Comparative study of membrane-stabilizing activities of kolaviron, *Dryopteris filix-mas* and *Ocimum gratissimum* extracts

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Received 27 July 2015
Accepted 15 March 2016

Egyptian Pharmaceutical Journal
2016, 15:6–9

Background
Diseases associated with inflammation have been one of the major concerns in medicine. Some anti-inflammatory drugs such as aspirin have been found to exert side effects on the gastrointestinal tract. Therefore, it is necessary to seek new chemotherapy agents from plant sources with little or no side effects that are capable of preventing inflammation-related disorders.

Aim
This study aims to investigate the effects of methanol extracts, aqueous and chloroform fractions of *Dryopteris filix-mas* (DF), *Ocimum gratissimum* (OG) leaves and kolaviron on membrane stabilization; acetyl salicylic acid was used as a reference drug.

Materials and methods
Whole blood of rats weighing 150–200 g was assessed using hypotonic solution-induced haemolysis of albino rats, which was determined spectrophotometrically.

Results
Of the three extracts, kolaviron showed the highest haemolysis inhibition capacity (49.6, 56 and 66.56%) in a concentration-dependent manner (2, 4 and 6 mg/ml, respectively) compared with acetyl salicylic acid, with a haemolysis inhibition capacity of 61.45, 67.22 and 70% in the order of increasing concentrations. This was followed by the aqueous fraction of OG leaves at 6 mg/ml, with percentage haemolysis inhibition of 61.1%, whereas the aqueous fraction of DF was 56.49% at 6 mg/ml.

Conclusion
The above result suggested that kolaviron, aqueous fractions of OG and DF could serve as excellent alternative anti-inflammatory therapy agents.

Keywords:
anti-inflammatory therapy, *Dryopteris filix-mas*, haemolysis, kolaviron, *Ocimum gratissimum*

Egypt Pharm J 15:6–9

1687-4315

Introduction

Recently, attention has shifted to the protective biochemical roles of naturally occurring elements found in plants for the treatment of inflammatory diseases. NSAIDs have been shown to prevent inflammation by blocking the conversion of arachidonic acid into prostaglandin. This is achieved by inhibiting cyclo-oxygenase (COX-2) enzymes [1]. However, the long-term administration of NSAIDs has been implicated in ulceration and bleeding in the gastrointestinal tract because of inhibition of the COX-1 and COX-2 isoforms [2]. As a result of these side effects, it is of paramount importance to develop potent chemotherapeutic agents of plant origin with little or no side effects. Therefore, this study aims to evaluate the effect of kolaviron, *Dryopteris filix-mas* (DF) and *Ocimum gratissimum* (OG) on hypotonic solution-induced inflammation in the erythrocyte membrane. Moreover, the erythrocyte membrane is similar to the lysosomal membrane and as such, erythrocyte membrane stabilization could be used to extrapolate the effect of drugs on the lysosomal membrane [3].

Materials and methods

Collection and identification of plants

OG leaf was collected from and authenticated in the Department of Pharmacognosy Bodija Market, Ibadan, University of Ibadan, Oyo State, with specimen voucher number DPUI No. 1504, whereas DF leaf and kolaviron (from *Garcinia kola*) were obtained from and authenticated in the Department of Bioscience, Salem University community, Lokoja, Kogi State, with specimen voucher numbers SU 11087 and 12914, respectively.

Extraction and phytochemical screening of plants

The extracts of OG and DF were obtained using the cold extraction method with methanol in a 1 : 10 (w/v) ratio. After thorough mixing, it was allowed to stand for 72 h and filtered using a sterile Whatman No. 1 filter paper. The green-coloured filtrate (extract) was concentrated using a rotary evaporator. The resulting crude extract was evaporated to dryness using a water bath. The crude methanol extract was partitioned successively between *n*-hexane, chloroform, ethyl acetate and distilled water.

Kolaviron is a defatted methanol extract of *G. kola* obtained by Soxhlet extraction in a 1 : 10 (w/v) ratio.

Kolaviron, extracts and fractions of DF and OG leaf were screened for various phytochemicals [4,5]. Flavonoids were detected with ethanolic KOH/ethyl acetate and alkaloids were discovered using Mayer's, Wagner's, Dragendorf reagents and picric acid. Also, saponins were identified using froth and haemolytic tests and cardiac glycosides were identified using chloroform/H_2SO_4. Tannins and phenols were identified using ferric chloride reagent, whereas ethanolic NaOH was used to determine the presence of anthraquinones.

