ORIGINAL RESEARCH ARTICLE

Effect of Evodiamine in the prevention and treatment of 5-FU induced diarrhea in Swiss Albino rats: A preliminary study

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Authors' contributions

XZ conceptualized, designed, monitored, carried out experiments and supervised the whole study. CQ contributed in the design of the study and carried out experiments. XC, QL, HW, MMRS and SA carried out experiments and involved in manuscript preparation and data analysis. XZ and CQ wrote the manuscript draft and they contributed equally in planning, designing of the study, performing experiments and writing manuscript draft. All authors read the manuscript and agreed to be accountable for all aspects of the work and approved the final manuscript.

Running title: Effect of Evodiamine on 5-FU induced diarrhea in rats

Abstract

The chemotherapeutic drugs used for the treatment of cancers induce various types of toxicities and severe side-effects including diarrhoea, constipation, nausea, vomiting, ulceration, bloating, hair loss, bone marrow suppression, loss of immunity, and cardiotoxicity. Chemotherapy-induced diarrhea (CID) badly interferes with the alterations of chemotherapy treatment of patients, dose reductions, dose delays and discontinuation of treatment of patients along with the deterioration of the Quality-Of-Life of cancer survivors. CID can even produce life-threatening toxic symptoms such as, electrolytes imbalance, dehydration and leading to death, if emerging steps not taken immediately. Currently available antidiarrheal drugs have several limitations with adverse-effects and contraindications including the development of drug-resistance, specifically against antibiotics used in diarrheal treatment. Evodiamine is an important quinolone alkaloid type bioactive compound obtained from the fruit of a traditional Chinese medicine *Evodia rutaecarpa* Benth. The fruit extract is traditionally used for several therapeutic purposes including diarrhea. Scientific studies on Evodiamine reported anticancer, antidiabetic, cardiovascular, antihyperlipidemic, anti-inflammatory, anti-microbial, and anti-Alzheimer's activities. However, no study for the antidiarrheal activity of Evodiamine has yet been conducted. Hence, the present study aimed to evaluate antidiarrheal effect of Evodiamine in the prevention and treatment of chemotherapy-induced diarrhea in experimental rats. Diarrhea was induced to Swiss albino female rats (8-12 weeks) with the administration of 5-FU (50 mg/kg/day, i.p.) for 7 consecutive days. The rats were then treated with Evodiamine during, 3 days pre- and 3 days post-treatment of 5-FU. At the end of 13 days experiments, all rats were sacrificed, and thymus and spleen weights were measured. Body weight and diarrhea rate and score were recorded every day. Our study resulted that Evodiamine significantly prevented and reduced the rate and intensity of diarrhea, body weight and thymus/spleen indexes in a dose dependent manner. The highest effects were observed with Evodiamine 50 and 100 mg/kg which exhibited similar effect with that of loperamide (3 mg/kg). Thus, our data demonstrate potential antidiarrheal activity of Evodiamine for the prevention and treatment of 5-FU induced diarrhea. To the best of our knowledge, this is the first ever study reporting to the antidiarrheal potential of Evodiamine against chemotherapy induced diarrhea.

Keywords:

Evodiamine, Chemotherapy, Diarrhea, TCM, Bioactive compound, Phytomedicine, Swiss albino rat, *In-vivo*, Adjuvant therapy

1. Introduction

Cancer is a leading cause of death worldwide (McQuade et al, 2016). According to the information of International Agency for Research on cancer, 18.1 million new cases were diagnosed and 9.5 million cancer-related deaths were recorded worldwide in the year 2018 (National Cancer Institute, 2020). Although chemotherapy has greatly improved the overall survival rate and duration of cancer patients, the chemotherapeutic drugs used for the treatment of cancers induce various types of toxicities and severe side-effects, such as diarrhoea, constipation, nausea, vomiting, ulceration, bloating, hair loss, bone marrow suppression, loss of immunity, and cardiotoxicity, etc. (Wu et al 2019; McQuade et al, 2016). Chemotherapy-induced toxicities and adverse-effects badly influence to compromise the clinical application of anticancer drugs (Iwamoto, 2013). Chemotherapy-induced diarrhea very badly interferes with the alterations of chemotherapy treatment of approximately 60% of patients, dose reductions in 22% of patients, dose delays in 28% of patients and discontinuation of treatment in case of 15% of patients (Arbuckle et al., 2000; Dranitsaris et al., 2005) along with the deterioration of the Quality-Of-Life (QOL) of cancer survivors (Benson et al., 2004; Stringer et al., 2007, 2009; Denlinger and Barsevick, 2009).

