



Evaluation of safety and protective effects of *Potentilla fulgens* root extract in experimentally induced diarrhea in mice

Vareishang Tangpu, Khirod Deori, Arun Kumar Yadav

ABSTRACT

Aim: The roots of *Potentilla fulgens* Wall. ex Hook. (Rosaceae) have been used in the indigenous system of medicine in Northeast India to treat diarrhea. The aim of this study was to investigate the safety and protective effects of *P. fulgens* root extract in experimentally induced diarrhea in mice. **Materials and Methods:** The protective effects of *P. fulgens* root extract was investigated against experimentally induced diarrhea in mice, using four experimental models, that is the measurement of fecal output, castor oil model, prostaglandin E2 (PGE2) enteropooling assay, and gastrointestinal transit test. The safety assessment of root extract was done in mice on the basis of general signs and symptoms of toxicity, food water intake and mortality of animals following their treatment with various doses of extract (100-3200 mg/kg). In addition, the serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, cholesterol and total protein of experimental mice were also monitored to assess the toxicity of root extract. **Results:** In the safety assessment studies, *P. fulgens* root extract did not showed any visible signs of toxicity, but mortality was observed in a single animal at 3200 mg/kg dose of extract. The extract also did not showed any adverse effects on the studied serum parameters of experimental animals. In the antidiarrheal tests, administration of 800 mg/kg dose of extract to mice showed 50% protection from diarrhea evoked by castor oil. In addition, the extract also showed 29.27% reduction in PGE2-induced intestinal secretion as compared with 30.31% recorded for loperamide, a standard antidiarrheal drug. **Conclusions:** The results of this study indicate that *P. fulgens* root extract possesses significant antidiarrheal properties. Therefore, the roots of this plant can be an effective traditional medicine for protection from diarrhea.

KEY WORDS: Acute oral toxicity, antidiarrheal, gastrointestinal, *Potentilla fulgens*, prostaglandin E2 enteropooling

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INTRODUCTION

Diarrhea is one of the principal causes of morbidity and mortality among children in developing countries [1]. Of India's more than 2.3 million annual deaths among children, about 334,000 alone are attributable to diarrheal diseases [2]. In order to combat the problems of diarrhea globally, the World Health Organization in its diarrheal disease control program has given a special emphasis on the use of traditional folklore medicines in control and management of diarrhea [3]. Medicinal herbs constitute an indispensable component of the traditional medicine practiced worldwide due to their easy accessibility. The use of herbal medicines in the treatment of diarrheal diseases is a common practice in many developing countries [4]. In India, the use of herbal medicines for the treatment of diarrheal diseases is particularly more common in the North-Eastern region, which is inhabited by several indigenous tribes [5-7].

Potentilla fulgens Wall. ex Hook. (Rosaceae) [Figure 1] is a yellow herb of 1-2 ft height. In India, it grows at altitudes of 1500-2000 msl in Sikkim, Assam, Meghalaya, Manipur and Nagaland states. Previous experimental studies on this plant have revealed that its root extract possesses antitumor [8], antioxidant [9], gastroprotective [10], and anthelmintic [11] activities. Whereas, the phytochemical studies on *P. fulgens* have revealed that it contains a few terpenes, namely potentene A, potentene B, and hyptadienic, tormentic and rosamultic acids, besides phenolic compounds viz. epicatechin and epiafzelchin, a flavonoid potifulgene and a glycoside viz. rutin [12,13].

During the course of our ethnopharmacological studies in Manipur, India, it came to our notice that the fresh roots of *P. fulgens* (vernacular name: *Ngarunri*) are either chewed or taken in the decoction form in the treatment of diarrheal disorders by the native people. A literature survey revealed no reports on antidiarrheal effects of this plant species. Therefore,



Figure 1: *Potentilla fulgens*: Whole plant and a flower

considering the ethnomedicinal use of this plant against diarrhea in Manipur, India, in this study, we were interested to investigate the safety and protective effects of *P. fulgens* root extract in experimentally induced diarrhea in mice.