Erythrocyte suspension

Blood was collected by a jugular puncture from albino rats under anaesthesia (chloroform) into a test tube containing an anticoagulant (EDTA). The blood was washed three times with 0.9% saline by centrifugation at 3000 rpm. After washing, it was measured and reconstituted (40%, v/v) with an isotonic buffer solution (154 mmol/l NaCl and 10 mmol/l sodium phosphate buffer, pH 7.4), which served as the stock erythrocyte solution. Approval was obtained from the institutional animal ethics committee before carrying out this research.

Hypotonic solution-induced haemolysis

The membrane-stabilizing activity of the extract was assessed using a hypotonic solution-induced rat erythrocyte haemolysis model designed by Sikder *et al*. [6]. The test sample was prepared by suspending 0.5 ml of stock erythrocyte solution in 5 ml of hypotonic solution that contained (50 mmol/l NaCl in 10 mmol/l sodium phosphate buffer, pH 7.4). The test contained the extracts/acetyl salicylic acid as a standard. The control sample consisted of 0.5 ml of red blood cells stock solution with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at 3000*g* and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

$$\%\text{inhibition of haemolysis} = 100 \times (OD_1 - OD_2/OD_1),$$

where OD_1 is optical density of the hypotonic-buffered saline solution alone (control) and OD_2 is optical density of the test sample in the hypotonic solution.

Statistical analysis

The data were analysed using one-way analysis of variance and Duncan's multiple-range tests. Data were expressed as mean ± SD of at least three independent measurements (assays) at *P* value less than 0.05.

Results and discussion

The inflammatory mediators such as cytokines, neutrophils, lymphocytes, etc. produced during

Table 1 Absorbance of different extracts of *O. gratissimum* leaves on haemolysis

Concentration (mg/ml)	Extracts	Methanol	Aqueous	Standard drug
	Chloroform			Acetyl salicylic acid
2.0	0.313 ± 0.002	0.274 ± 0.007	0.38 ± 0.002	0.281 ± 0.003
4.0	0.251 ± 0.001	0.247 ± 0.003	0.337 ± 0.001	0.239 ± 0.001
6.0	0.242 ± 0.003	0.242 ± 0.001	0.295 ± 0.002	0.218 ± 0.001
Control	0.397 ± 0.001	0.482 ± 0.005	0.758 ± 0.004	0.729 ± 0.005

Values are means of three replications ± SD.

Table 2 Absorbance of different extracts of *D. filix-mas* leaves on haemolysis

Concentration (mg/ml)	Chloroform	Methanol	Aqueous	Acetyl salicylic acid
2	0.416 ± 0.02	0.393 ± 0.04	0.346 ± 0.01	0.281 ± 0.003
4	0.371 ± 0.01	0.375 ± 0.018	0.339 ± 0.08	0.239 ± 0.001
6	0.343 ± 0.05	0.35 ± 0.03	0.332 ± 0.03	0.218 ± 0.001
Control	0.491 ± 0.03	0.452 ± 0.07	0.763 ± 0.01	0.729 ± 0.05

Values are means of three replications ± SD.

inflammation to attack pathogens may also cause damage to normal cells. Lysosomal hydrolytic enzymes are also released into the extravascular milieu, causing a variety of disorders. Some of the various methods used to assess the inflammatory potentials of drugs and phytotherapy include inhibition of denaturation of protein, uncoupling of oxidative phosphorylation, erythrocyte membrane stabilization and lysosomal membrane stabilization, among others [7,8].

Phytochemical screening of DF showed the presence of alkaloids and flavonoids, whereas saponins, phenols, quinones and phlobatannins were absent among others. OG showed the presence of alkaloids, flavonoids, saponins and tannins. Flavonoids, alkaloids and phenols, among others, were present in kolaviron. Tables 1, 2 and 3 show the absorbance used in the calculation of the various percentage inhibitions of haemolysis.

Table 4 shows the membrane-stabilizing activities of the extract and fractions of OG leaf ranging from 2 to 6 mg/ml. Chloroform fraction showed the least inhibition, with minimum and maximum inhibitions of 21 ± 0.32 and 39 ± 0.19%, respectively. Methanol extract showed a percentage inhibition of 43.15 ± 0.41 and 49.79 ± 0.45% for minimum and maximum, respectively. The aqueous fraction showed the lowest inhibition of 49.87 ± 0.3%, with the highest inhibition of 61.1 ± 0.2%, whereas the standard drug (acetyl salicylic acid) showed a maximum inhibition of haemolysis of 70 ± 1.2% in concentration dependent manner.