Moreover, the rate of chronic post-treatment CID among the cancer survivors has been estimated to be as high as 49% that has been reported to last up to 10 years after the ending of chemotherapy treatment (McQuade et al, 2016; Kim et al., 2012; Denlinger and Barsevick, 2009; Schneider et al., 2007). Although the underlying mechanisms of chemotherapy induced diarrhea could not understand clearly, it is assumed that the mucositis with inflammation and ulceration of the intestinal epithelium is the main contributor for the chemotherapy induced diarrhea (McQuade et al, 2016). However, the chemotherapy induced diarrhea can even produce life-threatening toxic symptoms including electrolyte imbalances, dehydration and leading to death, even in patients in good physical condition, if emerging steps have not been taken immediately.

The general approach for the treatment of diarrhea is to administer antimotility, antisecretory agents, opioids and their derivatives, such as diphenoxylate and loperamide, and antimicrobial agents such as, fluoroquinolones and third-generation cephalosporins in case of empirical treatment of severe acute infectious diarrhea (Brunton et al 2008; DuPont et al 2001; Diniz-Santos et al 2006). But currently available antidiarrheal drugs have several limitations with adverse effects and contraindications including the development of drug-resistance, specifically against antibiotics used in diarrheal treatment [Stein 2010; Alam and Bhatnagar, 2006].

Traditional Chinese medicine has a long history of use for the treatment of different types of ailments for more than 3000 years back (Fang 2020). Recently traditional Chinese medicines have attracted the attention of researchers for the investigations of antidiarrheal drugs, more specifically for the treatment of chemotherapy induced diarrhea. Evodiamine is an important bioactive compound (quinolone alkaloid) of the fruit of a traditional Chinese medicine *Evodia rutaecarpa* Benth of the genus Evodia (Yu et al, 2013). The fruit is traditionally used for the treatment of diarrhea among others. The chemical name of Evodiamine is ((+)-(S)-8,13,13b,14-tetrahydro-14-methylindolo[2′,3′:3,4] pyrido[2,1-b] quinazolin-5(7H)-one) indoloquinazoline alkaloid (Gavaraskar et al., 2015). The plant is traditionally used for the treatment of headache, abdominal pain, postpartum hemorrhage, dysentery and amenorrhea (Fang et al 2020; Lee et al 2008). Scientific studies demonstrated its anticancer, antidiabetic, protection and treatment of cardiovascular diseases, antihyperlipidemic, anti-inflammatory, anti-microbial, anti-nociceptive activity and anti-neurodegenerative activity including anti-Alzheimer's activities (Jiang 2009; Fang 2020; Yu et al., 2013; Yu et al 2016; Wang, Z.Y. et al., 2016; Cai, Q.Y. et al., 2014). The scientific investigations for the antidiarrheal activity of evodamine has not yet been explored. Therefore, we aimed to evaluate the pharmacological potential and toxicological studies of evodamine, the main bioactive compound of the fruit of *Evodia rutaecarpa* Benth, a popular traditional Chinese medicine, for the prevention and treatment of chemotherapy-induced diarrhea in experimental rats.

2. Materials and Methods

2.1 Chemicals and reagents

Loperamide was purchased from Shandong Yihong Chemical Co. Ltd, China. 5-Flurouracil (5-FU), Ketamine HCl and xylazine HCl were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other analytical grade reagents were locally procured and purchased.

2.2 Evodiamine as bioactive compound of TCM

Evodiamine was purchased as Evodia extract containing 80% evodiamine from Bolise Co. Ltd., Xiamen, China.

