due to these properties of loperamide, it is used to induce constipation. Laxatives are substances that have the ability to stimulate intestinal

secretion and increase the frequency of bowel movement. The use of synthetic drugs such as senokot for the treatment of constipation has been the common practice of the people in the past. However, these chemical substances sometimes present setbacks such as high cost, accessibility, stomach pain and cramps among others. Other laxative drugs currently used for the treatment of constipation include lubiprostone, linaclotide, prucalopride (Jiang *et al*., 2015) alvimopan, and methylnaltrexone (Coss-Adame and Rao, 2012). These drugs are classified under serotonin ($5\text{-}HT_4$) agonists that act by releasing acetylcholine which in turn improves bowel movement. Aside the cost and ease of availability of these drugs to the locals, they have been reported to cause diarrhea, nausea and headache (Jiang *et al*., 2015). Based on this, the study aimed to research on alternative means of treatment for constipation through the use of *Securinega virosa* plant.

Securinega virosa is one of the great African medicinal plants described as "cure all" plant (Amenu *et al*., 2019). It is a shrub that has a free height of about 5 m commonly found in most part of East and West Africa (Amenu *et al*., 2019). It is a shrub with dense, many low branches and evergreen or deciduous in nature (Magaji *et al*., 2015[b]). It is widely distributed throughout tropical Africa, but can also be found in India, Malaya, China and Australia (Uzama *et al*., 2013). It is commonly called white berry bush, snowberry tree or simple leaf bush (Amenu *et al*., 2019) and locally referred to as "tsuwaawun karee, gussu, gwiiwar karee" (Hausa), "iranje" (Yoruba), "njisinta" (Ibo), "shim" (Kanuri) and "kartfi-kartfi" (Shuwa Arabs) in Nigeria. The plant is commonly used in the management of inflammatory conditions and tumour (Magaji *et al*., 2015). Furthermore, the leaves of the plant are used in the management of ulcer (Salawu *et al*., 2019) and cancer in the South Western Nigeria (Soladoye *et al*., 2010). Ethnobotanical survey has reported the use of the plant in the management of constipation (Magaji *et al*., 2007). However, the scientific validation of the plant in the management of constipation is still elusive, thus, the objective of this study was to evaluate the laxative potential of *Securinega virosa* in experimental rats with constipation induced with loparamide.

MATERIALS AND METHODS

Study design and location

A Senokot drug, a product of Aspar Pharmaceutical Limited, South Africa was procured from Lamed Pharmaceutical Limited, Jos. Other analytical chemicals and reagents used were of good grade and standard.

Plant Materials

Securinega virosa leaves was obtained from Ibadan, Oyo State, Nigeria. The plant was authenticated at the herbarium of Forestry Research Institute of Nigeria by the herbarium Mr. Odewo, where it was assigned with the voucher number FHI-112798, and voucher specimen was deposited.

Preparation of Ethanol Leaf Extract

The leaves were properly washed in clean water and drained. They were dried at room temperature and then pulverized using an electric blender (Master Chef, with model number MC-BL 3302). Exactly 50 g of the material was extracted by percolating in 500 mL of ethanol with constant shaking for 24 hours on an orbital shaker. The mixture was filtered by passing through whatman No 1 filter paper (70 mm) and then concentrated in a water bath at 40^{o}C to obtain yield of 7.1 g. The extract was then reconstituted into the various doses used in this study.

Experimental Animals

Adult albino Wistar rats (*Rattus norvegicus*) weighing 153±4.7g were obtained from the National Vertinary Research Institute (VOM), Jos, Nigeria. The animals were housed in aluminum cages with saw dust as bedding at the animal house of Federal College of Forestry, Jos. They were allowed free assess of clean water *ad libitum* and animal pellet feeds (Grand Cereal and Oil Mills Limited, Jos). They were acclimatized for a duration of 14 days before commencement of the study. Proper guideline was followed on the use of experimental animals in accordance with the international ethical guidelines and the National Code for Health Research Ethics (NCHRE). Ethical clearance was obtained from the University of Jos Ethical Committee Animal Experimental Unit where reference number (F17-00379) was issued.

Constipation Induction

All animals except those in the control group were given oral administration of 1 ml loperamide (3 mg/kg body weight in 0.9 % sodium chloride for a period of 3 days). The control groups were given normal saline solution. Constipation was confirmed in the rats through the passage of hard and dry fecal pellets in a diminished number.