MATERIALS AND METHODS

Preparation of Plant Extracts

The plant material for this study was collected from Manipur, India and authenticated by Dr. Gurung, Herbarium Curator, Department of Botany, North-Eastern Hill University (NEHU), Shillong. A herbarium record of the material was also prepared and assigned a voucher number, i.e., AKY 221, which has been deposited in the Department of Zoology, NEHU, Shillong. The roots were dried under shade and pulverized into powder. Known amounts of the powdered materials were suspended in methanol and engaged for refluxing using Soxhlet fractional distillation apparatus at 40-50°C for 4-6 h. The resulting suspension was decanted out discarding the remnants and the filtrate was further concentrated in a rotatory evaporator under reduced temperature and pressure for removal of the solvent. The percentage yield of final extract was 5.15% w/w.

Drugs and Chemicals Used

Loperamide (Lopax, Axar Pharmaceuticals, Baroda, India) was used as a standard antidiarrheal drug. Castor oil (Fine, Mumbai, India) and prostaglandin E2 (PGE2) (Sigma-Aldrich Chemical Pvt. Ltd., USA) as diarrhea-inducing agents, activated charcoal (Merck, India) as an intestinal transit marker, and Gum Acacia (Fine Chem, Boisar, India) and Tragacanth powder (Central Drug House Pvt. Ltd., Bombay, India) as suspension agents were used in this study.

Experimental Animals

Male and female Swiss albino mice of 6-8 weeks of age (20-30 g) were procured from Pasteur's Institute, Shillong, Meghalaya, and housed singly in polycarbonate cage with free access to standard rodent diet and tap water *ad libitum*. The animals were acclimatized to laboratory condition for 7 days prior to the experiments and maintained at 25 ± 3°C under a light/dark cycle of 12 h. All procedures in this study were performed

according to the Institutional Ethics Committee (animal model) guidelines of the NEHU, Shillong and Committee for the Purpose of Control and Supervision of Experiments on Animals Guidelines of Indian Council of Medical Research, New Delhi.

Acute Oral Toxicity Test

Determination of median lethal dose (LD₅₀)

Mice were selected randomly and divided into seven groups of six animals' in each. Group I served as control and II-VII were given a single oral dose of test extract with a dose progression factor of two at 100, 200, 400, 800, 1600, and 3200 mg/kg, respectively. Animals were fasted for 3 h from food, but not water, prior to administration of doses. The general signs and symptoms of toxicity, food water intake and mortality rates of animals were observed for 72 h post-treatment. From these observations, LD₅₀ was calculated using SPSS software.

Serum Biochemical Tests

In another experiment, mice were divided into two groups of six animals in each for studying the effects of test extract on various serum biochemical profiles. Group I served as control and Group II was given 800 mg/kg single oral dose of extract (the highest dose tested for antidiarrheal activity in this study). Animals were fasted for 3 h from food, but not water, prior to the administration of doses. At the end of the experiment, i.e., 24 h post-treatment, animals were sacrificed by cervical dislocation. The blood samples were collected by cardiac puncture and kept for 30 min without disturbing and then centrifuged for 15-20 min at 2000 rpm to separate the serum. From this processed serum, levels of serum glutamate oxaloacetate transaminase (Enzyme Commission [EC], 2.6.1.1), serum glutamate pyruvate transaminase (EC 2.6.1.2), cholesterol and total protein were estimated by standard methods [14-16], using a semi-automated biochemical analyzer (Bayer).

Antidiarrheal Activity

The antidiarrheal efficacy of extract was assessed using the following four experimental models of diarrhea in mice.

Measurement of Fecal Output (FOP)

Fecal output was measured following methods of Bass [17] and Pillai [18] with modifications. Animals were divided into six groups ($n = 6$), Group I served as the control and received 2% gum acacia (0.5 ml); Groups II-V were treated with 100, 200, 400 and 800 mg/kg of plant extract, respectively, while Group VI received 0.5 ml of standard antidiarrheal drug loperamide at 5 mg/kg. After 8 h post-treatment, fecal materials were collected, dried in an incubator and their weights were recorded. The percentage FOP was calculated and expressed in terms of percentage reduction as follows:

$$\% \text{ FOP} = \frac{f_t \times 100}{f_c}$$

Where, f_t is the mean fecal weight of each treatment group, and f_c is that of the control group [19].