2.3 Experimental animals

Experiments were conducted using female Swiss Albino rats, age between 8-12 weeks. The rats were purchased from Shanghai Laboratory Animal Center (SLAC, Shanghai, China) and housed six per plastic cages provided with wood chip bedding, maintained with 12/12 h light-dark cycle and allowed free access to standard rodent diet and water *ad libitum*. Environmental changes were strictly controlled and prior to any experiment, all the animals were kept for 1 week to adjust with the new housing environment.

2.4 Ethical Approval of Experimental Protocol

The ethical approval was obtained from the Animal Ethics Committee of Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, Henan, China (Approval Number: 202015-41). All the experiments were conducted according to the approved Animal Use Protocol by the Ethics Committee and following the rules, regulations and laws of Henan prefecture of China, and in accordance with the Guidelines for Care and Use of Laboratory Animals published by the US National Institutes of Health. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines and recommendations were followed to reduce the pain and stress of the experimental animals. At the end of the experiments, the rats were sacrificed with anesthesia overdose: Ketamine HCl (100 mg/kg) and xylazine (10 mg/kg) through intra-peritoneal route (Davis, 2001).

2.5 Determination of Acute toxicity level of Evodiamine

Oral acute toxicity study (LD_{50} determination) of evodiamine 80% extract was performed following the OECD (Organization for Economic Cooperation and Development) by Fixed Dose Procedure (OECD protocol no. 420) as followed by (Kifayatullah et al 2015). Briefly, Swiss Albinon female (nulliparous and non-pregnant) rats, age: 8 weeks, were acclimized to

laboratory conditions 7 days prior to experiment. The rats were divided into five groups, each comprising 5 animals. Group-1 served as the untreated control (received water only), group-2, 3 and 4 received Evodiamine doses 300 mg/kg, 1000 mg/kg, and 2000 mg/kg, respectively. The rats were overnight fasted for food (not water) before dosing and fasted for food 3-4 hours after the administration of doses. The animals were observed individually during the first 30 minutes after dosing, special attention was given during the first 4 hours, then to observe periodically during the first 24 hours to see any toxic effect in the animals. During the entire period of observation for 14 days, the animals were observed and monitored for any changes in the behavior, body weight, urinations, food intake, water intake, respiration, convulsion, tremor, temperature, constipation, changes in eye and skin colors, and mortality of the animals.

2.6 Experiment design

Experimental method was designed as described by Wang et al, 2019 with some modification. Forty Swiss Albino female rats were randomly divided into eight groups, each group contained 5 rats:

Group-1 : Non-diarrheal control (NDC) - rats received saline only

Group-2 : Diarrheal control (DC) - rats received 5-FU (50 mg/kg/day, i.p.) for 7 days to induce diarrhea but no treatment

Group-3 : Rats received 5-FU for 7 days + Evodiamine (12.5 mg/kg) treatment for 13 days

Group-4 : Rats received 5-FU for 7 days + Evodiamine (25 mg/kg) treatment for 13 days

Group-5 : Rats received 5-FU for 7 days + Evodiamine (50 mg/kg) treatment for 13 days

Group-6 : Rats received 5-FU for 7 days + Evodiamine (100 mg/kg) treatment for 13 days

Group-7 : Rats received 5-FU for 7 days + Loperamide (3 mg/kg) treatment for 13 days

The rats were administered with the prescribed above mentioned doses of Evodiamine or loperamide for the first 3 days of experiments before the induction of diarrhea. Then 5-FU (50 mg/kg/day, i.p.) was injected to rats for 7 days. Evodiamine was suspended in 0.5 % carboxymethylcellulose sodium (CMC-Na) aqueous solution. Rats were orally administrated with Evodiamine or loperamide 30 minutes before the administration of 5-FU chemotherapy. At the end of 5-FU therapy, the rats were further treated with Evodiamine for 3 more consecutive days.

