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IN VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY OF THE FLOWER EXTRACTS OF *PEDALIUM MUREX L.*

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ABSTRACT

To evaluate the antioxidant and anti-inflammatory activities of the solid powder obtained from the ethyl acetate fraction from the flower Pedalium murex L. The flower extract was evaluated for antioxidant activity by 1,1–diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay and reducing power assay was carried out by FRC (Ferric Reducing Capacity) assay method. The in vitro anti-inflammatory activity was evaluated using human peripheral blood mononuclear cells (PBMC) were stimulated by lipopolysaccharide (LPS) to evaluate nitric oxide (NO) production method. The solid powder obtained from the ethyl acetate fraction from the flower Pedalium murex L showed a good antioxidant activity in scavenging DPPH radical and FRC assay with compared standard sample. This solid powder also showed good anti-inflammatory activity in cell viability (LPS induced PBMCs) assay and nitric oxide (NO) assay. These results suggest that the solid powder obtained from the ethyl acetate fraction from the flower Pedalium murex L have significant antioxidant and anti-inflammatory activities.

KEY WORDS

Anti-inflammatory, Ethyl acetate, FRC, Pedalium murex.

INTRODUCTION

Numerous higher plants are real wellsprings of regular items utilized as pharmaceuticals, agrochemicals, flavor and scent fixings, sustenance added substances and pesticides [1]. The look for new plant metabolites ought to be a need in present and future endeavors towards manageable protection and usage of biodiversity. Restorative plants are generally utilized as option helpful apparatuses for the counteractive action of numerous ailments [2, 3]. Herbal based drugs remain an important source because of the availability, relatively cheaper cost and no side effects when compared to modern medicine [4]. The field of free radical science is increasing more consideration nowadays. Free radicals are responsive oxygen and nitrogen species which are produced by different physiological procedures in the body. Uncontrolled era of free radicals prompts assault on layer lipids, proteins, catalysts and DNA causing oxidative anxiety and at last cell demise. These ROS are dependable for some degenerative human illnesses like diabetes mellitus, tumor, neurodegenerative scatters, Alzheimer's malady, Parkinson's ailment, atherosclerosis, maturing and provocative diseases [5]. In human body there are different chemical frameworks with the expectation of complimentary radical rummaging yet micronutrients like vitamin E, beta-carotene and vitamin C are the significant antioxidant. These must be given in consume less calories as body can't deliver these nutrients [6]. Assurance against free radicals can be upgraded by taking adequate measures of exogenous cell reinforcements. Cell reinforcement is a steady atom which gives an electron to a rampaging free radical and ends the chain response before essential particles are harmed. Free radical rummaging property of cancer prevention agents delays or hinders

cell damage [7]. Inflammation is the central feature of many physiopathological conditions in response to tissue injury and as part of host defense against microorganisms [8]. In inflammatory processes, macrophages have a key part in providing a prompt guard against outside operators. Upon enactment with an inflammatory jolt, for example, lipopolysaccharyde (LPS), macrophages produce pro-inflammatory go betweens, including nitric oxide (NO). Therefore, the search of effective non-toxic natural compounds with antioxidative activity has been identified in recent years [9]. Plants containing polysaccharides are the most potent in curing inflammatory diseases [10].

Pedalium murex L is an individual from sesame family, Pedaliaceae. It is found in various piece of the world, for example, tropical range, Srilanka, India, Mexico and Pakistan. In India, it happens fundamentally in the Western and Corommandal drifts as weed of waste places and is for the most part called under the Hindi name "" Gokhru or gokhar'' and in Sanskrit as ''gaja-daunstraka, gokshura or titta –gokshura". Its name shifts from one district to another extending from North to South and from East to West piece of the nation. Its likewise brought in Kannada (doddaneggilu), Malayalam (motha-malvi-gokharu), Tamil (Yanainerunjil &Ananerinnil), Marathi (Gokhara), Gujarati (Gokhura), Oriya(Yanainerunjil) ,Arabic (Khasakekabir) and Singapore (Atineranchi) [11-13]. A mixture or concentrate arranged from the diverse piece of the plant in frosty water is utilized as demulcent, diuretic , and furthermore observed to be best in the treatment of clutters of urinary frameworks, for example, gonorrhea, dysuria, incontinence of the pee and the other way around [14,15].This plant is likewise utilized by the neighborhood individuals as pain relieving and antipyretic exercises [16,17]. The leaves, root and fruits of this plant had anti-inflammatory and antioxidant action [18, 19]. Since there is an absence of logical assessment of the antioxidant and anti-inflammatory movement of this plant. Keeping this in see, the present examination has been attempted to explore the antioxidant and anti-inflammatory capability of the solid powder got from the ethyl acetate portion from the flower *Pedalium murex L*.

