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Results of Chemical and Pharmacological Study of the Mongolian Traditional Prescription "Indra-4"

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CC, UB, DB and AA conducted pharmacological experiments and performed statistical analysis on the results. Authors NT and MU identified and quantified the biologically active compounds present in the traditional medicine Indra-4. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aims to identify the biologically active ingredients in the Indra-4 prescription and evaluate their effects on rats with acute inflammatory bowel disease (IBD) induced by lipopolysaccharide (LPS).

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Methodology: Thin-layer chromatography (TLC) was used to identify bioactive compounds in the prescription, while UV/Vis spectrophotometry measured total phenolic compounds, flavonoids, triterpene saponins, and coumarins. Forty adult Wistar rats were divided into four groups: Normal, LPS (control group), Diarex (reference treatment), and Indra-4 (test group). The study investigates how these components impact the condition of IBD in the rats. Statistical analysis was performed using GraphPad Prism version 9.0.
Results: The study is the first to analyze the chemical and pharmacological properties of the traditional Indra-4 prescription, revealing that it contains polyphenolic compounds 2.38 ± 0.031% (gallic acid equivalent), flavonoids 0.28±0.034% (quercetin equivalent), coumarins 1.61±0.27% (isofraxedin equivalent), and triterpene saponins 0.9 ± 0.03% (oleanolic acid equivalent). In an enteritis model, a 300 mg/kg dose of Indra-4 reduced diarrhea by 68.9% compared to a control group treated with LPS ($p<0.01$). It also increased plasma sodium, potassium, and chloride plasma levels and lowered serum prostaglandin E2 levels by 30.8%, indicating potential anti-inflammatory effects ($p<0.05$). These findings suggest that Indra-4 may help alleviate diarrhea and inflammation in LPS-induced enteritis, providing a basis for its possible use in treating acute inflammatory bowel disease (IBD).
Conclusions: We conducted the first chemical and pharmacological analysis of the traditional Indra-4 prescription and discovered that it primarily contains polyphenolic compound derivatives, including flavonoids, coumarins, and triterpene saponins. These biologically active compounds exhibit various pharmacological actions and therapeutic effects. In the acute inflammatory enteritis disease model induced by lipopolysaccharide, the Indra-4 prescription demonstrates an antidiarrheal effect by decreasing prostaglandin E2 levels and preventing electrolyte loss, as confirmed through laboratory and histological analyses.

Keywords: Indra-4 prescription; traditional medicine; lipopolysaccharide.

1. INTRODUCTION

Each year, approximately 1.7 billion individuals worldwide are affected by diarrhea from various causes, leading to around 525,000 fatalities (Parcoportofino, 2021). In recent years, extensive research has concentrated on pharmacological agents and medicinal plants that can effectively treat and alleviate symptoms of inflammatory bowel disease, which are becoming increasingly prevalent (World Health Organization, 2017). Mongolian traditional medicine, with a history spanning approximately 5,000 years, has developed a diverse array of prescriptions for treating various diseases. These prescriptions primarily utilize natural raw materials, including plants, animals, and minerals. However, I would like to point out that not all traditional prescriptions have undergone comprehensive scientific study or validation (Bold, 2002).

Mongolian traditional medicine uses antidiarrheal prescriptions, with the Indra-4 prescription being particularly effective in treating diarrhea from various causes. However, further research is needed to identify the biologically active compounds in Indra-4 and understand the mechanisms behind their therapeutic effects. This highlights the need for more in-depth studies to comprehend its action fully (Jambal Choiji Danzan Perenlei, 2014).

Prescriptions used in traditional medicine for the treatment of various diseases are often multi-ingredient. This is because the raw materials included in the prescription are selected to reduce each other's negative effects and support positive beneficial effects, serving multiple purposes. Natural-based therapeutic preparations are characterized by their low side effects and compatibility with the human body. Scientists refer to natural-based medicinal preparations as the 'healing remedy of the 21st century' and are producing them in purified, complex forms (Dagvatseren et al., 2005).

The Indra-4 recipe consists of four herbs: *Holarrhena antidysenterica*, *Aconitum kuznezoffii*, *Polygonum bistorta*, and *Clematis tangutica*. The prescription shares common characteristics, including a brown color, a bitter taste, and cooling properties (Oldokh et al., 2013; Dagvatseren et al., 2014).

To establish the scientific basis for the efficacy of the Indra-4 prescription in treating diarrhea, we focused on identifying its bioactive compounds and analyzing their specific effects on the condition.

This study seeks to identify the biologically active compounds in Indra-4 and scientifically validate their traditional use for diarrhea treatment.

2. MATERIALS AND METHODS

2.1 Materials

The study employed the Indra-4 prescription, produced by the Traditional Medicine Factory at the Institute of Traditional Medicine and Technology.

Chemical reagents: Gallic acid (≥98%), quercetin (≥98.1%), umbelliferone (≥98%), and oleanolic acid (≥98%) were sourced from suppliers in Shanghai, China. The Folin-Ciocalteu phenol reagent (≥98%) was obtained from Sangon, China. All other reagents used were of analytical grade. Lipopolysaccharides from Escherichia coli 055: B5 (L2880-100MG) were procured from SIGMA-ALDRICH, USA. MLBio Co., Ltd, China, supplied the PGE2-rat ELISA kit, while the Na-rat, K-rat, and Cl-rat test kits were provided by Biobase Biodustry (Shandong) Co., Ltd, China.