2.7 Development of chemotherapy-induced diarrhea in rats

Swiss Albino female rats (age: 8-12 weeks) were treated intraperitoneally (i.p.) with 5-fluorouracil (5-FU) doses at 50 mg/kg dissolved in saline for 7 consecutive days for the induction of diarrhea.

2.8 Clinical observations and diarrheal assessment

Both body weights and diarrhea states were recorded every day. All animals were checked 4-times daily and diarrhea recorded according to the grading mentioned by Stringer et al 2006. The grading was as follows based on clinical symptoms of diarrhea:

No diarrhea = 0

Mild diarrhea (staining of anus) = 1

Moderate diarrhea (staining over top of legs and lower abdomen) = 2

Severe diarrhea (staining over legs and higher abdomen, often with continual anal leakage) = 3

All diarrhea assessments were conducted in a blinded fashion by 2 investigators (XZ and CQ).

The rate of diarrhea was calculated based on the following formula:

Diarrhea rate (%) = Number of diarrheal rats/the number of rats in each group × 100%.

Twenty-four hours after the last treatment, fecal sample (4 g) of each rat was collected in sterile plastic tube and stored at -80 °C. The rats were sacrificed by anaesthesia overdose and then thymuses and spleens were collected. Thymus and spleen index of each rat was calculated according to the following formula:

Thymus/spleen index = thymus/spleen weight/body weight (mg/g).

2.10 Statistical analysis

The data were analyzed by one way analysis of variance (ANOVA) using Statistical Package SPSS (version 25.0) software of IBM (International Business Machines) Corporation, USA, followed by Dunnett's-T3 test to determine statistical significance between groups. The data are means ± S.E.M. (standard error mean) of five animals. The p-value, $p < 0.05$ was considered as statistically significant.

3. Results

3.1 Five -Fluorouracil (5-FU) induced diarrhea in Swiss albino rats

The administration of 5-FU at the dose of 50 mg/kg through intra-peritoneal (i.p.) route successfully induced mild diarrhea to 40% rats of diarrheal control group 8 hours after administration of 5-FU. The peak incidence of diarrhea was induced to 100% of test animals

in diarrhea control group on 4th day of 5-FU administration (experiment day7) which continues up to 7th day of 5-FU induction (experiment day 10). However, after stopping of 5-FU treatment, the rate of diarrhea reduced to 80% on next day and ended to 60% on experiment day 13 (Table 2).

3.2 Evodiamine improved 5-FU induced body weight loss in experimental rats

Treatment of experimental Swiss albino rats with 5-FU (50 mg/kg) decreased body weight of rats in diarrheal control group every day for seven days of 5-FU treatment. However, after the ending of 5-FU treatment, the weight loss has stopped and the rats gradually started to regain weight on day 13 of experiment. The effect of Evodiamine on the 5-FU induced weight loss in Swiss albino female rats have presented in Table 1 and Figure 2.

Everyday weights were recorded and the weight records before starting 5-FU treatment (experiment day 3), on 5-FU ending day and the whole experiment ending day were statistically analyzed. As we can see in Table 1 and Fig. 2, 5-FU treatment significantly reduced body weight of rats on day 7 of 5-FU treatment (**$p<0.01$, weight loss 13.72%) and 3 days after stopping of 5-FU treatment (experiment day10) (**$p<0.01$, weight loss 14.33%) compared to that of 0 day of 5-FU administration. However, the administration of different doses of Evodiamine (12.5, 25, 50 and 100 mg/kg) and loperamide (50 mg/kg) prevented the remarkable reduction of body weight in 5-FU induced experimental rats. However, a marginal insignificant reduction of body weight was observed in Evodiamine treated groups which has be reversed on next day just after the ending of 5-FU treatment.

