ejpmr, 2018,5(6), 306-314

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTIDIARRHOEAL, ANTI-INFLAMMATORY AND CYTOTOXIC EFFECTS OF AQUEOUS AND METHANOLIC LEAVES EXTRACT OF *DISSOTIS THOLLONII* Cogn. (Melastomataceae)

Herve Tchoumbou Tadjoua[1*], Gilbert Ateufack[1], William Nana Yousseu[1], Olivette Laure Matafack Dongmo[3], Simjee Usman Shabana[2], Donatien Gatsing[4] and Choudhary Iqbal Muhammad[2]

[1]Department of Animal Biology, Dschang University, Laboratory of Animal Physiology and Phytopharmacology, P.O Box 67, Dschang, Cameroon.

[2]Hussain Ebrahim Jamal Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan.

[3]Department of Biochemistry, Dschang University, Laboratory of Biochemistry of Medicinal Plants, Food Sciences and nutrition, P.O Box 67, Dschang, Cameroon.

[4]Department of Biochemistry, Dschang University, Laboratory of Microbiology and Antimicrobial Substances, P.O Box 67, Dschang, Cameroon.

***Corresponding Author: Herve Tchoumbou Tadjoua**
Department of Animal Biology, Dschang University, Laboratory of Animal Physiology and Phytopharmacology, P.O Box 67, Dschang, Cameroon.

Article Received on 20/12/2017 **Article Revised on 02/05/2018** **Article Accepted on 23/05/2018**

ABSTRACT

Background: In Africa, principally in Cameroon, the aqueous extract of *Dissotis thollonii* is traditionally used for the diarrhoeal and inflammatory diseases. However, no scientific study was used to evaluate the safety of its use. This study aim to assess the anti-diarrheal and anti-inflammatory activities of the plant extracts on rats and mice. **Methods**: anti-diarrheal activity was studied on induced diarrheal by castor oil and intestinal motility in rat. *In vivo* anti-inflammatory effect was study using carrageenan induced peritonitis in mice. ROS production from phagocytes, TNF-α, antiproliferative activity on HeLa cell lines and cytotoxic activity were performed by using chemiluminescence technique. **Results**: The results indicated that aqueous and methanolic leaves extracts of *Dissotis thollonii* had, after 6h of observation, significantly ($p<0.001$) inhibited curatively diarrhoea induced experimentally by castor oil by increasing the latency time and reducing the frequency of defecation and water content at all the doses, compared to negative control which received distilled water. These extracts also reduced peristaltic movement in castor oil-induced intestinal transit. Results were also compared with loperamide used as anti-diarrheal drug. Additionally, *Dissotis thollonii* decreased significantly the number of leukocytes migration in peritoneal fluid *in vivo*, and *in vitro*, inhibited significantly ($p< 0.001$) the intracellular ROS production. *Dissotis thollonii* also possess significant ($p< 0.001$) inhibitory effect on the proliferation of Hela cell lines, on the reduction of TNF-α production and showed a brine shrimp larvicidal activity. **Conclusions**: *Dissotis thollonii* show anti-diarrheal and anti-inflammatory properties in rats and mice respectively, and anti proliferative activity *in vitro*. The results confirm traditional use of *Dissotis thollonii* in Cameroon concerning the treatment of diarrhea and inflammation.

KEYWORD: *Dissotis thollonii*, Anti-diarrheal, Anti-inflammatory, cytotoxicity.

1. INTRODUCTION

Diarrhea is a physiopathology characterized by a discharge of wet stool for several days. This physiopathology has led to a significant increase in infant mortality and morbidity particularly in poor country.[1] Diarrhea is a symptom of inflammatory bowel disease and represents the first observed manifestation of intestinal inflammation that can cause inflammatory diarrhea, weight loss, fever and anemia.[2,3] Nowadays, many plants are used for the treatment of diarrhea. These plants can be largely useful and lead to synthetic modifications of biological activity. For example, the World Health Organization promotes the development of traditional medicine that partly uses medicinal plants.[4] Among the many species exploited, *Dissotis thollonii (D. thollonii)* is traditionally used in the treatment of several illnesses. Thus, it is used by the traditional healer in Senegal on eczematous wounds and in the western region of Cameroon for the treatment of digestive disorders, inflammatory diseases, wounds, jaundice and anemia.[5] The leaves are recommended for the treatment of gastrointestinal disorders (obstruction, amebiasis, diarrhea, vomiting and constipation) and ulcers. An earlier study of this plant showed that *D. thollonii*

significantly inhibited fluid accumulation in intestine induced by Prostaglandin E2.[6] This study aim to evaluate the antidiarrhoeal activities of aqueous and methanolic leaves extracts of *D. thollonii* on induced-diarrhoea in rats, anti-inflammatory activities of the same extracts on carrageenan induced peritonitis *in vivo*, antiproliferative activity on HeLa cell lines, ROS generation and cytokine inhibition.

2. MATERIALS AND METHODS

2.1. Collection and identification of plant material

The fresh leaves of *D. thollonii* were collected in September 2016 in the Bafou village, Menoua division, West Region of Cameroon. A sample was identified at the National Herbarium of Cameroon (Yaoundé) by comparison with existing voucher specimen No.13292/SRF Cam.

2.2. Preparation of the plant material

The collected plant parts (leaves) were separated from undesirable materials. They were dried under shade, ground and stored in an airtight container prior to extraction.

2.3. Preparation of plant extract

The aqueous extract was prepared by boiling 800 g of powder in 5L distilled water for 15 min as indicated by the traditional healer. The decoction was cooled during 1 h and filtered using filter paper (Whatman No.1), and the filtrate was evaporated in a regulated drying oven at 35 °C to give 51.6 g of the aqueous extract corresponding to an extraction yield of 6.45% (w/w). The other portion of leaf powder (900 g) was macerated in 6.75 L of methanol for 72 h, filtered and the solvent removed from the extract under reduced pressure, using a Büchi (R-200) rotary evaporator at 65 °C. This gave 63.8 g of the methanolic extract, corresponding to a yield of 7.09% (w/w).