Castor Oil Model

The method was modified from Jacoby *et al.* [20], Otshudi *et al.* [21] and has been described previously by Tangpu and Yadav [6]. Overnight-fasted mice were randomly divided into six groups ($n = 6$). Group I received 0.5 ml of 2% gum acacia suspension; Groups II-V were treated with 100, 200, 400 and 800 mg/kg of plant extract, respectively; Group VI mice were given 0.5 ml of 5 mg/kg of loperamide. One hour later, diarrhea was induced in all groups by inoculating castor oil (0.5 ml/mouse, p.o.). The numbers of diarrheal episodes were recorded for each time, and cumulative values were calculated for 4 h post-induction of diarrhea and the numbers of animals devoid of diarrheal droppings at 4 h were considered as a percentage protection from diarrhea.

PGE2-Enteropooling Assay

The protocol was adopted from Robert *et al.* [22] with modifications as described previously by Tangpu and Yadav [6]. Overnight-fasted mice were randomized into seven groups ($n = 6$). The animals received PGE2 as diarrheal agent (0.5 ml of 100 $\mu\text{g/kg}$ PGE2 in 5% ethanol in saline). Group I served as control (0.5 ml; 2% gum acacia); Group II served as a vehicle control (100 $\mu\text{g/kg}$ PGE2 + 2% gum acacia); Groups III-VI received 100, 200, 400, and 800 mg/kg of plant extract, respectively; Group VII received 5 mg/kg dose of loperamide. All these treatments were done 1 h prior to PGE2-diarrheal induction. 30 min later, animals were sacrificed, and their small intestines were ligated from pyloric sphincter to ileocaecal junction and assessments of the accumulation of intestinal fluid secretion induced by PGE2 were made and calculated in terms of percentage reduction.

Gastrointestinal Transit Test

In this test, mice were divided into six groups ($n = 6$) and allowed to starve for 16 h prior to the experiment. Group I served as the control, Groups II-V were treated with test extract at 100, 200, 400, and 800 mg/kg oral dose, respectively. Group VI animals received 5 mg/kg loperamide. 5 min later, 0.5 ml of charcoal meal was orally inoculated to each mouse. All the mice were sacrificed 30 min later, their small intestines from the pylorus to caecum cut out and distance travelled by the charcoal marker was measured, and expressed as a percentage of the total length of small intestines. The percentage inhibition of the marker transit in the intestine was calculated as described by Akah and Offiah [23].

Analysis

All the data are represented as mean \pm standard error of the mean. The significance of the difference between the control and treated groups were determined by the Student's *t*-test, and $P < 0.05$ was considered as significant.

RESULTS

Acute Toxicity Test

LD_{50}

Oral administration of plant extract starting from 100 to 3200 mg/kg caused mortality in single mice at the dose of 3200 mg/kg, observed within 72-h post-treatment; however, no visible clinical signs were observed in the rest of experimental animals. Using SPSS software, the LD_{50} of extract was found to be 5355.97 mg/kg in mice.

Serum biochemical profile

The different serum parameters of mice following treatment with 800 mg/kg dose of plant extract are shown in Figure 2. There were no significant differences observed in the levels of any serum parameters from the extract-treated after 24-h post-treatment as compared with the control group.

Antidiarrheal Activity

Measurement of FOP

The plant extract moderately reduced the FOP as compared to standard drug loperamide in a dose-dependent fashion. At 800 mg/kg dose, the percentage reduction in FOP was found to be 26.37%, while loperamide at 5 mg/kg concentration showed 41.76% reduction in FOP [Figure 3].