Experimental Design

A total of 36 experimental animals were used in which they were distributed 6 animals per group. Group 1 (control) and group 2 (constipated control) received distilled water, while groups 3, 4 and 5 received 50 mg/kg, 100 mg/kg and 200 mg/kg body weight of the plant extract respectively. Group 6 animals were administered with senokot, the reference drug.

The extracts and the drugs were administered using metal oropharyngeal cannula for a 7 days experimental period and for onward treatment of constipation. Parameters recorded include feed intake, number of faeces (pellets counted), water content of faeces, weight of faeces, body weight gain and water intake.

Constipation Parameters

The faeces from each of the experimental groups were carefully collected at 9.00 am every day and counted, their weights were then evaluated. The water intake from each group and the water content of the faeces was also evaluated. Dry faeces weight was deducted from the fresh faeces weight and the difference was considered as the water content of faeces.

Gastrointestinal Motility Test

The method of (Nagakura *et al*., 1999) was employed in calculating the gastrointestinal (GIT) motility ratio. This signifies the rate of reduction of the intestinal content in the small intestine of the rats. The GIT motility was carried out on the last day of the extract administration by administering 1 ml of carmine solution (3 g of the carmine suspended in 0.5 % carboxymethyl cellulose solution) orally. An hour later, animals were sacrificed and the intestines removed. The distance traveled by the carmine and the total length of the intestine was measured. The GIT ratio was calculated as the distance traveled by the carmine in relation to the total length of the small intestine.

Statistical Analysis

All data were expressed as mean ± standard deviation (S.D). Comparison of the experimental data from the test control group was analyzed using One Way Analysis of Variance (ANOVA), using 95% confidence interval. Least Significant Difference (LSD) was used to determine the level of significant in all the parameters used and differences between groups were considered statistically significant at $p \leq 0.05$.

RESULTS

Loperamide Administration and Effects

The results obtained showed a significant ($p \leq 0.05$) reduction in the water intake, faeces weight, number of faeces as well as the water content of faeces in the constipated rats compared to the control (Table 1). However, no significant difference occurred in the feed intake between the control and the constipated animals. This is an indication that constipation was successfully induced by the administered drug.

Table 1. The effect of loperamide induced constipation on the water intake, feed intake, faeces weight, number of faeces and water content of faeces in albino rats.

Parameters	Water intake (mL)	Feed intake (g)	Faeces weight (g)	Number of faeces	Water content of faeces (g)
Normal	18.53±0.60[a]	15.17±0.89	6.17±0.05[a]	75.39±0.86[a]	1.83±0.08[a]
Constipated	8.16±0.31[b]	16.23±0.31	2.77±0.02[b]	21.03±0.02[b]	0.64±0.06[b]
L.S.	*	N.S	*	*	*

Values are expressed as means ± S.D; n = 6. N.S indicates values are not significantly different from the normal control, * indicates values are significantly different from the normal control. All values are at $p \leq 0.05$.

Treatment of Constipation with *Securinega virosa* Extract

At the onset of the treatment of constipation, the untreated constipated group exhibited reduction in water intake level while the groups treated with 50, 100 and 200 mg/kg body weight of the extract as well as the reference drug showed significant difference in water intake level. Also, significant difference was observed on the body weight gain, weight of faeces, water content of faeces and number of faeces (Table 2). The result showed that feed intake remained intact across all the treatments and are not significantly altered.

Table 2. Effect of Ethanol Leaf Extract of *Securinega virosa* on the Water Intake, Feed Intake, Body Weight Gain and Faecal Properties of Loperamide-induced Constipated Rats

Parameters	Water intake (ml)	Feed intake (g)	Body weight gain (g)	Weight of faeces (g)	Number of Feces	Water content of faeces (ml)
Normal	48.53±0.04^{a}	32.08±1.22^{a}	16.15±0.06^{a}	6.52±0.10^{a}	82.63±5.84^{a}	1.35±0.07^{b}
Loperamide Constipated	37.63±0.72^{d}	32.06±1.00^{a}	23.11±0.09^{c}	3.56±0.03^{c}	57.64±2.43^{c}	0.97±0.06^{a}
50 mg/kg b.w	46.52±0.28^{c}	32.19±2.51^{a}	16.55±0.08^{b}	4.85±0.03^{b}	64.30±2.04^{bc}	1.25±0.01^{b}
100 mg/kg b.w	43.68±0.76^{c}	31.69±2.04^{a}	15.79±0.53^{a}	5.41±0.06^{a}	73.14±4.77^{ab}	1.26±0.05^{b}
200 mg/kg b.w	47.11±0.24^{b}	30.34±0.94^{a}	15.90±0.03^{a}	5.62±0.01^{a}	82.16±1.56^{a}	1.69±0.05^{d}
Senokot	47.37±0.15^{ab}	32.49±0.03^{a}	16.58±0.08^{b}	5.38±0.18^{a}	78.39±4.02^{a}	1.52±0.05^{c}
L.S.	*	N.S	*	*	*	*