2.4 . Experimental animal

Wistar Albino male and female rats of 2 to 2.5 months and weighing on average 130 g were used for anti-diarrhoeal activity whereas male (6–8 weeks old) mice, weighing on average 35 g, were used for the anti-inflammatory test. They were bred in the Animal House Facility, Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Sciences. Prior to experimental protocol, the rats and mice were acclimatized for 48 h to laboratory conditions for minimizing any nonspecific stress.

2.5. Brine shrimp lethality assay

This experimentation was performed, as described by.[7] 20 mg of extracts were dissolved in 2 ml of distilled water to make a stock solution and from this solution, 2.5, 25 and 250 µl were transfer in different vials (3vials/ concentration) to have final concentration of 5, 50 and 500 µg/ml respectively and then 5, 50 and 500 µl were also transfer in different vials (3vials/ concentration) to have final concentration of 10, 100 and 1000 µg/ml respectively. These were left for evaporation of the solvent overnight. After 2-days of hatching and maturation as nauplii, 10 larvae were introduced per vials, using a Pasteur pipette, and the volume was completed to 5 ml with seawater, incubated at 25º C for 24 h under illumination. Data were analyzed with Finney computer program to determine LD_{50} values with 95% confidence intervals.

2.6. MTT Cytotoxicity assay

In vitro cytotoxicity of extracts was evaluated by MTT assay described by.[8] 96 welled flat bottom plates containing 100 µl of cell suspension (6×10^4 cells/ml) were incubated (24 h, 37 °C) in CO2 (5%). After removing the media in each well, extracts (12.5–100 µg/ ml) and complete DMEM were added (200 µl final volume). Positive control wells contained cells (100 µl) and complete DMEM and in negative control 0.5% triton X100 (2 µl) were add. After incubation (48 h, 37 °C) in CO2 incubator, the supernatant was removed, MTT (50 µl, 0.5 mg/ml) diluted in PBS (5 mg/ml) added and plates were incubated again (4 h). MTT was carefully aspirated and DMSO (100 µl) was added with agitation (10–15 min) in an orbital shaker. The spectrophotometer was used at 540 nm for absorbance. The percent inhibition or decrease in cells viability was obtained by the formula.

$$\%\text{Inhibition} = 100 - \frac{OD_{\text{test group}} - OD_{\text{blank}}}{OD_{\text{Control group}} - OD_{\text{blank}}} \times 100 \quad \text{where OD= optical density}$$

2.7. Antiproliferative activity on Hela cells

Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay.[9] For this purpose, HeLa cells (Cervical Cancer) were cultured in Minimum Essential Medium Eagle, supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of $6x10^4$ cells/ml was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of extracts (1-100µg/ml). After 48 hrs, 200 µL MTT (0.5 mg/ml) was added to each well and incubated further for 4 hrs. Subsequently, 100µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC_{50}) for HeLa. The percent inhibition was calculated by using the following formula.

% inhibition = 100-((mean of O.D of test compound – mean of O.D of negative control)/ (mean of O.D of positive control – mean of O.D of negative control)*100). Where OD= optical density
The results (% inhibition) were processed by using Soft-Max Pro software (Molecular Device, USA).

2.8. Oxidative Burst Assay (ROS generation assay)

This experiment was performed as described by[10] with slight modifications. Briefly 25 µL HBSS ++ diluted whole blood (Hanks Balanced Salt Solution containing calcium chloride and magnesium chloride) were incubated with 25 µL of three different concentrations of compounds (1, 10 and 100 µg / mL), each in triplicate. Control wells received HBSS ++ and cells, but no compounds. The test was performed in 96-well half-surface white plates, which were incubated at 37 ° C for 15 minutes in the luminometer thermostat chamber. After incubation, 25 µL of serum opsonized zymosan (SOZ) and 25 µL of intracellular reactive oxygen species detecting probe, luminol, were added to each well, with the exception of blank wells (containing only HBSS ++). The ROS level was recorded in the luminometer in terms of relative light units (RLU). The following formula was used to calculate the percent inhibition (%) for each compound:

$$\text{Inhibition (\%)} = \frac{(RLU_{control} - RLU_{sample}) \times 100\%}{RLU_{control}}$$

2.9. Determination of TNF-α level

The level of TNF-α was detected by ELISA.[11] Briefly, the microtiter plates were coated overnight at 4 ° C with an immunoaffinity-purified polyclonal sheep antibody against TNF-α (2 mg mL-1). After plates blocking, recombinant murine TNF-α standard at various dilutions and samples were added in triplicate and incubated overnight at 4 ° C. Rabbit biotinylated immunoaffinity-purified pAb anti-TNF-α (1:500) was added, followed by incubation at room temperature for 1h. Fifty microliters of avidin-HRP (1: 5000 dilution, DAKO A / S, Denmark) were added to each well; after 30 minutes, the plates were washed and the OPD colored reagent (200 mg / well, Sigma) was added. After 15 minutes, the reaction was stopped with 1M H2SO4 and the optical density (OD) was measured at 490 nm. The results were expressed as pg/mL of TNF-α, based on the standard curves.