Effect on Castor Oil Model

There were significant differences in percentage protection of diarrhea induced by castor oil as compared to the control. In

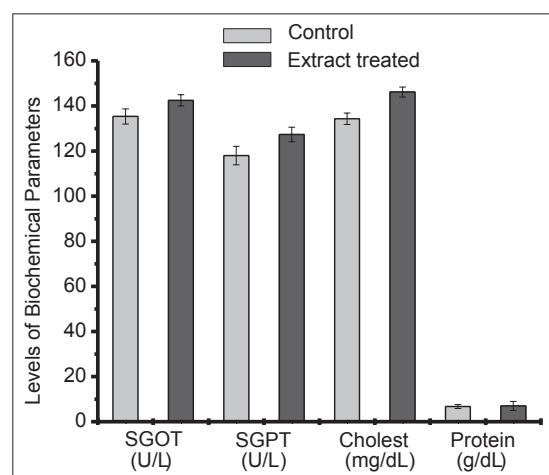


Figure 2: Effects of *Potentilla fulgens* root extract on the levels of SGOT, SGPT, cholesterol and protein after 24-h post-treatment of mice with 800 mg/kg dose of extract. SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, Cholest: Cholesterol. Values are plotted as mean \pm standard deviation, Student's *t*-test ($n = 6$). There was no significant change in serum biochemical parameters with the control mice during treatment