MATERIALS AND METHODS

Collection of flowers

The new flowers of *Pedalium murex.L* were gathered from Z. Suthamalli, Ariyalur (Dt), Tamil Nadu, India. This plant was legitimately recognized by Dr.S.John Britto, Director, The rapinat Herbarium and Center for Molecular Systematics (Authentication No. DP002 dated: 22/01/2016). St.Joseph's College (Campus), Tiruchirappalli, Tamil Nadu, India.

Extraction and fractionation

Crisp flowers (3 kg) of *Pedalium mure L*. were removed with 90% ethanol (5x500ml). The consolidated alcoholic concentrate was amassed in vacuo and the aqueous concentrate was progressively fractionated with petroleum ether (60-800C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether division and diethyl ether division did not yield any isolable substance. Ethyl acetate fraction on focus yielded a dry powder which was broken up in DMSO to get different fixations and were utilized for additionally examines.

DPPH scavenging assay

DPPH radical searching action of the sample was decide as indicated by the technique detailed by Blois (1958). An aliquot of 0.5 ml of test arrangement in methanol was blended with 2.5 ml of 0.5 mM methanolic arrangement of DPPH. The blend was shaken energetically and hatched for 30 min oblivious at room temperature. The absorbance was estimated at 517 nm utilizing UV spectrophotometer. Ascorbic acid was utilized as a positive control. DPPH free radical rummaging capacity (%) was ascertained by utilizing the recipe [20, 21, 22].

% Inhibition= [{Abs $_{control}$- Abs $_{sample}$}/Abs $_{control}$] ×100

Where Abs $_{control}$ is the absorbance of the DPPH radical+ ethanol, Abs $_{sample}$ is the absorbance of DPPH radical+ test separate**/**standard.

Ferric Reducing Capacity (FRC) assay

The FRC assay is like the FRAP assay with the exception of that it utilizes O-Phenanthroline rather than TPTZ. Phenanthroline shapes a Fe^{III}-$(Phen)_3$ complex that is diminished to an orange-red—shaded Fe^{II}-$(Phen)_3$ complex. The response blend containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM), 20 ml of acetate buffer and 2 ml of different focuses extending from 10 to 250µg was brooded at room temperature for 10 min and the absorbance of the same was estimated at 510 nm. Newly arranged FRC

reagent clear perusing was taken at 510 nm. In this assay lessening limit of the samples tried and compared and that of ascorbic acid. All estimations were done in triplicate and the average was taken [23, 24, 25].

% Reduction = A_{510} sample / A_{510} blank reagent

Isolation of Human PBMC and Culture

PBMC (peripheral blood mononuclear cells) from sound givers were disengaged from EDTA tube blood by Ficoll-Hypaque angle centrifugation. To put it plainly, peripheral blood from the givers was weakened with sterile phosphate buffer saline and overlaid on the Ficoll-Hypaque arrangement, and centrifugation was performed at 350 x g for 10 min at room temperature. The recuperated PBMC were refreshed in RPMI-1640 and brooded at 37°C 95%O_2 + 5% CO_2 for 30 min before playing out the investigations [26].

The cell viability of PBMCs with LPS Stimulation

After the PBMC isolation and pre-incubation period, 1×10^5 cells/ml PBMC were cultured in a 96-well polypropylene plate in serum-free RPMI-1640 medium with LPS at a final concentration of 100 ng/ml and various concentrations of the sample in dimethyl sulfoxide (DMSO). Cells in all conditions were incubated at 37°C, 95%O_2 + 5% CO_2 for 24 h. Supernatants were removed from the treated cells to investigate nitrous oxide (NO) scavenging activity. The viability of the cells was performed to evaluate the cytotoxicity of the sample using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay. In the control wells, cells were incubated with DMSO vehicle and without LPS. In the positive control wells, cells were incubated with LPS and DMSO vehicle. The cell viability was determined by measuring the absorbance at 570 nm by a micro plate reader [26].

% Cell viability = (OD of the sample/OD of the control) ×100

Nitric oxide (NO) inhibition assay

Nitric oxide discharged in the supernatants was explore utilizing Griess reagent. The supernatants (100 µl) were blended with 20 µl of 1% sulfanilamide in 5% phosphoric acid,

hatched for 10 min at room temperature, 20 µl of 0.1% naphthyl ‐ ethylenediamine dihydrochloride were included and brooded for 10 min at room temperature. After that the absorbance of the response blend was perused at 540 nm. Sodium nitrite was utilized for the adjustment bend [27, 28, 29].