2.10. Carrageenan-induced peritonitis in mice

BALB/c mice were divided into 6 groups (n = 6). Inflammation was induced by modification of the technique as described by.[12] The extracts were administered orally at doses of 125, 250 and 500 mg/kg and indomethacin at a doses of 10 mg/kg *p.o.* and carrageenan (0.25 ml, 0.75% in saline) was injected intraperitoneally 1 h later and after 4 h, the mice were euthanized by cervical dislocation and the peritoneal cavity cells were harvested by washing the cavity with 3mL of PBS containing EDTA 1mM at pH: 7.4. The total leukocyte count was determined in a Neubauer chamber. The percentage of the leukocyte inhibition was calculated by the following equation: % = (1-T/C) ×100, where T represents the treated group leukocyte counts and C represents the control groups leukocyte counts.

2.11. Castor oil-induced diarrhea in rats

The animals were initially screened by observing stool's aspect. Those not showing diarrheic stools were selected for the final experiment. Thus, forty-eight rats were randomly divided into eight equal group (n=6) divided into controls, standard and test groups. The negative control groups received distilled water (1 mL/100 g b.w). The positive control group received loperamide at the dose of 2.5 mg/kg orally. The test groups received aqueous and methanolic extracts of *D. thollonii* at doses of 125, 250 and 500 mg/kg orally. Each animal was placed in individual cage, the floor of which was lined with filter paper, changed every hour. Diarrhoea was induced by oral administration of 1 mL/100 g b.w castor oil to each rat, and after observed the first diarrheic defecation, the animals were treat with different doses of extract and reference drug. During an observation period of 6 h, parameters such as the latency time, the frequency of defecation and the water content of feces were recorded. Water content of feces was expressed in terms of percentages using the formula.

$$Wc\ (\%) = \left(\frac{Fw\text{-}Dw}{Fw}\right) \times 100$$

Where, Wc = Water content of feces; Fw = Fresh weight (g); Dw = Dry weight (g).

2.12. Castor oil induced intestinal transit

The choice of animals was made as described above. Forty-eight rats were randomly divided into eight equal group (n=6) divided into controls, standard and test groups. The positive control groups received distilled water (1 mL/100 g b.w). The negative control group received atropine sulfate at the dose of 5 mg/kg orally. The test groups received aqueous and methanolic extracts of *D. thollonii* at doses of 125, 250 and 500 mg/kg orally. After 1 h, all animals received 1 ml of castor oil. Another 1 h after, all animals received deactivated charcoal (1 mL/100 g b.w). 30 min later, all animals were euthanized by cervical dislocation, and the small intestines were removed. The distance traveled by the charcoal from the pylorus to the caecum and the total length of the small intestine were recorded. The intestinal transit percentage was determined using the formula below.

$$\text{\% transit} = \frac{\text{Distance traveled by charcoal}}{\text{Total length of the small intestine}} \times 100$$

2.13. Statistical analysis

The mean ± standard Error of Mean (SEM) was used to express the results of the study. For statistical analysis, the measurement data were analyzed by One-way analysis of variance (ANOVA), follow by post test of Tukey-Kramer was used. P< 0.05 was statistically significance acceptable. GraphPad prism was used for all data analysis.

3. RESULTS

3.1. Brine shrimp larvicidal activity of aqueous and methanol extracts

The leave extracts of *D. thollonii* showed a brine shrimp larvicidal activity. The lethality concentration (LC50) of aqueous and methanol extracts were 500 µg/mL and 520 µg/mL respectively (table 1). The degree of lethality was directly proportional to the concentration of the extract. Maximum mortalities (100%) were observed at a concentration of 1000 µg/mL only with aqueous extract. Based on the results, the brine shrimp lethality of the aqueous and methanol leave extracts were found to be concentration-dependent.

Table 1: The number of shrimp nauplii that survived after treating with aqueous and methanolic extracts and the percentage mortality.

Plant Extracts	Concentrations (µg/ml)	Number of Surviving Nauplii After 24h			Total number of Survivors	% Mortality	LC_{50} (µg/ml)
		T1	T2	T3			
Aqueous extract	5	10	10	10	30	0	500
	10	9	10	8	27	10	
	50	8	9	10	27	10	
	100	9	8	8	25	16.66	
	500	5	6	4	15	50	
	1000	0	0	0	0	100	
Methanolic extract	5	10	10	10	30	0	520
	10	10	10	10	30	0	
	50	9	10	9	28	6.66	
	100	10	9	9	28	6.66	
	500	7	7	8	22	26.66	
	1000	2	1	2	5	83.33	

The lethality concentration (LC_{50}) was assessed at 95% confidence intervals using Finney software analysis. *T=Trial.

3.2. Cytotoxicity of *D. thollonii* extracts on 3T3 Cells

Aqueous and methanolic extracts of *D. thollonii* were tested for their possible toxicity effects. Methanolic extract showed toxic effect whereas aqueous extract showed low level of toxicity on this cell line (IC50 >100 µg/ml) compared to the cyclohexamide (IC50 = 0.8 ± 0.20 µg/ml) used as the standard cytotoxic drug (Table 2).

3.3. Effect of *D. thollonii* extracts on Hela cells proliferation assay

On this assay, methanolic extract showed a notable level of antiproliferative effect, at a maximum inhibition with IC50 = 20.15 ± 3.57 µg/ml, whereas aqueous extract showed a low level of antiproliferative effect with an IC50 >100 µg/ml compared to Doxorubicin (IC50 = 1.2 ± 0.40 µg/ml) (Table 2).

3.4. Effect of *D. thollonii* extract on intracellular ROS production

To evaluate myeloperoxidase dependent effect of extracts, luminol was used as probe. On human whole blood phagocytes, results showed that, methanolic extract of *D.thollonii* possess significant inhibitory activity on intracellular ROS production; with IC50 values of 13.4 ± 0.8 µg/ml while with aqueous extract, IC50 >250 µg/ml (Table 2). Concerning neutrophils, both extracts showed potent inhibitory effect with an IC50 value of 12.2 ± 0.7 µg/ml (methanolic extract) and 25.3 ± 1.5 µg/ml (aqueous extract) (Table 2), all compare to Ibuprofen, used as reference product, which showed an IC50 of 11.2 ± 1.9 and 2.5 ± 0.6 µg/ml, respectively for whole blood and polymorpho neutrophils.