2.2 Animals

An adult Wistar rat weighing 180-220 grams was obtained from the experimental animal vivar of the Institute of Traditional Medicine and Technology. The vivar maintained a temperature of 20±1°C, 50-60% humidity, a 12-hour light/dark cycle, and air circulation of 8-15 times per hour. Forty rats were used in the study, and they were provided with fresh water and a specific animal feed.

2.3 Chemical Analysis

UV/Vis spectroscopy: Absorbance measurements were performed using a UV-2102 UNICO spectrophotometer manufactured in China.

Thin Layer Chromatography (TLC) method: Weigh 0.5 g of the Indra-4 prescription and extract it with ethyl acetate. Filter the extract and allow it to sit at room temperature for 24 hours. Subsequently, the supernatant was collected using a glass tube and applied to a silica gel glass plate alongside a 1 mg/ml solution of the standard substance (gallic acid). For thin-layer chromatography (TLC), utilize a solvent system composed of benzene, ethyl acetate, formic acid, and acetone in a ratio of 5:5:1:1 for spot extraction. Spray the plate with a 2% ferric chloride alcohol solution and place it in a drying oven at 100°C-105°C for 1-2 minutes. The spots will appear the same color as the standard substance under normal light.

To detect flavonoids, use a solvent system of toluene, ethyl acetate, chronic acid, and methanol in a ratio of 6:6:1.6:0.4. For coumarins, employ a mixture of toluene, ethyl acetate, and acetic acid in a ratio of 4.5:5:0.5. To detect triterpenes and saponins, utilize a benzene-acetone system in a ratio of 8:2. The detection solutions include 1% $FeCl_3$, 3% $AlCl_3$, 5% KOH, and a 5% vanillin-H_2SO_4 alcohol solution (Hildebert & Sabine, 1996; Pharmacopoeia of Raw Materials and Drugs Used in Traditional Mongolian Medicine, 2023; Nyamdemberel & Chimedragchaa, 2020).

2.4 Ultraviolet Spectrophotometric Method

Method for the Quantitative Determination of Total Phenolic Compounds in the Indra-4 Prescription: Measure 0.5 ml of a 70% ethanol solution of the research sample, 10 ml of distilled water, and 1 ml of the Folin-Ciocalteu selective reagent in a 25 ml volumetric flask. Subsequently, dilute the mixture with a 10.75% sodium carbonate solution. Allow the mixture to stand at room temperature for 30 to 40 minutes before measuring its absorbance using a UV spectrophotometer at a wavelength of 760 nm. Gallic acid equivalents will be utilized to quantify the polyphenolic compounds present in the Indra-4 prescription (Hildebert & Sabine, 1996; Pharmacopoeia of Raw Materials and Drugs Used in Traditional Mongolian Medicine, 2023; Nyamdemberel & Chimedragchaa, 2020).

Quantitative determination of total flavonoid content: Transfer 4 mL of a 70% ethanol solution of Indra-4 into a 25 mL volumetric flask. Add 0.5 mL of concentrated hydrochloric acid, mix thoroughly, and heat the mixture in a water bath at 40°C for 20 minutes. After heating, add 2 mL of 1% aluminum chloride and dilute the solution to the mark with 70% ethanol.

Allow the solution to equilibrate at room temperature for 20 minutes. Subsequently, the absorbance was measured using a UV spectrophotometer at 430 nm.

For the blank solution, transfer 4 mL of the 70% ethanol solution of the Indra-4 formulation into a 25 mL volumetric flask and dilute to the mark with 70% ethanol.

The total flavonoid content of the Indra-4 prescription was quantified in quercetin equivalents (Zhang, 2012; Wh et al., 1976).

Determination of the quantitative content of total triterpene saponins: Measure 0.4 mL of a 70% ethanol solution containing the sample (10 mg/mL), 0.4 mL of a 5% vanillin-acetic acid solution, and 2.4 mL of perchloric acid into a 10 mL volumetric flask. Stir the mixture thoroughly and heat in a water bath at 70°C for 15 minutes (Suparman, 2014). After cooling, add ethyl acetate to adjust the final volume to 10 mL. For the blank solution, combine 0.4 mL of the 70% ethanol solution, 0.4 mL of the 5% vanillin-acetic acid solution, and 2.4 mL of perchloric acid in a 10 mL volumetric flask. Stir well and heat in a water bath at 70°C for 15 minutes. After cooling, add ethyl acetate to achieve a total volume of 10 mL. Measure the absorbance using a UV spectrophotometer at a wavelength of 550 nm. The Oleanolic Acid Equivalent (OAE) represents the total triterpene saponins in the traditional Indra-4 prescription.

Determination of total quantitative content: In a 10 mL volumetric flask, add 1 mL of a 70% ethanol solution containing the sample at 10 mg/mL concentration (Suparman, 2014). Introduce 0.1 mL of concentrated hydrochloric acid and stir thoroughly. Heat the mixture in a water bath at 40°C for 20 minutes (MenidiMedica Biotechnology, n.d.). Subsequently, dilute to the mark with 70% ethanol. Allow the solution to equilibrate at room temperature for 15 minutes, then measure the absorbance using a UV spectrophotometer at 336 nm, using 70% ethanol as the blank (Sobha, 2013). The Isofraxcedin Equivalent (IFE) quantifies the total coumarin content in the traditional Indra-4 prescription (Pharmacopoeia of Raw Materials and Drugs Used in Traditional Mongolian Medicine, 2023).