Values are expressed as means ± S.D; n = 5. N.S indicates values are not significantly different from the normal control. Mean with different superscripts are significantly different from each other. All values are at $p \leq 0.05$

Gastro-intestinal Motility

The administration of ethanol leaf extract of *Securinega virosa* brought about a significant increase in the gastrointestinal transit. The extract-treated groups have higher carmine movement than the constipated control. The gastrointestinal movement ratio in the extract treated groups was similar to that of the normal control and at higher doses, the effect was seen to be better than senokot standard drug (Fig. 1).

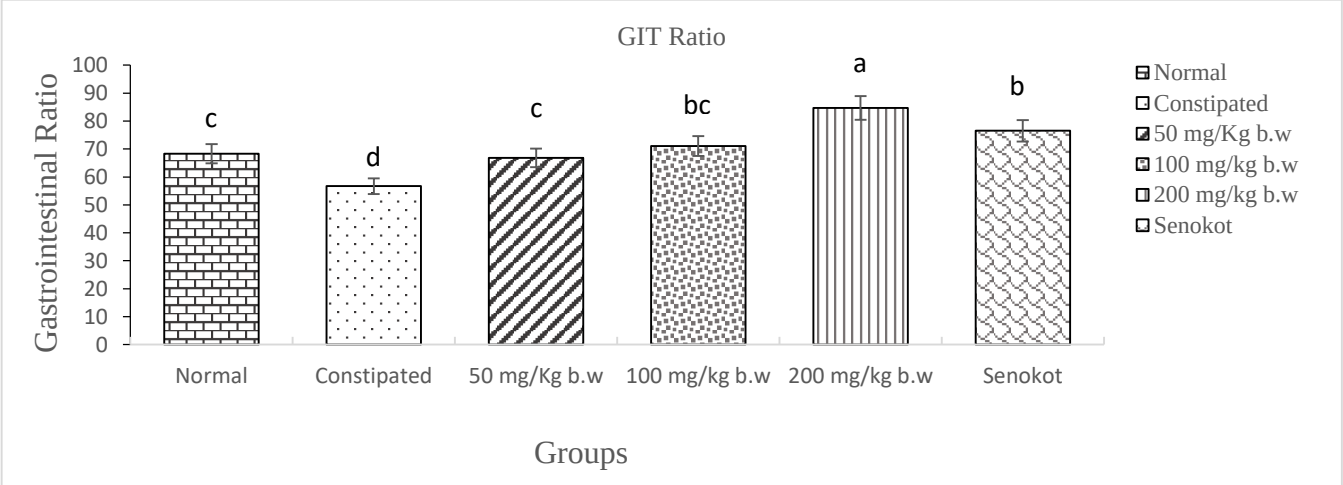

Figure 1. Effects of Ethanol Leaf Extract of *S. virosa* on the Gastrointestinal Motility Ratio on Loperamide-induced Constipated Rats. Alphabets that are different on different bars show significant difference, while alphabets that are the same on different bars indicate statistical similarity among different treatments.

DISCUSSION

Constipation is the inability of individuals to excrete faeces as and when necessary due to lack of fluid retention by the intestines. Laxatives are drugs, substances or compounds that stimulate bowel movement and enhance the excretion of faeces (Werth and Christopher, 2021). In this study, the *in vivo* laxative potential of *S. virosa* was investigated and the activity was compared with a reference drug. The reduction of the water intake in the constipated rats is attributed to the action of loperamide. This effect showed clearly in the reduction of the water content of faeces, number of faeces and faeces weight. The stimulation of the opioid in the periphery elicited the inhibition of bowel movement. This results in decrease peristalsis and hence, less bowel movement and reduction in faecal pellets excreted (Okokon *et al*., 2021). The ethanol extract of *S. virosa* was observed to be effective on the constipated rats in a dose-dependent

manner. The 100 mg and 200 mg/kg body weight doses were comparable with the reference drug. The extract was able to bring about an increase in the number of faeces, weight of the faeces, amount of water intake and water content of faeces.