Table 2: Inhibition percentage and IC50 value of aqueous and methanolic extracts of *Dissotis thollonii* on human whole blood evaluated by luminol amplified chemiluminescence.

	Oxidative brust (250 µg/ml)				Antiproliferative activity on Hela cells line (100 µg/ml)		Cytotoxicity on 3T3 Cells line (100 µg/ml)	
	Whole Blood		PMNs					
	Inhibition %	(IC_{50} µg/ml)	Inhibition %	(IC_{50} µg/ml)	Inhibition %	(IC_{50} µg/ml)	Inhibition%	(IC_{50} µg/ml)
Aqueous extract	0.9 ± 0.6	>250	98.2 ± 0.2	25.3 ± 1.5	29.93	>100	30.23	>100
Methanol extract	97.3 ± 3.1	13.4 ± 0.8	86.4 ± 0.6	12.2 ± 0.7	66.90	20.1 ± 3.5	71.59	19.5 ± 1.6
Ibuprofen	73.2 ± 0.2	11.2 ± 1.9	89.1± 0.1	2.5 ± 0.6	-	-	-	-

Doxorubicin	-	-	-	-	92.20	1.2 ± 0.4	-	-
cycloheximide	-	-	-	-	-	-	80.01	0.8 ± 0.2

The IC50 values are presented as mean ± SD of triplicates. Where WB whole blood, PMNs Polymorpho neutrophils.

3.5. Effect of *D. thollonii* extracts on Production of Pro-Inflammatory Cytokine (TNF-α)

The effect of extracts on the release of TNF-α by activated THP-1 cells was evaluated with concentrations of 2–50 µg/ml of the extracts (Fig. 1). The aqueous and methanolic extracts of *D.thollonii* significantly ($p < 0.001$) decreased the level of TNF-α with 73.19 ± 4.6 % and 93.16 ± 0.0 % inhibitions corresponding to IC50 of 2.3 ± 0.03 and 2.2 ± 0.1 µg/ml respectively. Control samples did not show any significant ($P > 0.05$) variations.

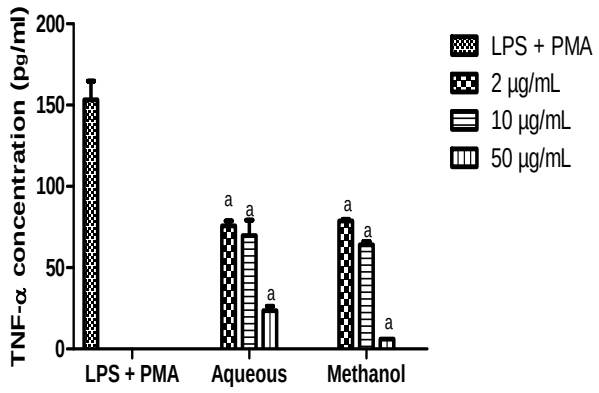

Fig. 1: Effect of crude extracts of *D.thollonii* on TNF-α secretion level by THP-1 activated by LPS. Data represent mean ± SD of triplicates. [a]P < 0.001 when compared with that of the control: PMA + LPS.

3.6. Effects of *D.thollonii* extracts on carrageenan-induced peritonitis in mice

In carrageenan induced peritonitis activity, the leukocytes migration in peritoneal fluid and percentage of leukocytes inhibition of the control, standard and test compounds are shown in Table No: 3 and Fig. No: 2. The aqueous and methanolic extracts of *D.thollonii* significantly ($p < 0.001$) decreased the number of leukocytes at all the doses compared to control received only carrageenan. The dose of 500 mg/kg of aqueous extract as well as 500 and 250 mg/kg of methanolic extract reacted like indomethacin, used as standard drug. Generally, the extracts inhibited leukocytes migration in peritoneal fluid in a dose-dependent manner.

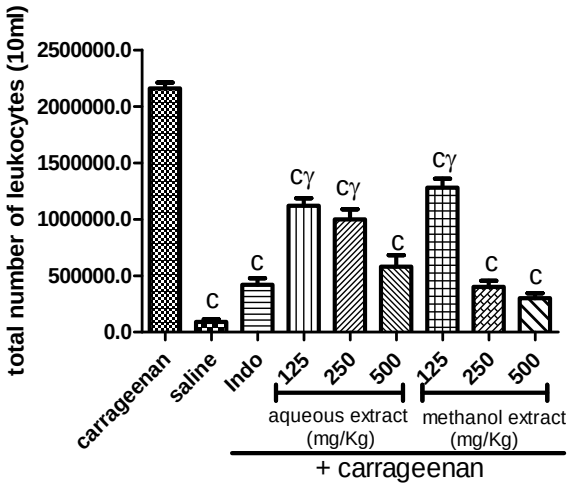

Fig. 2 Effect of crude extracts of *D.thollonii* on the leukocytes migration in peritoneal fluid. Each histogram represents the mean ± ESM (n = 6). [c]p <0.001: significant differences from the negative control (carrageenan). [γ]p <0.001: significant difference from the positive control (indomethacin).

Table 3: Effect of crude extracts of *D.thollonii* on the inhibition percentage of leukocytes in peritoneal fluid.