2.5 Effect of the Traditional Indra-4 Prescription Enteritis Model in Rats

Detection of the inhibitory effect of the Indra-4 prescription on LPS-induced diarrhea in rats: The standard group, LPS group, Indra-4+LPS group, and Diarex+LPS group, with ten rats in each group. Before LPS exposure, the rats in the standard and LPS groups were administered distilled water. In contrast, the Indra-4+LPS group received Indra-4 at a dose of 300 mg/kg, while the Diarex+LPS group was administered Diarex at a dose of 500 mg/kg twice daily for five days (Sobha, 2013). Following this administration, the rats were provided with water only for 12 hours. One hour after the final drug administration, all groups, except for the standard group, were treated with LPS at a dose of 30 mg/kg (Liu et al., 2009; Haixia, et al., 2024).

Frequency of diarrhea detection before specimen collection: After treatment with LPS, each cage of rats had a filter paper couch that was changed hourly for four hours. The frequency of diarrhea was evaluated by counting the fecal deposits on the filter paper. Experiments were conducted with groups of 10 rats, and each experiment was replicated once. Following an overnight fast, the animals received an intraperitoneal injection of 250 µL of sterile 0.9% saline (vehicle), either with or without 1 mg/kg of E. coli 0111: B4-derived LPS (Sigma). The test compounds were administered orally two hours before the LPS injection. Six hours after LPS administration, the rats were euthanized, and jejunal segments were collected for intestinal permeability measurement using Ussing chambers and for evaluating inflammatory tone through myeloperoxidase (MPO) activity assay.

Histopathological analysis: An enteritis pathological model was established, and microstructural analysis of small intestine tissue was conducted using standard techniques for animal tissue and organ samples. The tissues were fixed in a 10% buffered formalin solution for 24 hours, washed with running water, and processed through ethanol and xylene before being embedded in paraffin (Peng, 2021). Sections were cut to a 2-5 µm thickness using a sled microtome (Yamato Kohki Industrial Co., Ltd), stained with hematoxylin-eosin, and prepared for microscopic examination with a Nikon Eclipse Ci microscope. Furthermore, histopathological analyses of small intestine tissue samples from the animals will be performed, and the results will be compared across different groups.

3. RESULTS AND DISCUSSION

3.1 TLC Result

Biologically active compounds in the traditional Indra-4 prescription were identified utilizing thin-layer chromatography (TLC). For the analysis of phenolic compounds, gallic acid was employed as a standard in a solvent system consisting of benzene, ethyl acetate, formic acid, and acetone (5:5:1:1). Following the application of a 2% ferric

chloride alcohol solution to the chromatogram, a dark blue spot was observed at the same level as the standard (Rf=0.51).

In the analysis of flavonoids, quercetin was used as the standard in a toluene, ethyl acetate, formic acid, and methanol (6:6:1.6:0.4) solvent system. The chromatogram was treated with a 3% AlCl3 alcohol solution, detecting a yellow spot exhibiting fluorescence at the same level as the standard (Rf=0.37). Additionally, coumarin was compared with umbelliferone in a toluene, ethyl acetate, and acetic acid (4.5:5:0.5) solvent system. Triterpene saponin was analyzed alongside oleanolic acid in a benzene and acetone (8:2) solvent system. Upon applying a detection solution of 5% KOH and 20% H2SO4, colors comparable to those of the standard substances were observed, with umbelliferone exhibiting blue fluorescence (Rf=0.65) and oleanolic acid displaying a purple-brown color (Rf=0.88).

3.2 Results from the UV/Vis Spectrophotometer

The polyphenolic compounds, total flavonoids, triterpene saponins, and total coumarins in the traditional Indra-4 prescription were quantified using a UV spectrophotometer. The polyphenolic content concerning gallic acid was determined. To establish the gallic acid's linearity, we measured the solutions' light absorption at concentrations of 0.24, 0.72, 1.2, 1.68, and 2.16 µg/mL (n=5, RSD=0.06445%). A standard curve was generated, resulting in the linear equation (Y=0.0849x+0.001, R^2=0.9994), and the results were expressed as a percentage (Fig. 1).

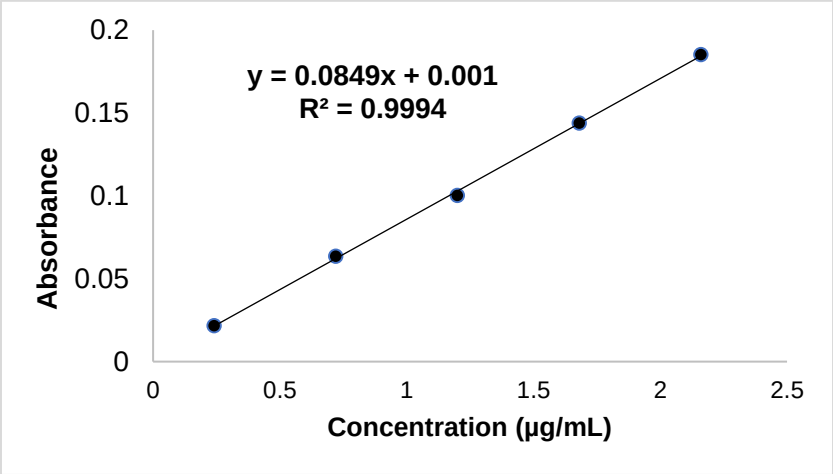

Fig. 1. Standard curve generated using a Gallic acid solution with concentrations ranging from 0.24 to 2.16 µg/mL