3.3 Evodiamine ameliorates the incidence of 5-FU induced diarrhea in rats

The incidences of diarrhea have been presented in Table 2 and Fig. 3. Administration of 5-FU induced diarrhea among 40% of experimental rats on the 1st day of treatment and the incidence of diarrhea has increased everyday of 7 days treatment with 5-FU. The highest incidence (rate of diarrheal case: 100%) of diarrhea was observed on day 4 of 5-FU treatment which continues for 7 days up to end of 5-FU treatment. However, treatment of diarrheal rats with Evodiamine (12.5, 25, 50 and 100 mg/kg) dose dependently reduced the rate of diarrheal cases. After withdrawal of 5-FU administration, the diarrheal cases started to disappear and no cases of diarrhea was found on day 12 and 13 of experiment in Evodiamine 50 and 100 mg/kg, as well as loperamide (3 mg/kg) treatment.

3.3 Evodiamine improved 5-FU induced diarrhea score in Swiss albino rats

The effect of Evodiamine on 5-FU induced diarrhea score has been presented in Table 3 and Figure 3. The rate and score of diarrhea have been recorded everyday with the onset of 5-FU treatment. As shown in the Table 3 and Fig. 3(A), administration of 5-FU on the first day of 5-FU treatment (4^{th} day of experiment) caused 40% cases of diarrhea in experimental rats with score 1 of each. Evodiamine at doses 12.5 and 25 mg/kg treatment groups were observed to have 20% cases of diarrhea with a score of 1 in both of the cases; whereas Evodiamine doses 50 and 100 mg/kg treatment prevented the induction of diarrhea with a score of zero (0). Loperamide 3 mg/kg also exhibited similar effect on rats with diarrhea score of zero (0). The diarrhea score has continued to increase everyday with 5-FU treatment group and on day 4 and 5 of 5-FU treatment (experiment day 7), 100% incidence of diarrhea was observed among the treated group with diarrhea score of 2 for all the animals (Table 3, Fig. 3D and 3E). On day 4^{th} and 5^{th} of 5-FU treatment, both of the Evodiamine at 50 and 100 mg/kg prevented the incidence of diarrhea to 20% with a diarrhea score of 1, similar to that of loperamide treatment group. On day 6 and 7 of 5-FU treatment (experiment day 9 and 10), 100% incidence of diarrhea was observed among the animals of 5-FU control group with a diarrhea score 2 (80%) and 3(20%) (Fig. 3F and 3G, and Table 3). Evodiamine at the dose of 50 mg/kg prevented the incidence of diarrhea to 40% with a score of 1 on experiment day 9 and 10. Whereas, Evodiamine at the dose of 100 mg/kg on the same days similarly but more potentially prevented the diarrhea incidence to 40% and 20%, respectively with a diarrhea score of 1 on both of the days. No case of diarrhea was observed in case of experimental rats treated with Evodiamine 50 and 100 mg/kg one day after the withdrawal of 5-FU treatment (Fig. 3I and 3J). Our investigation resulted with the dose-dependent improvement of the incidence and score of diarrhea similar with that of loperamide 3 mg/kg.

3.4 Evodiamine improved thymus and spleen indexes in 5-FU induced diarrheal rats

The effect of Evodiamine on thymus and spleen indexes has been presented in Fig. 4(A) and Fig. 4(B), respectively. The rats were pre-treated with Evodiamine for 3 days before, during and after the administration of 5-FU (50 mg/kg/day, i.p.) for 7 consecutive days. At the end of 13 days experiment period, the rats were sacrificed and thymus/spleen index (thymus/spleen weight in mg/weight of rat in g) was calculated as mentioned in the Methodology section. Our study resulted that treatment of rats with 5-FU significantly reduced thymus index (### p <0.001) and spleen index (## p <0.01) after 7 days of 5-FU treatment. However, treatment of rats with Evodiamine at the doses of 25 mg/kg (*p <0.05), 50 mg/kg (*p <0.05), 100 mg/kg (**p <0.01) and loperamide 3 mg/kg (**p <0.01)

significantly increased the thymus index (Fig. 4A). Similarly, Evodiamine treatment at the doses of 50 mg/kg (*p <0.05), 100 mg/kg (*p <0.05) and loperamide 3 mg/kg (*p <0.05) significantly enhanced spleen index (Fig. 4B).