Treatments	Parameters	
	Leukocytes (10^5 ml^{-1})	Leukocytes inhibition %
1- Control	21.60 ± 0.0034	-
2- Saline	0.90 ± 0.0023[c]	-
3- Indomethacin	4.20 ± 0.0036[c]	80.56
4- AQ E 125 mg/kg	11.20 ± 0.0039[cγ]	48.15
5- AQ E 250 mg/kg	10.00 ± 0.0045[cγ]	53.71
6- AQ E 500 mg/kg	5.80 ± 0.0047[c]	73.15
7- MOHE 125mg/kg	12.80 ± 0.0042[cγ]	40.75
8- MOHE 250 mg/kg	4.00 ± 0.0035[c]	81.49
9- MOHE 500 mg/kg	3.00 ± 0.0031[c]	86.12

Each histogram represents the mean ± ESM (n = 6). [c]p <0.001: significant differences from the negative control (carrageenan). [γ]p <0.001: significant difference from the positive control (indomethacin). AQE= Aqueous extract; MOHE= Methanol extract.

3.7. Effects of *D.thollonii* extract on inhibition of castor oil-induced diarrhoea

Sixty minutes after administration of castor oil, diarrhea was effective in all the animals. Immediate treatment of

rats after appearance of first diarrheal defecation with aqueous and methanol extracts of *D.thollonii* show at dose 500 mg/kg, highly significantly ($p<0.001$) prolonged the latency time, reduced the frequency of defecation and the water content of feces when compared to the negative control group received distilled water. In addition, this dose of plant extracts reacted like loperamide, used as standard drug. Generally, the extracts inhibited diarrhoea in a dose-dependent manner (Fig. 3-5).

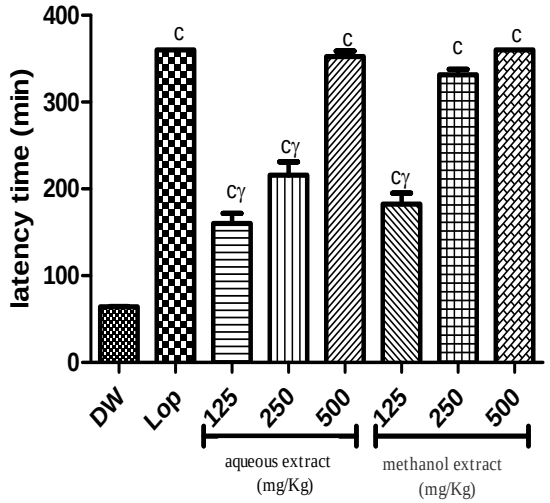

Fig. 3: Effect of aqueous and methanolic leaves extracts of *D. thollonii* on the latency time in castor oil induced diarrhea. These values represents the mean ± ESM (n=6) ; $^{c}p<0,001$: significant differences relative to the negative control (distilled water). $^{\gamma}p<0,001$: significant differences relative to the positive control (loperamide). DW= Distilled Water.

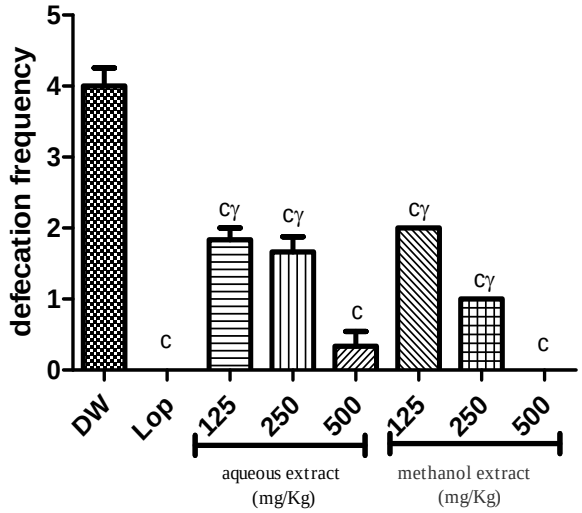

Fig. 4 Effect of D. *thollonii* extracts on the defecation frequency in castor oil induced diarrhea. These values represents the mean ± ESM (n=6) ; $^{c}p<0,001$: significant differences relative to the negative control (distilled water). $^{\gamma}p<0,001$: significant differences relative to the positive control (loperamide). DW= Distilled Water.

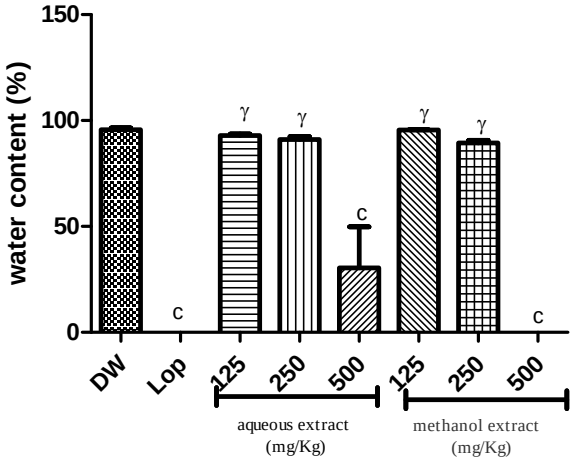

Fig. 5 Effect of D. *thollonii* extracts on water content in castor oil induced diarrhea.

These values represents the mean ± ESM (n=6) ; $^{c}p<0,001$: significant differences relative to the negative control (distilled water). $^{\gamma}p<0,001$: significant differences relative to the positive control (loperamide). DW= Distilled Water.

3.8. Effects of *D.thollonii* extracts on gastrointestinal motility test

Compared with the control group distilled water, all the doses of extract significantly ($p<0.001$) decreased the propulsive movement and transit of charcoal meal to the gastrointestinal tract, as well as peristaltic index. The standard antidiarrheal drug, atropine sulfate (5 mg/kg p.o.) produced greater antimotility effect as well as the higher dose (500 mg/kg *p.o.*) of methanol extract (Fig.6-8).