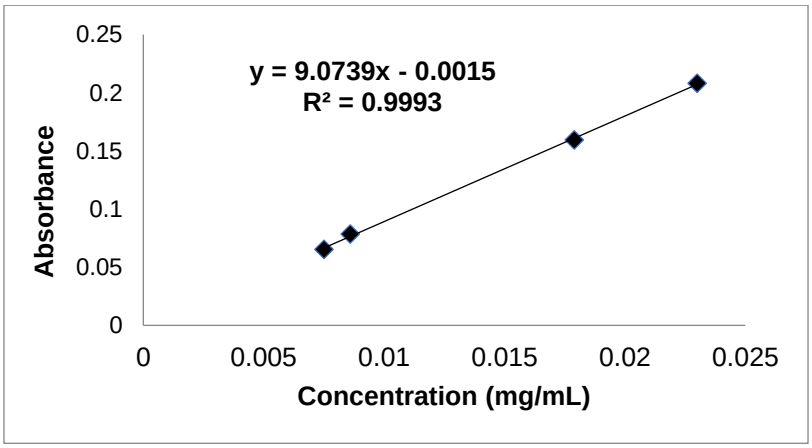

Fig. 2. The standard curve was generated using a quercetin solution with concentrations ranging from 0.0075 to 0.023 mg/mL

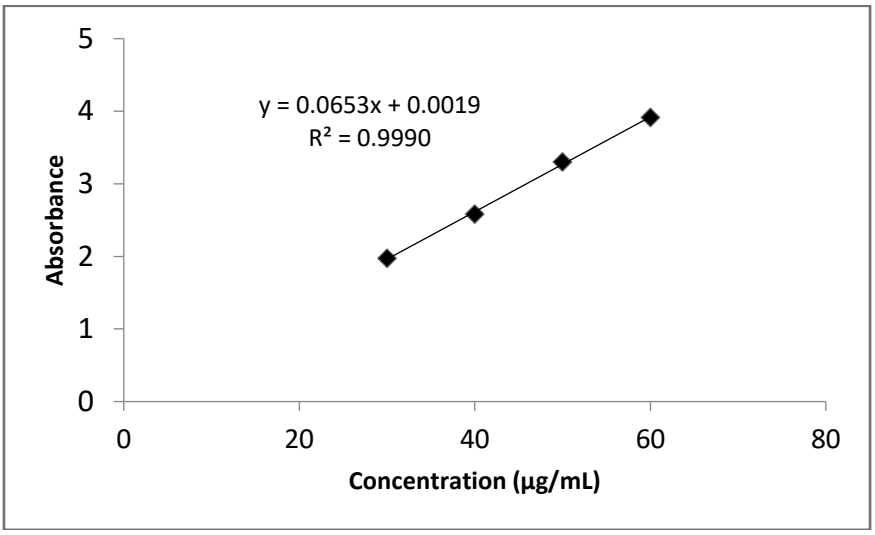

Fig. 3. The standard curve was generated using an isofraxedin solution with 30 to 60 µg/mL concentrations

The Indra-4 prescription contains a polyphenolic compound concentration of 2.38 ± 0.031%, as determined using the line equation in Fig. 1. A standard curve was established to evaluate the linearity of quercetin, measuring light absorption in solutions with concentrations of 0.0075, 0.0086, 0.0179, and 0.023 mg/mL (n = 4, RSD = 0.0074%). The linear equation obtained was Y = 9.0739x - 0.0015, with an R^2 value of 0.9993; this data is illustrated in Fig. 2.

The total flavonoid content was measured at 430 nm using a UV spectrophotometer, calculated in terms of quercetin equivalents using the equation Y=9.0739x−0.0015 (with R2=0.9993), and found to be 0.28±0.034%.

Researchers discovered that the traditional Indra-4 prescription, containing Polygonium bistorta and Clematis tangutica Korsh, includes phenolic compounds like gallic and tannic acids, phenolic carboxylic acids such as caffeic and chlorogenic acids, flavonoids including quercetin and kaempferol, and vitamin C.

The coumarin content in the Indra-4 prescription was measured using isofraxedin as a reference compound. Based on their light absorption, a calibration curve was created with isofraxedin solutions at 30, 40, 50, and 60 µg/mL concentrations. The resulting equation was y=0.0653x+0.0019, with a high correlation (R^2 = 0.9990). A relative standard deviation (RSD) of 0.84% (n=4) indicated the method's precision (Fig. 3).

The coumarin content in the Indra-4 prescription was measured using a UV spectrophotometer at a 336 nm wavelength. By comparing it to the reference substance isofraxedin (calibration equation: Y=0.0653x+0.0019), the coumarin content was calculated to be 1.61±0.27%.

The traditional Indra-4 prescription includes *Holarrhena antidysenterica* Wall, which contains coumarin compounds, particularly furacoumarin derivatives, that are significant in medicine. Coumarin is believed to help protect plants from parasites like fungi and bacteria. Furacoumarins have antispasmodic, vasodilating, and muscle-relaxing effects. However, some coumarins can increase skin sensitivity to UV rays, potentially causing inflammation.