3.5 Oral acute toxicity study of Evodiamine in laboratory rats

Oral acute toxicity study of Evodiamine was carried out following the OECD guideline-Fixed Dose Procedure (OECD protocol no. 420) as mentioned in the Methodology section. No case of Mortality was observed during the 14 days of treatment with a limited dose of 2000 mg/kg body weight of Evodiamine. The treated animals could tolerate the Evodiamine doses and there was no statistically significant difference in body weight between the treated and untreated groups. The animals did not exhibit any abnormalities or major behavioural changes such as respiratory distress, abnormal locomotion, tremors, salivation, diarrhoea, sleep, walking backwards, reactions to handling, catalepsy, coma or any toxic symptoms either immediately or during the post-treatment observational period of 14 days. Thus, we can say that the LD_{50} for oral administration of Evodiamine is higher than 2000 mg/kg B.W. Therefore, the used doses of Evodiamine (12.5 - 100 mg/kg) were well tolerated by the animals.

4. Discussion

Chemotherapy induced diarrhea is one of the major complications in the treatment of cancer patients which retards the effective treatment regimen, hindrances to reduce the chemotherapy dose below the therapeutic level and ultimately causes failure of chemotherapeutic treatment.

Loperamide is an agonist on opioid receptor in the GI tract which decreases peristalsis and increases fluid reabsorption (Kornblau et al., 2000). High dose loperamide (a synthetic opiate derivative) alleviates diarrhea associated with chemotherapeutic administration. However, loperamide is used as the first line standard drug for the treatment of chemotherapy induced diarrhea despite of but exerts several side-effects including severe constipation, abdominal pain, dizziness, dry mouth, rashes, drowsiness, dizziness and worsening of bloating, nausea, vomiting (McQuade et al., 2016; Stein et al., 2010; Lenfers et al., 1999; Sharma et al., 2005; Richardson and Dobish, 2007). Loperamide can cause a paralytic ileus, and patients should be routinely monitored while using high-dose loperamide (Kornblau et

al., 2000). Besides, the effectiveness of loperamide as monotherapy for severe diarrhea is limited (Saltz, 2003; Yang et al., 2005). Due to the severe side-effects of currently available drugs for the treatment of chemotherapy-induced diarrhea and other complications, the scientists all over the world are in search for the novel agents, specially phytomedicines for the treatment of chemotherapy related complications.

Recently, traditional Chinese medicine (TCM) has attracted the attention and interest of investigators and medical professionals to control cancer-associated side-effects and to decrease the chemotherapy-related toxicities. Evodiamine is an indoloquinazoline alkaloid is a major bioactive compound isolated from the fruits of a Traditional Chinese medicinal plant *Evodia rutaecarpa* Benth under Rutaceae family. Evodiamine and its derivatives have been scientifically investigated and established for its different pharmacological actions including against weight management, as anti-cancer, anti-diabetic and anti-inflammatory properties (Gavaraskar et al., 2015). However, the therapeutic potential of Evodiamine against chemotherapy induced diarrhea has not been investigated by any researcher.

The present study evaluated the preventive and treatment potential of Evodiamine (the bioactive compound from the fruit of a Traditional Chinese medicinal plant- *Evodia turaecarpa* Benth) against chemotherapy induced diarrhea (CID). Our investigation resulted that Evodiamine dose-dependently prevented the incidence and improved the conditions of 5-FU (50/kg) induced diarrhea with the reduction of diarrhea rate and score in Swiss albino rats (Table 2 and 3, and Fig. 3). Besides, Evodiamine treatment significantly improved chemotherapy induced body weight, and thumus and spleen indexes in rats (Fig. 2 and 4). Evodiamine thus prevented and improved the rate and intensity of diarrhea as well as diarrhea related other factors such as loss of body weight and organ weight (thymus and spleen).