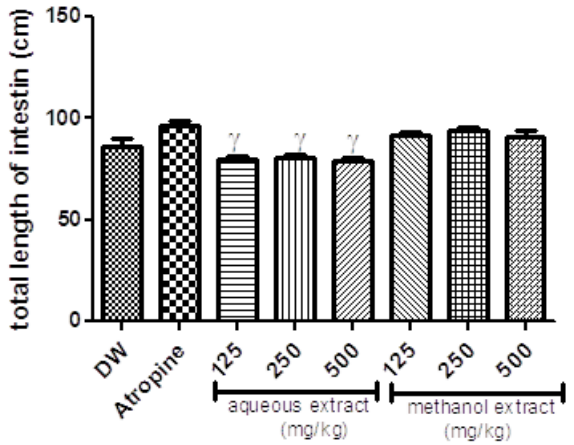

Fig. 6 Total length of intestine of different test group on castor oil induced intestinal transit. Each histogramme represent the mean ± ESM (n = 6). $^{\gamma}p<0,001$: significant difference relative to the positive control (atropine sulfate). DW= Distilled Water.

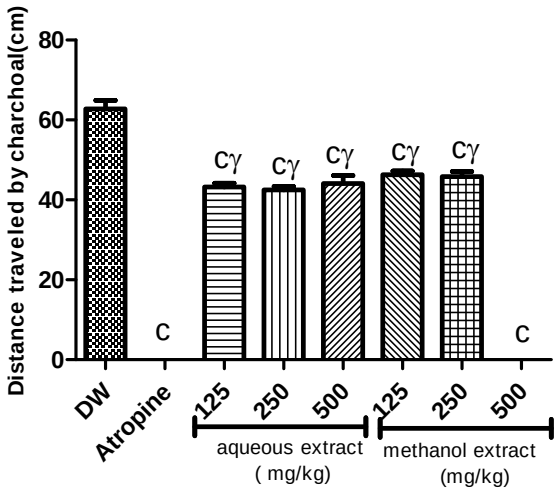

Fig. 7 Effect of D. *thollonii* extracts on the distance traveled by charchoal in castor oil induced intestinal transit. Each histogramme represent the mean ± ESM (n = 6). ^{c}p<0,001: significant differences relative to the negative control (distilled water). $^{\gamma}$p<0,001: significant differences relative to the positive control (atropine sulfate). DW=Distilled Water.

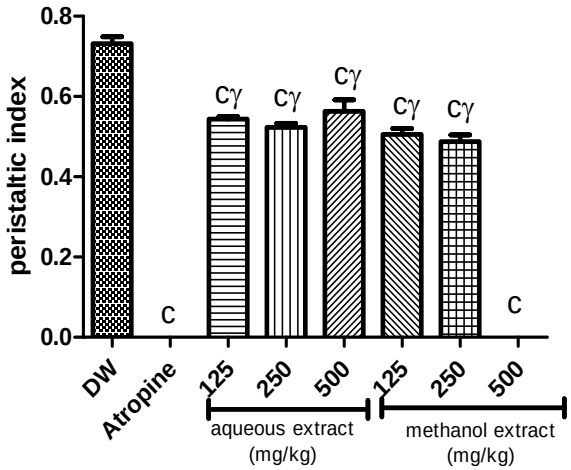

Fig. 8 Effect of D. *thollonii* extracts on the peristaltic index in castor oil induced intestinal transit Each histogramme represent the mean ± ESM (n = 6). ^{c}p<0,001: significant differences relative to the negative control (distilled water). $^{\gamma}$p<0,001: significant differences relative to the positive control (atropine sulfate). DW=Distilled Water.

4. DISCUSSION

In this study, the observed lethality of these plant extracts in brine shrimp indicated the presence of potent cytotoxic and possible antitumor components of these extracts. According to[13], when the LC50 value is less than 1000 µg/mL, the crude plant extract is toxic while it is nontoxic if the LC50 is greater than 1000 µg/mL. This strong cytotoxic effect was demonstrated on the 3T3 cell line, which showed that the aqueous extract had a low level of toxicity on this cell line (IC50 >100 µg/ml) whereas the methanol extract showed cytotoxic effect (IC50 = 19.51±1.67µg/ml). To determine and identify toxic effect of plant extracts and products in humans, cytotoxicity tests *in vitro* are usually used.[14,15] Cytotoxicity tests may give different results depending on the cytotoxicity test used and the test agent.[16] Therefore, it is important to conduct more than one test to determine the viability of cell an *in vitro* test, which would increase the reliability of the results obtained.

HeLa cancer cells that were also treated with *D. thollonii* extracts at 100 µg/ml were studied to prove the plant extracts effect on cell growth. Our observations showed that cell growth was inhibited by the methanol extract (IC50 = 20.15 ± 3.57 µg/ml). Based on the fact that our extracts have larvicidal activity on shrimp and are cytotoxic on the 3T3 cell line, it can be said that the inhibitory effect of *D. thollonii* on cell growth is probably due to the induction of cell death probably caused by the presence in these plants of secondary metabolites such as flavonoids, phenols and polyphenols. These groups of secondary metabolites that have already been described in these plants extract (tannins, flavonoids, sterols, anthraquinones, phenols and polyphenols)[17] are known to possess anti-inflammatory properties. Some of these metabolites have been described as natural painkillers in the literature[18] and have chemopreventive and antitumor effects.[19] Flavonoids are known to be plant polyphenolic compounds that possess analgesic and anti-inflammatory properties by inhibiting the enzymes involved in inflammation. In inflamed tissue, flavonoids can inhibit cyclooxygenase, prevent the formation of prostaglandins and the secretion of TNF.[20] This can support the results obtained on the Oxidative brust test with the inhibition of whole blood with methanol extract (IC50 = 13.4 ± 0.8µg/ml) and Polymorpho neutrophils with both extracts (IC50 = 25.3 ± 1.5 and 12.2 ± 0.7 µg/ml respectively), as well as the significant (p< 0.001) decreased of the level of pro-Inflammatory Cytokine (TNF-α).