The laxative property exhibited by the leaf extract could be attributed to phytochemicals present in the plant as reported by Uzama *et al*. (2012). Specifically, secondary metabolites such as alkaloids and anthraquinones are majorly present as glycosides and are reported to exert laxative action through direct stimulation of the musculature of the colon (Clementi and Weber-Schöndorfer, 2015). Consistently, another proposed mechanism of action of these phytochemicals includes alterations in the colonic motility, which leads to an accelerated large intestinal transit. Some researchers have shown that phytochemicals like aloin (a major component of glycosides) changing to aloe-emodin in the colonic flora is responsible for the laxative properties of some plants such as *Aloe ferox*, *Aloevera* and *Acacia ataxacantha* (Tosan *et al*., 2014).

Measurement of the colonic transit time is a useful tool in diagnosing constipation, abdominal bloating and refractory irritable syndrome (Tessema *et al*., 2020). In this study, the ethanol leaf extract of *S. virosa* significantly increased the intestinal motility. This could be due to the laxative effect expressed by the extract, which brought about increased peristalsis bowel movement and accumulation of fluid in the intestine, leading to intestinal transit and increased colonic movement as expressed by senokot. This result corroborates with the other plants such as *Grewia ferruginea* reported to exhibits laxative action (Tessema *et al*., 2020).

CONCLUSION

Conclusively, ethanol leaf extract of *S. virosa* exhibited laxative properties in loperamide-induced constipated wistar rats. The laxative effects of the extract were recorded to be dose-dependent. The effects of the extract also showed in the gastrointestinal motility ratio by promoting higher bowel movement in the treated groups. Thus, the plant could be used in the management of constipation induced by loperamide.

AUTHORS' CONTRIBUTIONS

The entire work was carried out with collaboration among all authors. Author SMO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AO managed the literature searches and corrected the first manuscript. Author MUH carried out the statistical analysis and also performed literature searches while author JKB managed the analysis and carried out the laboratory works. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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REFERENCES

Amenu, J.D., Neglo, D. and Abaye, D.A. (2019) Comparative study of the antioxidant and antimicrobial activities of compounds isolated from solvent extracts of the roots of *Securinega virosa*. *Journal of Biosciences and Medicines*, 7, 27-41.

Ashafa, A.O.T., Sunmonu, T.O., Abass, A.A. and Ogbe, A.A. (2011). Laxative potential of the ethanolic leaf extract of *Aloevera* (L.) Burm. F. in Wistar rats with loperamide-induced constipation. *Journal of Natural Pharmaceuticals*, 2(3), 158-162.

Clementi, M. and Weber-Schöndorfer, C. (2015). Gastro-intestinal medications, hypolipidemic agents and spasmolytics. *Drugs During Pregnancy and Lactation*, 93- 113. https://10.1016/b978-0-12-408078-2.00006-8.

Coss-Adame, C. and Rao, S.C. (2012). Pharmacologic management of chronic constipation: Current treatment options. *Medicina Universitaria*, 14(55), 90-98.

Griffith, J.I., Kim, M., Bruce, D.J., Peterson, C.D., Kitto, K.F., Mohammed, A.S., Rathi, S., Fairbanks, C.A., Wilcox, G.L. and Elmquist, W.F. (2022). Central Nervous System distribution of an Opioid Agonist Combination with Synergistic Activity. *Journal of Pharmacology and Experimental Biology*, 380(1), 34-46.

Heidelbaugh, J., Stelwagon, M., Miller, S.A., Shea, E.P. and Chey, W.D. (2015). The Spectrum of Constipation-Predominant Irritable Bowel Syndrome and Chronic Idiopathic Constipation: US Survey Assessing Symptoms, Care Seeking, and Disease Burden. *American Journal of Gastroenterology*, 110(4), 580-587.

Higgins, P.D. and Johnson, J.F. (2004). Epidemiology of constipation in North America: a systematic review. *American Journal of Gastroenterology*, 99(4), 750-759.