Chemotherapy-induced diarrhea may be classified as uncomplicated (grade1–2 with no complications) or complicated (grade 3–4 with one or more complicating signs or symptoms), early on set (<24 h after administration) or late onset (>24 h after administration) and may be categorized as persistent (present for >4 weeks) or non-persistent (present for <4 weeks) according to The National Cancer Institute's Common Terminology Criteria for Adverse Effects grading system (McQuade et al., 2016; Stein et al., 2010;). Uncomplicated CID may be managed by modification of the diet and administration of standard anti-

diarrheal drugs, but complicated diarrhea requires aggressive high dose anti-diarrheal drugs and hospitalization (McQuade et al., 2014, McQuade et al., 2016).

Our result showed that prior to inducing diarrhea with 5-FU, Evodiamine had no negative effect on bodyweight, rather a gradual increase of weight has been marked. However, treatment with 5-FU resulted in a significant reduction in bodyweight in 5-FU control group when compared between before and after 5-FU treatments (**$P < 0.01$) (Fig. 2, Table 1). Evodiamine attenuated the 5-Fu induced body weight loss of rats in a dose dependent manner, indicating improvements in food intake and nutrition in addition to the loss of intestinal contents due to reduction of incidence and severity of diarrhea. The chemotherapy induced diarrhoea might be associated with the altered gut motility, impairing water absorption and intestinal microflora (Gibson and Keefe, 2006; Gibson and Stringer, 2009). Bleeding is usually accompanied with diarrhoea in patients undergoing chemotherapy (Gibson and Keefe, 2006; Gibson and Stringer, 2009). We have observed that faecal blood has come out in grade 3 diarrhea in rats challenged with 5-FU at day 6 and 7 (Fig. 3F and 3G), and Evodiamine treatment at doses 25, 50 and 100 mg/kg dose of CS treatment prevented the stool bleeding. This finding suggested that Evodiamine also has a capacity for reducing the ulcerative lesions in gastrointestinal track.

Previous studies showed that administration of 5-FU significantly decreased feed intake and bodyweight (Bajic et al., 2016; Mashtoub et al., 2013; Torres et al., 2008). A reduction in feed intake and bodyweight is observed in cancer patients because of nausea and pain associated with chemotherapy treatment (Green et al., 2010; Smith et al., 2008). In our study, the daily administration of Evodiamine to 5-FU treated rats significantly protected the loss of body weight and maintained the bodyweight nearly the normal level (Table 1 and Fig. 2).

5-FU is frequently used as a potential chemotherapeutic agent specially for treating gastrointestinal malignancy. However, approximately 80% of patients receiving 5-FU develop chemotherapy-induced mucositis including diarrhea (Smith et al., 2008). This adverse-effect can worsen the quality of life in patients undergoing chemotherapy and can cause an early cessation of chemotherapy. Therefore, effective preventive and therapeutic agents against chemotherapy-induced diarrhea are required.

In this study, all the model rats showed the clinical symptoms of diarrhea. We also found that thymus and spleen indexes in the model rats decreased significantly compared with those of the control group. Thymus and spleen indexes, whose levels depend on the extent of lymphocyte proliferation, can be used to partly reflect host immune function (Yuan et al., 2009; Wang et al., 2019). The results indicated that the immune system of the model group was affected by 5-Fu chemotherapy.

5. Conclusion

Our study clearly demonstrated that Evodiamine, in a dose-dependent manner, prevented and potentially attenuated the incidence and severity of 5-FU induced diarrhea in experimental rat model. Treatment of chemotherapy induced rats with Evodiamine also significantly prevented and retarded the loss of body weight, and thymus and spleen weight indexes as well. This is the first report of Evodiamine for the effect on chemotherapy induced diarrhea. From our findings we can conclude that Evodiamine can be a potential candidate for the prevention and treatment of chemotherapy-induced diarrhea. However, as this is a preliminary report, further investigations are encouraged.

List of abbreviations

CID: Chemotherapy induced diarrhea*;* OECD: Organization for Economic Cooperation and Development; TCM: Traditional Chinese Medicine; 5-FU: 5-Fluorouracil; Evo: Evodiamine; NDC: Non-diarrheal control; DC: Diarrheal control; ANOVA: Analysis of variance.

Consent of publication

All the authors of this manuscript have consented to publish the article and they don’t have any conflict of interest on this article.