The total triterpene saponins content in the Indra-4 prescription was measured using oleanolic acid as a standard. A reference curve was created with concentrations ranging from 2 to 10 µg/mL, showing a calibration equation of y=0.0821x−0.0207 and a high correlation (R^2 = 0.9997). The relative standard deviation was 0.12% across five measurements. (n=5, Fig. 4).

The total triterpene saponins were measured using a spectrophotometer at a 550 nm wavelength, with the results calculated as oleinolic acid. The equation used for calculation was Y = 0.0821x - 0.0207 (R^2 = 0.9997). The content of triterpene saponins was found to be 0.9 ± 0.03%. The statement attributes the therapeutic effects of *Clematis tangutica* Korsh to triterpene saponins, which are a vital component of its traditional medicinal use.

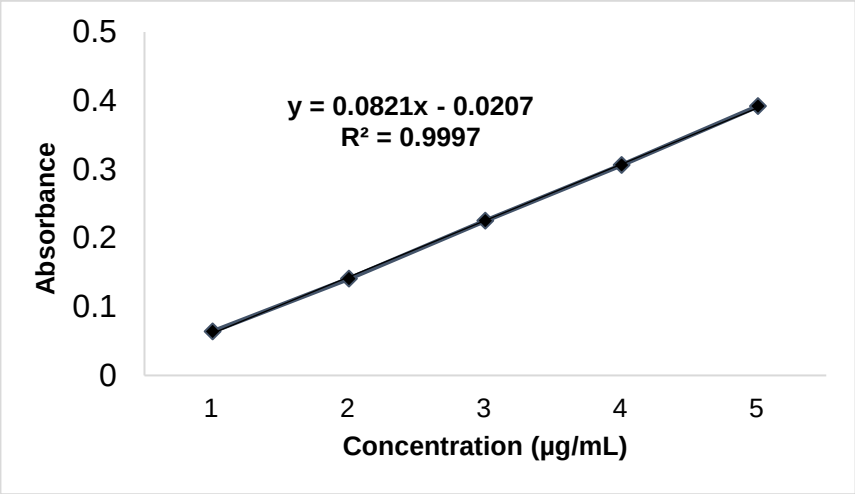

Fig. 4. The standard curve was generated using an Oleanolic acid solution with concentrations ranging from 2 to 10 µg/mL

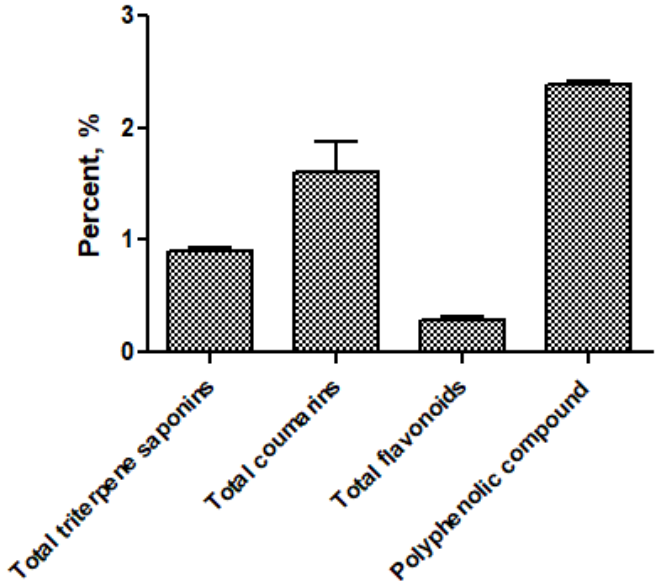

Fig. 5. Amount of biologically active compounds in Indra-4 traditional prescription

The phytochemical analysis of the traditional Indra-4 prescription reveals that polyphenols and coumarins are the most abundant biologically active compounds, while flavonoids and triterpene saponins are present in smaller quantities. This distribution corresponds directly to the medicinal raw materials used in the prescription.

3.3 Results of Anti-diarrhea Activity

The study by Jun Liu et al. (2009) involved creating an acute enteritis model using an intraperitoneal injection of 30 mg/kg lipopolysaccharide (LPS). We then performed biochemical analyses to measure sodium, potassium, and chloride levels. Additionally, we conducted an ELISA (enzyme-linked immunosorbent assay) to analyze prostaglandin E2 levels. Histopathological examination of the small intestine was also carried out to assess tissue changes associated with the disease.

Frequency of diarrhea after injection of LPS: The frequency of diarrhea was higher in the LPS, Indra-4+LPS, and Diarex+LPS groups compared to the standard group. The frequencies were as follows: LPS group (5.80±1.6), Indra-4+LPS

group (1.80±0.8), and Diarex+LPS group (3.50±1.0), compared to the standard group, which had a frequency of 0.60±0.5 P < 0.05).

The Table 1 shows that in the LPS-induced acute enteritis disease model, the number of animals in the control group increased by 89.6% compared to the healthy group at 4 hours, with the difference being statistically significant (p<0.01).

The study found that diarrhea was reduced by 68.9% in the group treated with Indra-4 at 300 mg/kg compared to the control group (p<0.01). Diarrhea was decreased by 39.6% in the group treated with Diarex at 500 mg/kg compared to the control group (p<0.05).