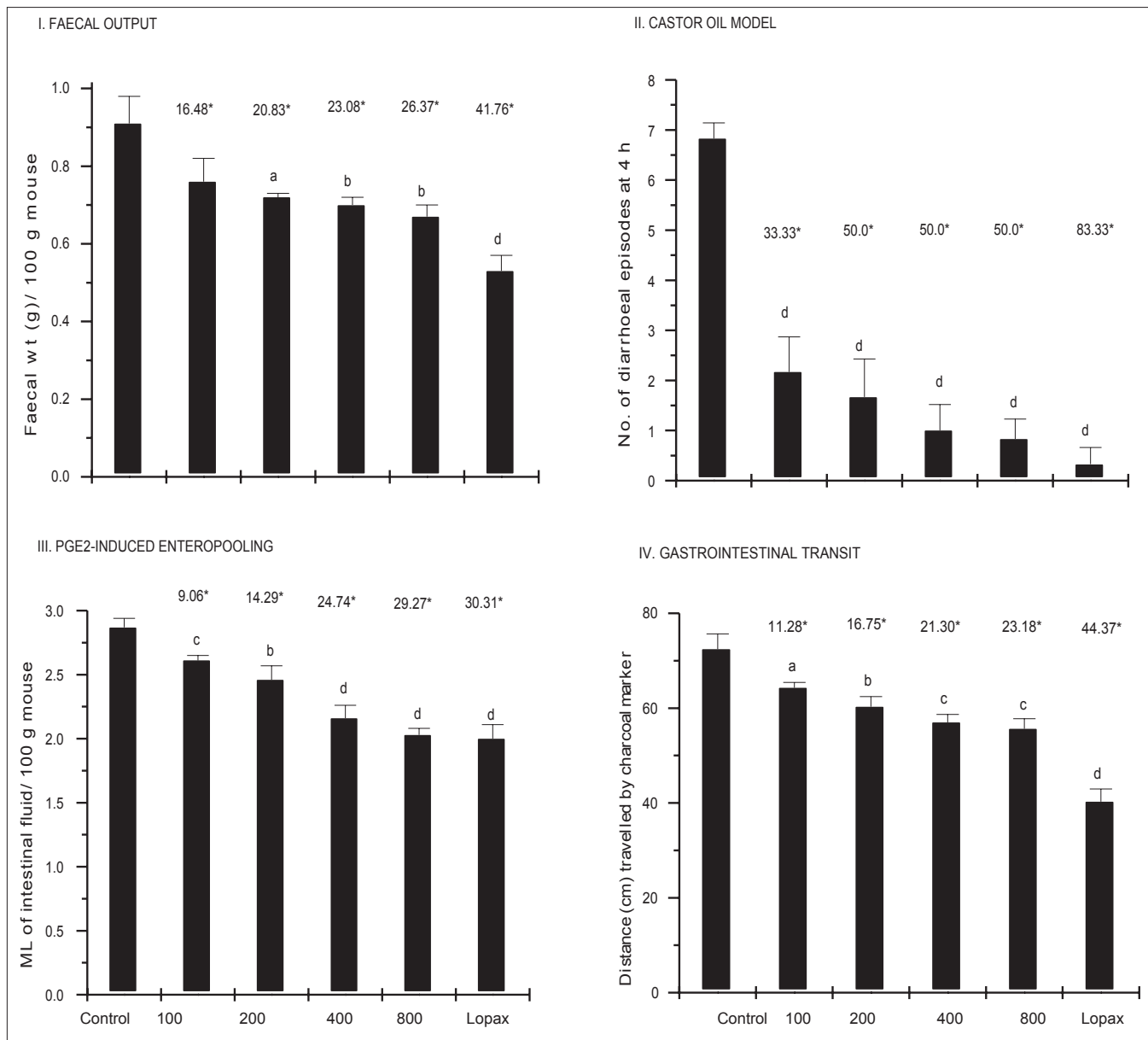


Figure 3: Antidiarrheal efficacy of *Potentilla fulgens* root extract, as represented by four different study parameters. Values are plotted as mean \pm standard error of mean ($n = 6$). *% reduction, or % inhibition, or % animal protection from diarrhea. Treatment (mg/kg, p.o.): Extract at 100, 200, 400, and 800; loperamide at 5. ^{a,b,c,d} $P < 0.05, 0.02, 0.01, \text{ and } 0.001$, versus control, Student's t -test

this case, the 800 mg/kg dose of extract could protect 50.00% of the animals from diarrheas evoked by castor oil. In comparison, loperamide at 5 mg/kg dose showed 83.33% protection of diarrhea [Figure 3].

Effect on PGE2-Enteropooling Assay

The differences in reduction of PGE2-induced intestinal secretion by the plant extract was found to be weakly significant in a dose-dependent fashion as compared with the control. The extract reduced 29.27% intestinal secretion at 800 mg/kg dose. The percentage reduction by 5 mg/kg dose of loperamide was showed 30.31%, which was almost comparable with 800 mg/kg dose of extract [Figure 3].

Effect on Gastrointestinal Transit Test

The inhibition of charcoal meal transit along the small intestine in treated group was found to be 23.18% at 800 mg/kg dose of extract [Figure 3]. Although the plant extract showed statistically significant inhibition of charcoal meal transit as compared with control, it was observed that inhibition by the highest tested dose of 800 mg/kg was comparatively low as compared with 44.37% recorded for 5 mg/kg dose of loperamide.

DISCUSSION

In different parts of the world, medicinal plants have been used traditionally for the treatment of various ailments, including

diarrheal diseases. Although, they offer various benefits in the healthcare system, still not much is known about the alleged effects and safety of many medicinal plants that are used as traditional medicines. This may be because of either traditional healers offer such medicines without taking into account the toxicity aspects of herbs or people believe that herbal medicines are natural and therefore they are devoid of any harmful effects. It is therefore important to properly justify the safety and efficacy of medicinal plants that are used as traditional medicines. During the course of our ethnopharmacological studies in Manipur, India, we identified nine medicinal plants that are used traditionally for the treatment of diarrhea by various tribal communities. Of these, a highest (73.33%) user response was recorded for *Rhus javanica* (Anacardiaceae) and a lowest (35.00%) for *Galinsoga parviflora* (Asteraceae). Herein, the user response for *P. fulgens* was recorded to be 50.00% [24]. Therefore, keeping in view the popularity of this plant among tribal communities, the present study was aimed to evaluate the antidiarrheal efficacy and safety of *P. fulgens* root extract in experimental models of diarrhea in Swiss albino mice.