Jiang, C., Xu, Q., Wenn, X. and Sun, H. (2015). Current developments in pharmacological therapeutics for chronic constipation. *Acta Pharmaceutica Sinica B*, 5(4), 300–309.

Koloski, N.A., Jones, M., Wai, R., Gill, R.S., Byles, J. and Talley, N.J. (2013). Impact of persistent constipation on health-related quality of life and mortality in older community-dwelling women. *American Journal of Gastroenterology*, 108, 1152-1158. https://doi.org/10.1038/ajg.2013.137

Magaji, M.G, Musa, A.M., Abdullahi, M.I., Ya'u, J. and Hussaini, I.M. (2015). Isolation of Bergenin from the Root Bark of *Securinega virosa* and Evaluation of its Potential Sleep Promoting Effect. *Avicenna Journal of Phytomedicine*, 5(6), 587-596.

Magaji, M.G., Ya'u, J., Musa, A.M., Anuka, J.A., Abdu-Aguye, I. and Hussaini, I.M. (2015[b]). *Securinega virosa* (*Euphorbiaceae*) root bark extract inhibits glioblastoma multiforme cell survival *in vitro*. *African Journal of Pharmacy and Pharmacology*, 9(27), 684-693.

Magaji, M.G., Yaro, A.H., Mohammed, A., Zezi, A.U., Tanko, Y. and Bala, T.Y. (2007). Preliminary anti-diarrhoeal activity of methanolic extracts of *Securinega virosa* (*Euphorbiaceae*). *African Journal of Biotechnology*, 6(24), 2752-2757. https://doi.org/10.5897/AJB2007.000-2440

Nagakura, Y., Naitoh, Y., Kamato, T., Yamano, M. and Miyata, K. (1999). Compounds processing 5-HT3 receptor antagonistic activity inhibit intestinal propulsion in mice. *European Journal of Pharmacology*, 311(1), 67-72.

Okokon, J.E., Brown, N.P., Udom, G., and Bankhede, H.K. (2021). Investigation of Leaves on Laxative Activity. *Advance Pharmaceutical Journal*, 6(4), 116-120.

Salawu, M.O., Yekeen, A., Nafiu, M.O. and Oloyede, H.O.B. (2019). Anti-ulcerogenic potential of aqueous extract of *Securinega virosa* leaf in indomethacin-induced ulcerated rats. *Notulae Scientia Biologicae*, 11(2), 1-9.

Sharma, A., & Rao, S. (2016). Constipation: pathophysiology and current therapeutic approaches. *Gastrointestinal Pharmacology*, 59-74

Soladoye, M.O., Amusa, N.A., Raji-Esan, S.O., Chukwuma, E.C., Taiwo, A.A. (2010). Ethnobotanical survey of anti-cancer plants in Ogun State, Nigeria. *Annals of Biological Research*, 1(4), 261-273.

Tessema, M.Y., Wubneh, Z.B., and Asrie, A.B. (2020). Laxative activities of 80% methanolic extract of theleaves of *Grewia ferruginea* Hochst Ex A Rich in Mice. *Journal of Evidence-Based Integrative Medicine*, 25, 1-8

Tosan, C.A., Obidola, S.M. and Folashayo, O.P. (2014). Loperamide induced constipated wister rats: Laxative role of aqueous extract of *Acacia ataxacantha* Leaves. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(12), 189-199.

Uzama, D., Bwai, M.D. and Sunday, A.T. (2012). The Phytochemicals, proximate and elemental analyses of *Securinega virosa* leaf extracts. *Research Journal in Engineering and Applied Sciences*, 1(6), 351-354.

Uzama, D., Bwai, M.D., Orijajogun, O.J., Olajide, O. and Sunday, A.T. (2013). The antioxidant potentials and phytochemical properties of the hexane, ethyl acetate and ethanolic extracts of *Securinega virosa* (*Euphorbiaceae*) leaves. *Journal of Applied Pharmaceutical Sciences*. 3(5), 131-133.

Werth, B.L. and Christopher, S.A. (2021). Laxative Use in the Community: A Literature Review. *Journal of Clinical Medicine*, 10(143), 1-13.

Wintola, O.A., Sunmonu, T.O. and Afolayan, A.J. (2010). The effect of Aloe ferox Mill. in the treatment of loperamide-induced constipation in Wistar rats. *BMC Gastroenterology*, 10(95), 1-5.