After evaluation of the anti-inflammatory activity on ROS (reactive oxygen species) generation and pro-Inflammatory cytokine (TNF-α), the aqueous and methanolic leaves extract of *D.thollonii* was tested on mice peritonitis induced by carrageenan, to evaluate its activity on cell migration processes. Carrageenan is a polysaccharide derived from algae[21] that has the ability to enhance vascular permeability, to promote the initial action of histamine and serotonin and to induce acute inflammation by the action of prostaglandins and bradykinin.[22] The production of pro-inflammatory mediators such as cytokines, reactive oxygen species and nitric oxide is stimulated by these hormones. However, tissue damage may occur if inflammation persists, and result in loss of function. The aqueous and methanolic leaves extract of *D. thollonii* showed a good anti-inflammatory effect in the peritonitis induced by carrageenan, by a significant (p< 0.001) reduction of the

number of leukocytes into the peritoneal cavity of mice at all doses tested and dose dependant manner. A study with *in vitro* experimental models conducted by[23] showed that chlorogenic acid has the capacity to modulate translocation from the intracellular to the extracellular environment, to reduce leukocyte migration, and also to inhibit the expression of CD-31 receptors. The extracts of *D. thollonii* could act by one of these mechanisms, which could explain the significant decrease in the number of leukocytes that transmigrate to the peritoneum of the mouse.

It is known that inflammatory bowel disease is a general group of inflammatory colon diseases and small intestine. Diarrhea is one of the symptoms of IBD.[24] The etiology of diarrhea is complex; there are certain mechanisms that can trigger the disease, such as increased release of electrolytes (secretory diarrhea), infectious or induced inflammatory mucosal injury (exudative diarrhea) and motility intestinal disturbed.[24] Many natural compounds are widely used in the treatment of gastrointestinal diseases.[25] In general, it is known that drugs that exhibit anti-inflammatory properties also have the inhibiting property of castor oil-induced diarrhea. Various laxatives substances, such as castor oil are used to induce diarrhea in animal models. In our study, castor oil was used to create a diarrhea model. Castor oil released ricinoleic acid, after it digestion by intestinal lipases, which stimulates excessive motility of the small intestine, thus change the electrolytes permeability of the intestinal mucus.[17] Curative treatment with aqueous and methanolic leaves extract of *D. thollonii* showed antidiarrhoeal effect at all the doses. These extracts significant inhibited diarrhea by increasing the latency time, reducing de defecation frequency as well as the water content (500 mg/kg), suggesting that the action of the extracts may be due to inhibition of prostaglandin-E2 biosynthesis, by increasing the reabsorption of electrolytes (Na +, K +, Cl-) and, consequently, by decreasing the secretion of fluids in the gastrointestinal tract. The aqueous and methanolic extract reacts like loperamide, a positive control drug that is an agonist of opioid receptor capable of acting on the μ-opioid receptors of the myenteric plexus to decrease intestinal transit.[26]

In the evaluation of intestinal motility, atropine sulfate was used as a standard drug. Atropine is known to inhibit intestinal transit, probably because of its anticholinergic effect.[27] *D. thollonii* extracts reduced intestinal propulsive movement in the charcoal meal treated model at all the doses, and only the dose of 500 mg/kg was comparable to the effect of atropine sulphate. Nevertheless, this is logical because atropine sulfate is pure compared to the extract which is a mixture of several compounds. Activated charcoal was used in the gastrointestinal motility test to discover the effects of these extracts on peristaltic movement. The results show that these extracts suppress the propulsion of deactivated charcoal and thus show a reduction in the propulsive movement of the small intestine after pretreatment with extracts of *D. thollonii*. All these results can be supported by the presence of secondary metabolites (tannins, flavonoids, sterols, anthraquinones, phenols and polyphenols) presents in these extracts.

5. CONCLUSION

This study demonstrates the anti-diarrheal, anti-inflammatory and anti-proliferative effects of aqueous and methanolic leaves extract of *Dissotis thollonii*. These activities probably result from the spasmolytic or can be due to a possible antisecretory effect of plant extracts on the intestinal smooth muscle, and also by release of pro-inflammatory mediators. All this results demonstrated its potential use as a phytotherapeutic agent.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to TWAS (The World Academy of Sciences) and ICCBS (International Center for Chemical and Biological Sciences) for Sandwich Postgraduate Fellowship Award to TADJOUA TCHOUMBOU Herve (FR number: 3240287195).

Funding

This manuscript research project was supported by the TWAS (The World Academy of Sciences) and ICCBS (International Center for Chemical and Biological Sciences), University of Karachi.

Conflict of interest

The Author has not declared any conflict of interest.

REFERENCES

1. Abdullah M, Firmansyah MA. Clinical approach and management of chronic diarrhea. Acta Med Indones, 2013; 45(2): 157-65.
2. Mazzolin LP, de Almeida Kiguti LR, da Maia EO, Fernandes LT, da Rocha LR, Vilegas W, Pupo AS, Di Stasi LC, Hiruma-Lima CA. Antidiarrheal and intestinal antiinflammatory activities of a methanolic extract of Qualea parviflora Mart. in experimental models. Journal of ethnopharmacology, 2013; 150(3): 1016-23.
3. Juckett G. and Trivedi, R. Evaluation of chronic diarrhea. American Family Physician, 2011; 84: 1119-1126.
4. Fischer A. Primary immunodeficiency diseases: an experimental model for molecular medicine, Lancet, 2001; 357: 1863-1869.
5. Maluma V. Les Antherotoma, Dissotis (inl. Heterotis) Melastomataceae endémiques d'Afrique centrale; 2005. [Online] Avaiable from: http://www.orchid-africa.net/PDF/ TAXONOMANIA%2015.pdf [Accessed on 23th May 2017]. French.
6. Tadjoua TH, Ateufack G, Shabana US, Dongmo MOL, Yousseu NW, Rukesh M, Muhammad IC and Gatsing D. Antidiarrhoeal, Antibacterial Activities and Growth Stimulatory Effect on Some Probiotic