Table 5 shows the membrane-stabilization activity of DF leaf extract/fractions. The chloroform fraction showed minimum and maximum membrane stability of 15.25 ± 0.05 and 30.14 ± 0.1%, respectively. The methanol extract showed a minimum activity of 13.05 ± 0.2% and a maximum inhibition of 22.57 ± 0.6%. The aqueous fraction showed the lowest and highest percentage haemolysis inhibition of 54.65 ± 0.1 and 56.49 ± 0.03%, respectively. The reaction of the red blood cell was found to be biphasic.

Table 6 shows the effect of kolaviron on membrane stability. Kolaviron showed the minimum inhibition activity of 49.6 ± 0.5% and membrane-stabilizing activity of 66.56 ± 0.78%.

The inhibition potential of the kolaviron was the highest among the extracts/fractions. The response on the erythrocyte membrane in concentration increasing order [9].

It was observed that all the extracts/fractions showed inhibition potential on the erythrocyte membrane in a concentration-dependent manner. However, none of the extracts and fractions showed membrane-stabilizing activity higher than that of the standard drug.

The mode of action of the extracts, fractions and standard anti-inflammatory drugs could be associated with their ability to bind to the erythrocyte membranes and the accompanying surface charge alterations of the cells, which might have prevented physical interactions with aggregating agents or enhanced dispersal by mutual repulsion of the like charges involved in erythrocyte haemolysis. Saponins and flavonoids were reported to exert a profound stabilizing effect on the lysosomal membrane both *in vivo* and *in vitro*, whereas tannins and saponins were capable of binding cations,

Table 3 Absorbance of kolaviron on haemolysis

Concentration (mg/ml)	Kolaviron	Acetyl salicylic acid
2	0.315 ± 0.02	0.281 ± 0.003
4	0.275 ± 0.02	0.239 ± 0.001
6	0.209 ± .005	0.218 ± 0.005
Control	0.625 ± 0.001	0.729 ± 0.05

Values are means of three replications ± SD.

Table 4 Effect of different extracts of *O. gratissimum* on inhibition of haemolysis (%)

Concentration (mg/ml)	Extracts	Methanol	Aqueous	Standard drug
	Chloroform			Acetyl salicylic acid
2	21.16 ± 0.32	43.15 ± 0.41	49.87 ± 0.3	61.45 ± 0.8
4	36.78 ± 0.4	48.76 ± 0.62	55.54 ± 1	67.22 ± 0.7
6	39 ± 0.19	49.79 ± 0.45	61.1 ± 0.2	70 ± 1.2

Values are means of three replications ± SD.

Table 5 Effect of different extracts of *D. filix-mas* leaves on inhibition of haemolysis (%)

Concentration(mg/ml)	Chloroform	Methanol	Aqueous	Acetyl salicylic acid
2	15.25 ± 0.05	13.05 ± 0.2	54.65 ± 0.1	61.45 ± 0.8
4	24.28 ± 0.03	17.04 ± 0.9	55.57 ± 0.07	67.22 ± 0.7
6	30.14 ± 0.1	22.57 ± 0.6	56.49 ± 0.03	70.00 ± 1.2

Values are means of three replications ± SD.

Table 6 Effect of kolaviron on inhibition of haemolysis (%)

Concentration (mg/ml)	Kolaviron	Acetyl salicylic acid
2	49.6 ± 0.5	61.45 ± 0.8
4	56 ± 0.35	67.22 ± 0.7
6	66.56 ± 0.78	70 ± 1.2

Values are means of three replications ± SD.

thus stabilizing erythrocyte membranes and other biological macromolecules [7,10,11].

The lowest activities observed with crude methanol extracts of the plants leaves could be associated with the chelating potentials of other phytoconstituents over the membrane-stabilizing components. Also, the reduced membrane-stabilizing activity in DF may be associated with the absence of tannins.

Conclusion

It could be inferred from the above results that OG leaf extract/fractions and kolaviron as well as DF leaf extract/fraction have some bioactive principles capable of stabilizing hypotonic solution-induced haemolysis of the erythrocyte membrane. Therefore, they could serve as an excellent alternative chemotherapy agent in the management and treatment of inflammation-related diseases and disorders.

Acknowledgements

The authors are very grateful to Professor S. Awe, the Dean of College of Natural and Applied Science, Salem University, Nigeria, who gave the permission to carry out the research in the Biochemistry Laboratory, Salem University.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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