Serum electrolyte levels in rats: The study found that the levels of sodium (Na+), chloride (Cl-), and potassium (K+) were significantly elevated in the Indra-4+LPS and Diarex+LPS treatment groups when compared to the LPS group. These findings suggest that the treatments with Indra-4 and Diarex had a notable impact on the electrolyte balance in the presence of lipopolysaccharide (LPS), which is known to induce inflammatory responses in the body.

Na+ (Sodium levels):

- In the Indra-4+LPS group, the sodium concentration was 508.4±91.6 mmol/l, and in the Diarex+LPS group, it was 500.0±41.7 mmol/l.
- In contrast, the LPS group showed a much lower sodium level of 351.1±70.3 mmol/l.

The significantly higher sodium levels in the Indra-4+LPS and Diarex+LPS groups suggest that both treatments may play a role in enhancing sodium retention or balance in the body during inflammation induced by LPS.

Cl- (Chloride levels):

- The chloride levels were also elevated in both the Indra-4+LPS group (104.7±1.29 mmol/l) and the Diarex+LPS group (105.9±1.13 mmol/l) compared to the LPS group (88.4±12.58 mmol/l).

Chloride is an important electrolyte that works alongside sodium to maintain proper fluid balance and nerve function. The increase in chloride levels in the treatment groups indicates a possible beneficial effect of Indra-4 and Diarex in regulating electrolyte homeostasis during inflammation.

K+ (Potassium levels):

- In the Indra-4+LPS group, the potassium level was significantly higher at 1.87±0.47 mmol/l.
- The Diarex+LPS group showed a potassium concentration of 0.80±0.39 mmol/l.
- In comparison, the LPS group had a very low potassium level of 0.28±0.08 mmol/l.

Potassium is crucial for muscle function, including heart muscle, and for maintaining the body's acid-base balance. The increased potassium levels in the treatment groups suggest that Indra-4 and Diarex might have protective effects on cellular function and help in maintaining potassium balance during inflammation.

Table 1. Frequency of diarrhea in different groups

Treatment	Dose (mg/kg)	Number of installations
Normal	10	0.60±0.5
LPS	10	5.80±1.6$^{\#}$
Diarex	500	3.50±1.0*
Indra-4	300	1.80±0.8**

*$^{\#}$p<0.01 vs Normal group; * p<0.05 vs LPS group; ** p<0.01 vs LPS group*

Table 2. Serum electrolyte levels in different groups (mean ± sd)

	Dose (mg/kg)	Na	K	Cl
Normal	10	524.4±47.9	2.17±0.60	104.2±1.98
LPS	10	351.1±70.3$^{\#}$	0.28±0.08$^{\#}$	88.4±12.58$^{\#}$
Diarex+LPS	500	500.0±41.7*	0.80±0.39*	105.9±1.13*
Indra-4+LPS	300	508.4±91.6*	1.87±0.47*	104.7±1.29*