Organisms of Aqueous and Methanol Leaves Extract of Dissotis Thollonii. Journal of Chemical and Pharmaceutical Research, 2017; 9(10): 95-105.
7. Carballo LJ, Hernandez-inda. L Z, Perez P and Gravalos M.D. A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. Bio Med Central Biotechnology, 2002; 2: 1-10.
8. Scholz G, Pohl I, Genschow E, Klemm M, Spielmann H. Embryotoxicity Screening Using Embryonic Stem Cells in vitro: Correlation to in vivo Teratogenicity. Cells Tissues Organs, 1999; 165: 203–11.
9. Sylvester PW. Optimization of the tetrazolium dye (MTT) colorimetric assay for cellular growth and viability. Drug Design and Discovery: Methods and Protocols, 2011; 157-68.
10. Haklar G, Sayin-Özveri E, Yüksel M, Aktan AÖ, Yalçin AS. Different kinds of reactive oxygen and nitrogen species were detected in colon and breast tumors. Cancer letters, 2001; 165(2): 219-24.
11. Taktak YS, Lee M. A solid phase enzyme immunoassay for serum amyloid A (SAA) protein: clinical evaluation. Journal of immunological methods, 1991; 136(1): 11-6.
12. Gupta M, Mazumdar UK, Sivakumar T, Vamsi ML, Karki SS, Sambathkumar R, Manikandan L. Evaluation of anti-inflammatory activity of chloroform extract of Bryonia laciniosa in experimental animal models. Biological and Pharmaceutical Bulletin, 2003; 26(9): 1342-4.
13. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, and McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Plant Medica, 1982; 45: 31-34.
14. Clemedson C, Ekwall B. Overview of the final MEIC results. I. The in vitro–in vitro evaluation. Toxicolgy In Vitro, 1999; 13: 657–63.
15. Scheers ME, Ba E, Dierickx JP. In vitro long-term cytotoxicity testing of 27 MEIC chemicals on HepG2 cells and comparison with acute human toxicity data. Toxicology In Vitro, 2001; 15(2): 153–61.
16. Weyermann J, Lochmann D, Zimmer A. A practical note on the use of cytotoxicity assays. International Journal Pharmaceutics, 2005; 288(2): 369–76.
17. Gilbert A, Herve TT, William YN, Leonard SF, Jules-Roger K, Albert K: Antidiarrhoeal and antibacterial activity of aqueous and methanolic leaves extracts of Dissotis thollonii Cogn.(Melastomataceae). Asian Pacific Journal of Tropical Biomedicine, 2014; 4: S672-S678.
18. Raygude KS, Kandhare AD, Ghosh P, Ghule AE, Bodhankar SL. Evaluation of ameliorative effect of quercetin in experimental model of alcoholic neuropathy in rats. Inflammopharmacology, 2012; 20(6): 331-41.
19. Rahman M, Selvarajan K, Hasan MR, Chan AP, Jin C, Kim J, Chan SK, Le ND, Kim YB, Tai IT. Inhibition of COX-2 in colon cancer modulates tumor growth and MDR-1 expression to enhance tumor regression in therapy-refractory cancers in vivo. Neoplasia, 2012; 14(7): 624IN17-33IN18.
20. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Molecular and cellular endocrinology, 2011; 335(1): 2-13.
21. Prajapati VD, Maheriya PM, Jani GK, Solanki HK. Carrageenan: a natural seaweed polysaccharide and its applications. Carbohydrate polymers, 2014; 105: 97-112.
22. Rauf A, Uddin G, Siddiqui BS, Khan A, Khan H, Arfan M, Muhammad N, Wadood A. In-vivo antinociceptive, anti-inflammatory and antipyretic activity of pistagremic acid isolated from Pistacia integerrima. Phytomedicine, 2014; 21(12): 1509-15.
23. Hebeda CB, Bolonheis SM, Nakasato A, Belinati K, Souza PD, Gouvea DR, Lopes NP, Farsky SH. Effects of chlorogenic acid on neutrophil locomotion functions in response to inflammatory stimulus. Journal of ethnopharmacology, 2011; 135(2): 261-9.
24. Gong XP, Sun YY, Chen W, Guo X, Guan JK, Li DY, Du G. Anti-diarrheal and anti-inflammatory activities of aqueous extract of the aerial part of Rubia cordifolia. BMC complementary and alternative medicine, 2017; 17(1): 20.
25. Sebai H, Jabri MA, Souli A, Rtibi K, Selmi S, Tebourbi O, El-Benna J, Sakly M. Antidiarrheal and antioxidant activities of chamomile (Matricaria recutita L.) decoction extract in rats. Journal of Ethnopharmacology, 2014; 152: 327–332.
26. Chittrakarn S, Sawangjaroen K, Prasettho S, Janchawee B, Keawpradub N. Inhibitory effects of kratom leaf extract (Mitragyna speciosa Korth.) on the rat gastrointestinal tract. Journal of Ethnopharmacology, 2008; 116: 173–8.
27. Izzo AA, Mascolo N, Capasso R, Germano MP, Depasquale R, Capasso F. Inhibitory effect of cannabinoid agonist on gastric emptying in rats.Archives of Pharmacology. Naunyn Schmiedebergs Archives of Pharmacology, 1999; 360(2): 221-223.