Availability of data and materials

All data generated or analyzed during this study have been included in the article and its supplementary files as Tables and Figures. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Further information on data will be made available on request through the Animal Ethics Committee of Affiliated Cancer Hospital of Zhengzhou University, China.

Competing interests

The authors declare that they have no competing interests on the research work and to publish the article.

Ethical approval

The ethical approval for experimental protocol of animals care and use in this study was obtained from the Animal Ethics Committee of Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, Henan, China (Approval Number: 202015-41).

Statement of human and animal rights

All the experiments involving animals were conducted according to the approved Experimental Animal Care and Use Protocol by the Animal Ethics Committee of Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, Henan, China, and in accordance with the Guidelines for Care and Use of Laboratory Animals published by the US National Institutes of Health. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines and recommendations were followed to reduce the pain and stress of the experimental animals.

Statement of informed consent

There are no human subjects in this article and informed consent is not applicable.

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Figure Legends

Fig. 1: Induction of diarrhea and treatment pattern of Evodiamine. Swiss albino female rats (8-12 weeks) were pre-treated with Evodiamine doses at 12.5, 25, 50 and 100 mg/kg or loperamide (3 mg/kg) orally for the first 3 days of experiments before the induction of diarrhea. Then 5-FU (50 mg/kg/day, i.p.) was injected to rats for 7 consecutive days. Evodiamine or loperamide was administered 30 minutes before the injection of 5-FU chemotherapy. At the end of 5-FU therapy, the rats were further treated with Evodiamine and loperamide for 3 more consecutive days. At the end of 13 days experiments, all the rats were sacrificed.

Fig. 2: Effect of Evodiamine on the change of body weight induced by 5-FU treatment. Swiss albino female rats (8-12 weeks) were pre-treated with Evodiamine (12.5, 25, 50 and 100 mg/kg) or loperamide (3 mg/kg) for the first 3 days. Then 5-FU (50 mg/kg/day, i.p.) was injected to rats for 7 consecutive days starting from day 4 to day10. At the end of 5-FU therapy, the rats were further treated with Evodiamine and loperamide for 3 more consecutive days up to day13. The body were recorded everyday but statistical analysis was performed for body weights on just before and after 5-FU treatment and at the end of the experiment. The data are S.E.M. of five animals in each group. **$p<0.01$ compared to day 4 among the group.

Fig. 3: Effect of Evodiamine on the rate and intensity of chemotherapy (5-FU) induced diarrhea. Swiss albino female rats (8-12 weeks) were pre-treated with Evodiamine (12.5, 25, 50 and 100 mg/kg) or loperamide (3 mg/kg) for the first 3 days. Then 5-FU (50 mg/kg/day, i.p.) was injected to rats for 7 consecutive days starting from day 4 to day10. At the end of 5-FU therapy, the rats were further treated with Evodiamine and loperamide for 3 more consecutive days up to day13. The rate and intensity of diarrhea was measured as described in the Methodology section. Mild diarrhea: staining of anus; Moderate diarrhea: staining over top of legs and lower abdomen; Severe diarrhea: staining over legs and higher abdomen, often with continual anal leakage.

Fig. 4: Effect of Evodiamine on the thymus and spleen index. Swiss albino female rats (8-12 weeks) were pre-treated with Evodiamine (12.5, 25, 50 and 100 mg/kg) or loperamide (3 mg/kg) for the first 3 days. Then 5-FU (50 mg/kg/day, i.p.) was injected to rats for 7 consecutive days starting from day 4 to day10. At the end of 5-FU therapy, the rats were further treated with Evodiamine and loperamide for 3 more consecutive days up to day13. At the end of the experiment, the rats were sacrificed and thymus and spleen weights were measured to calculate the thymus (Fig. 4A) and spleen index (Fig. 4B) as mentioned in the Methodology section. The data are S.E.M. of five animals in each group. ## $p<0.01$ (Fig. 4B), ### $p<0.001$ (Fig. 4A) compared to non-diarrheal control (NDC) group; *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to diarrheal control (DC) group. Evo: Evodiamine.