*$^{\#}$p<0.01 vs Normal group; * p<0.05 vs LPS group*

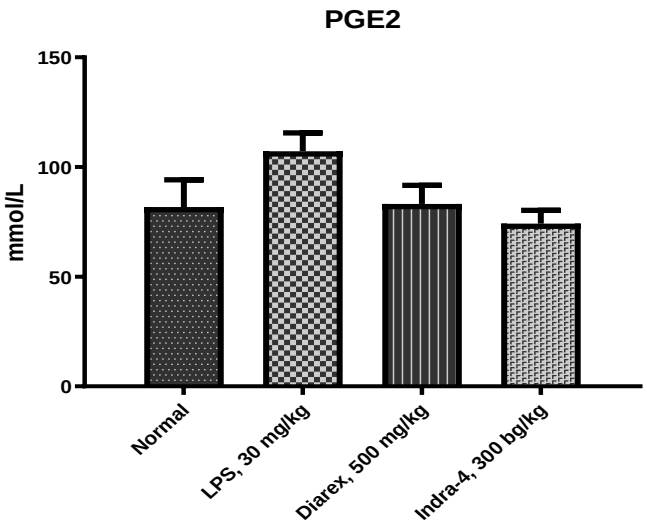

Fig. 6. Serum PGE2 production in response to LPS-induced diarrhea in rats

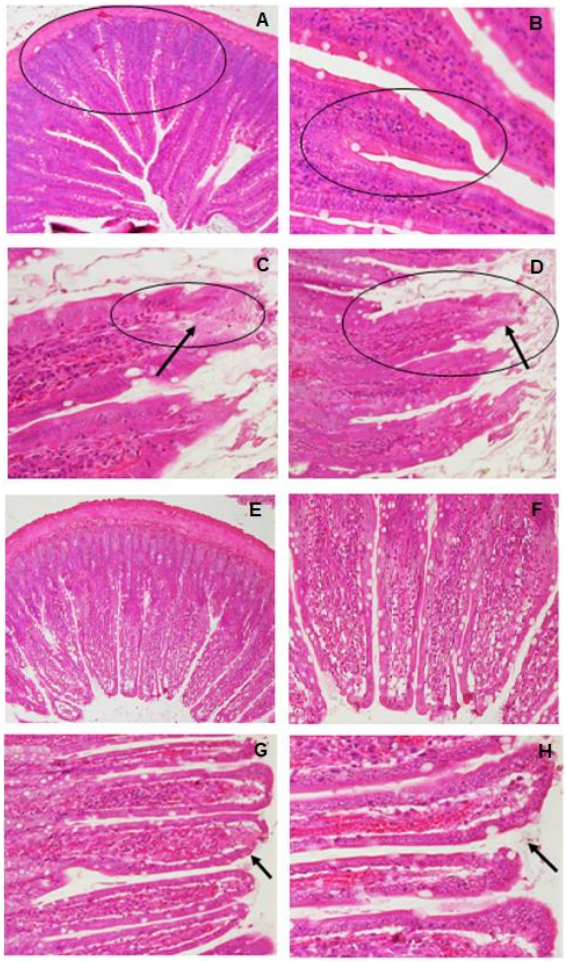

Fig. 7. Histological examination of the small intestine with hematoxylin and eosin, x10 and x20
A, B-Normal, C, D-LPS. E, F-Diarex+LPS, G, H-Indra-4+LPS

These findings highlight the potential of Indra-4 and Diarex as effective treatments in modulating electrolyte levels during inflammation induced by LPS. Both treatments led to higher levels of Na+, Cl, and K+ compared to the LPS group, suggesting that they might help in stabilizing electrolyte balance, which could be critical in managing inflammation-related disorders or conditions that affect electrolyte homeostasis. The statistical significance further supports the validity of these results.

Serum PGE2 levels in rats:

Measurement of PGE2 Levels using ELISA: An Enzyme-Linked Immunosorbent Assay (ELISA) was utilized to measure the levels of PGE2 (Prostaglandin E2) in the serum of Wistar rats. The serum levels of PGE2 were assessed four hours after stimulation with LPS (Lipopolysaccharide). This time frame was chosen to capture the early inflammatory response following LPS - induced stimulation.

Effects of Indra-4 prescription on the histology of duodenum in diarrheal rats:

Findings on PGE2 levels in wistar rats: The results showed a significant increase in the serum level of PGE2 following LPS administration, indicating that LPS induced a strong inflammatory response. Prostaglandin E2 is a key mediator in the inflammatory process, and its elevated levels in serum after LPS exposure confirm its involvement in the immune response triggered by LPS.

Role of wistar rats in PGE2 production: The findings also suggest that Wistar rats play an important role in the production of PGE2 as an essential enzyme when exposed to LPS. This makes them a valuable model for studying inflammation and the associated pathways, especially in relation to prostaglandin production during immune system activation.

Cytokine secretion and PGE2 levels in enteritis: The study further evaluated cytokine secretion and PGE2 levels in serum following the induction of enteritis (inflammation of the intestines). The results demonstrated a significant increase in the levels of PGE2 in the experimental group with enteritis compared to the healthy control group, highlighting the role of PGE2 as a marker of inflammation in the gut.

Effect of Indra-4 treatment on PGE2 levels: In the enteritis-induced rats, treatment with Indra-4 prescriptions led to a significant reduction in the elevated PGE2 levels ($p<0.05$). This suggests that Indra-4 treatment was effective in alleviating both the enteritis and mucosal injury in the rat model of small intestine inflammation. The reduction in PGE2 levels following Indra-4 treatment points to its potential as an anti-inflammatory agent, capable of mitigating the inflammatory response and tissue damage associated with enteritis.

Overall, this study highlights the important role of PGE2 in inflammation and demonstrates that Indra-4 could be a promising therapeutic option for reducing intestinal inflammation and promoting recovery from mucosal injury. By reducing the elevated PGE2 levels, Indra-4 may help in the management of conditions like enteritis and other inflammatory diseases of the digestive system.

The healthy rats duodenum exhibited standard (Fig. 7A, B). In untreated diarrheal rats, the duodenal histology showed mucosal destruction (Fig. 7C, D). On the other hand, the duodenal was normal in rats treated with Diarex 500 mg/kg (Fig. 7E, F) and Indra-4 300 mg/kg (Fig. 7G, H).

In the group of rats that received Indra-4 at a dose of 300 mg/kg, no pathological alterations such as inflammation, degeneration, or necrosis were detected in the various layers of the small intestine, including the intestinal mucosa, submucosa, and muscular layers. These findings suggest that the treatment with Indra-4 did not cause any noticeable damage or adverse effects to the structure of the small intestine.

Furthermore, when compared to the control group, the microstructure of the epithelial cells in the mucosal lining and the crypt cells appeared normal. This indicates that the integrity of the cellular architecture and function in these regions of the intestine was preserved, even after administration of Indra-4, suggesting that the compound did not induce any cellular changes or disruptions in the intestinal epithelium.

These observations provide evidence of the safety of Indra-4 at the specified dose, as it does not appear to induce any adverse histological changes in the small intestine, which is crucial for evaluating its potential therapeutic applications without causing significant tissue damage.

This version expands upon the findings, explaining the implications of the results in greater detail while maintaining the technical terminology and context.

In Mongolian traditional medicine, plants, and remedies with therapeutic properties have long been utilized to treat various conditions, including diarrhea (Bold, 2002). In many developing countries, the majority of people depend on herbal remedies to treat diarrhea (Mahmud & Rahmatullah, 2020). It is essential to explore natural drugs as alternatives to widely used synthetic antidiarrheal medications, which are linked to serious side effects.

The majority of the known chemical compounds in *H. antidysenterica* are present in the stem, bark, and leaves, with a few also found in the seeds. The primary compounds include steroidal alkaloids, flavonoids, triterpenoids, phenolic acids, tannins, resins, coumarins, saponins, and ergosterol (Rana et al., 2023).