In the acute toxicity study, the plant extract did not show any visible signs of toxicity, but mortality was observed in a single animal at 3200 mg/kg dose of extract. We assume that the cause of mortality only in single mice might be due to other physiological factors rather than the adverse effect of extract, as no visible signs of toxicity were observed in the rest of animals. A recent study also tested the acute toxicity of *P. fulgens* root extract and did not find any mortality or symptoms of toxicity in animals up to 4000 mg/kg [10]. The present study also did not record any significant differences in any of the studied serum biochemical parameters between the extract-treated (800 mg/kg dose) and control group of animals. On the basis of these findings, it may be said that *P. fulgens* root extract does not possess any toxic effects in experimental animals up to 3200 mg/kg dose.

In this study, the plant extract showed significant antidiarrheal activity in all four models of diarrheas tested. Diarrhea results from an imbalance between the absorptive and secretive mechanisms in the intestinal tract, accompanied by hypermotility and intestinal hurry, which results in an excess loss of fluid through the faces [25]. Therefore, most of the animal based antidiarrheal studies are mainly focused on absorptive, anti-secretory or anti-motility effects of the test substances [5,6].

This study revealed that the plant extract reduces the FOP of animals in a dose-dependent manner, which indicates the presence of an anti-secretory or pro-absorptive property in the extract. Laloo et al. [10] have also reported that 200 and 400 mg/kg, p.o. dose of *P. fulgens* root ethanolic extract significantly inhibits ethanol and pyloric ligation-induced gastric ulcers due to its anti-secretory properties.

The induction of diarrhea by castor oil is due to ricinoleic acid which is produced as a result of hydrolysis by lipases in the small bowel, which in turn stimulates PGE2 secretion and together acts primarily in the small intestine to stimulate secretion of fluids and electrolytes and speeds up the intestinal transit [26].

In castor oil model, the plant extract could protect up to 50% of animals from diarrheas. A similar pattern of diarrhea protection was reported for *Cymbopogon citratus* and *R. javanica* from our previous antidiarrheal studies [5,6]. This indicates that the secretion of intra-luminal fluid is perhaps blocked by the test extract, which slows down the intestinal transit. Loperamide, the standard antidiarrheal drug, which is known for its anti-motility and anti-secretory properties, also works on the similar mechanism of action [27]. PGE2 are known to be associated with changes in the bowel that stimulates diarrhea [28]. The extract also showed significant differences in reduction of intestinal secretion in PGE2-enteropooling assay in a dose-dependent manner. The reduction of intestinal secretion by plant extract was quite comparable with that of loperamide, which shows its potential in reducing bowel movements induced by PGE2. Further, a reduction in intestinal motility was observed in gastrointestinal transit test. The extract moderately inhibited the charcoal meal transition along the small intestine as compared with loperamide in a dose-dependent manner. Thus, it appears from these findings that the plant extract may mediate its effects more or less in a same way as the standard drug loperamide.

In our previous studies, we have reported some other antidiarrheal plants viz. *C. citratus* and *R. javanica* from the same region [5,6]. These two plants showed comparatively better antidiarrheal activities than what was observed for *P. fulgens* in the present study. Several antidiarrheal studies have shown that plant extracts containing tannins, flavonoids, alkaloids, saponins, and steroids usually possess significant antidiarrheal activity [29,30] and most frequently, tannin containing plants are used to treat diarrhea and dysentery in traditional medicines [31]. For example, Nsaka Lumpu et al. [32] evaluated the phytochemical and antidiarrheal property of *Alstonia congensis* and suggested that antidiarrheal effects of this plant were largely contributed by the presence of tannins. Tannins have also been known to be important constituents of *Potentilla* species and therefore it is most likely that the antidiarrheal effect of this plant is due to its tannins components [33].

CONCLUSION

The present study clearly suggests that the *P. fulgens* root extract possesses significant antidiarrheal activity and is safe to use as traditional medicine. Further studies, however, are necessary to isolate and identify the active ingredients of this plant to understand its precise mechanism of antidiarrheal action.

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