In our study, we evaluated the antidiarrheal effects of Indra-4, and the findings demonstrate notable antidiarrheal activity in rat models. Indra-4 prescription demonstrated a significant ($p<0.05$) reduction in LPS-induced diarrhea in animals. The inhibitory effect supports the traditional use of Indra-4 in treating diarrhea. The antidiarrheal effect of Indra-4 represents the first documented evidence of its antidiarrheal activity. The LPS-induced diarrhea model is frequently utilized to investigate the impact of natural medicines and raw materials in traditional treatments. We concentrated on examining the impact of Indra-4 on LPS-induced intestinal inflammation in rats, aiming to understand the underlying molecular mechanisms.

Research shows that interleukins, such as IL-1, IL-1β, IL-6, and IL-8, significantly drive inflammatory reactions. They act as indicators of acute inflammation triggered by LPS (Rahman et al., 2015; Hanino, 2011). The Indra-4 pathway prescription could be a key target for treating inflammation caused by LPS-induced diseases. DK Sharma's research on ethanolic extracts from *Holarrhena antidysenterica* Wall seeds showed a significant rise in fecal dry weight and a reduction in defecation frequency in rat models suffering from diarrhea induced by castor oil and *Escherichia coli* (Sharma et al., 2015). An *In vitro* antibacterial study was performed using the methanolic extracts of the bark, seed, and callus of *Holarrhena antidysenterica* against Staphylococcus aureus, Salmonella typhimurium, and Escherichia coli. The results showed that the bark extract exhibited the strongest antibacterial activity against Staphylococcus aureus, with an inhibition zone of 10.05 mm, while it showed weaker activity against Salmonella typhimurium (6.65 mm) and Escherichia coli (2.7 mm). The seed extract of *Holarrhena antidysenterica* demonstrated inhibition zones of 7.05 mm against Staphylococcus, 5.50 mm against Salmonella, and 3.95 mm against E. coli. The callus extract exhibited an inhibition zone of 4 mm against Staphylococcus and the least activity against E. coli, with an inhibition zone of 3.1 mm. Overall, the study indicates that all three types of extracts from *Holarrhena antidysenterica* possess notable antibacterial activity (Sharma et al., 2015; Rana et al., 2023).

Kutaja parfait vati, which contains *Holarrhena antidysenterica*, is commercially available and has been shown to significantly decrease diarrhea and intestinal motility in mice experiencing diarrhea induced by castor oil (Kumari et al., 2021). The antidiarrheal effect may also be attributed to the inhibition of ricinoleic acid release, leading to Na+ and K+ ATPase activity activating electrolyte absorption in the intestinal lining (Yous et al., 2018). Many studies have shown that certain chemicals in medicinal plants exhibit antidiarrheal effects by reducing gut motility, slowing intestinal transit, enhancing water absorption, or decreasing electrolyte secretion (Ali et al., 2015). Compounds such as flavonoids and tannins demonstrate antidiarrheal effects by promoting the reabsorption of electrolytes and water from the small intestine (Tiwari et al., 2011). *Clematis tangutica* Korsh contains flavonoids, a group of compounds comprising two benzene rings with hydroxyl groups linked by a central three-carbon atom. These compounds are typically found in plants as conjugates (flavonoid glycosides) or aglycones (flavonoids), with the molecular formula $C_{15}H_{10}O_8$. Flavonoids can produce pharmacological effects like preventing lipid peroxidation, inhibiting enzyme activity, and combating diarrhea, bacteria, viruses, allergies, and inflammation. They also protect the cardiovascular system and reduce aging in the body through their antioxidant, free radical scavenging, and chelating properties on divalent ions (Guo et al., 2023; Yuan et al., 2023).

Holarrhena antidysenterica Wall, *Clematis tangutica* Korsh, and *Polygonum bistorta* L, which are part of the Indra-4 formulation, contain

high levels of flavonoids, triterpenoids, phenolic acids, tannins, resins, coumarins, and saponins, all of which have been found to exhibit anti-diarrheal properties (McMurray et al., 2020). *Aconitum kuznezoffii*, included in the Indra-4 prescription, has been shown to treat diarrhea in a model of intestinal inflammation in experimental animals without disrupting the balance of normal microflora (Zhang et al., 2023; Zhou et al., 2024).

We will extract and identify additional chemical compounds from this traditional Indra-4 prescription and carry out further detailed studies on its chemical and pharmacological properties (Maxmedchem, n.d.; Mzena, 2020).

4. CONCLUSIONS

1. We conducted the first chemical and pharmacological analysis of the traditional Indra-4 prescription and discovered that it primarily contains polyphenolic compound derivatives, including flavonoids, coumarins, and triterpene saponins. These biologically active compounds exhibit various pharmacological actions and therapeutic effects.
2. In the acute inflammatory enteritis disease model induced by lipopolysaccharide, the Indra-4 prescription demonstrates an antidiarrheal effect by decreasing prostaglandin E2 levels and preventing electrolyte loss, as confirmed through laboratory and histological analyses.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby announce that generative AI technologies such as Large Language Model GPT have been used during the writing or editing of manuscripts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Mongolia's Ministry of Health Ethical Review Committee has approved the experimental protocol.

Animal Ethical Committee approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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