Statistical analysis

All the data were reported as the mean ± standard deviation (S.D.). All statistical analysis was performed by methods for one-way analysis of variance (ANOVA) and Student's t-test utilizing Graph Pad Prism statistical software package version7.02. The IC_{50} esteem was processed from nonlinear backslide examination using the Graph Pad Prism programming with the condition: $Y = 100[1 + 10^{(X'' \ LogIC_{50})}]$. Only a value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Anti-oxidant activity

DPPH assay is quick, simple and practical strategy to quantify an antioxidant effect, which includes the utilization of the free radical [30], 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is usually used to test the capacity of mixes to go about as free extreme foragers or hydrogen contributors and it depends on the capacity of DPPH to decolorize within the sight of antioxidant [31, 32]. The inhibition of the DPPH radical by the solid powder obtained from the ethyl acetate fraction from the flower *Pedalium murex L* was concentration dependent (Fig.1). The inhibition percentage of the solid powder at different concentrations (10, 50,100,200 and 250 µg/ml) ranged between 76.43 ± 0.10% to 82.41±0.13%. This solid fraction was capable of reducing the DPPH radical 50% with IC_{50} of <10 µg/ml compared to standard ascorbic acid which also have an IC_{50} <10 µg/ml. So the solid powder obtained from the ethyl acetate fraction from the flower *Pedalium murex L* showed a very good anti-radical activity in scavenging DPPH radical and showed a maximum % inhibition of 82.41±0.13% at 250µg/ml concentration. DPPH scavenging activity of *Pedalium murex L* was shown in table 1.

The decreasing furthest reaches of a compound may fill in as a basic pointer of its potential antioxidant activity. The science of iron-based assays might be stuffed with the response condition:

Fe (III)–L + antioxidant ↔ Fe (II)–L + oxidized antioxidant

Where L is the ferrous-specific chromogenic ligand delivering the shaded species Fe (II)–L because of the concerned redox response. Longer wavelengths quite often constitute an imperative preferred standpoint in spectrophotometric strategy determination, on the grounds that most plant pigments and additionally a few antioxidants demonstrate huge assimilation at shorter

wavelengths near the UV scope of the unmistakable range. This reasoning applies for L :O-Phenanthroline instead of TPTZ ligand for modern FRAP assay. The redox response with tris(phen)Fe (III) of a polyphenolic compound Ar(OH)n is given as:

$n\text{Fe(phen)}_3^{3+} + \text{Ar(OH)}n \leftrightarrow n\text{Fe(phen)}_3^{2+} + \text{Ar(=O)}n + n\text{H}^+$

The reduction values of the solid powder obtained from the ethyl acetate fraction from the flower *Pedalium murex L* at various concentration (10, 50,100,200&250 µg/ml) ranged between 18.42± 0.90% to 88.40±1.06% are depicted in Table 2. The IC_{50} values of this solid fraction were found to be 50.24±1.06µg/ml compared to standard ascorbic acid which has a 32.88±2.50µg/ml. In the concentration extend examined, all the solid powder exhibited reducing power that expanded straightly with concentration (Fig.2). So the solid powder obtained from the ethyl acetate fraction from the flower *Pedalium murex L showed* a very good anti-radical activity in FRC assay and showed a maximum % reduction 88.40±1.06 % at 250µg/ml.

Anti-inflammatory activity

The ideal condition for LPS-incited pro-inflammatory cytokines production was in PBMCs. LPS is a gram - negative microbes which has an endotoxin and a constituent of the external layer. LPS stimulates innate immunity by regulating the production of inflammatory mediators such as TNF-α in PBMCs. The results showed (Fig.3) that the solid obtained from the ethyl acetate fraction from the flower *Pedalium murex L* showed dose dependant. Examination of the cell viability of the fraction in PBMCs using the MTT assay had indicated that the powder at (25 to 200) µg/ml did not affect the viability of PBMCs. Thus, the inhibition of LPS- induced mediator inflammation by the solid powder was not the result of a possible cytotoxic effect on these cells. So these outcomes propose the anti-inflammatory impact of the solid powder (Table 3), focusing on pro-inflammatory cytokines generation, and using this solid fraction possibly did not bring about unfavorable impacts.

Nitric oxide (NO) is a pivotal biological messenger. As an inflammatory arbiter, NO assumes a part in a wide range of physiological and pathophysiological procedures, for example, macrophage-intervened cytotoxicity, vein dilatation, smooth muscle unwinding, and neurotransmission (33). PBMCs were refined with LPS (100 ng/ml) and the substance of the proved nitric oxide (NO) were measured in the supernatants as a component of macrophage actuation. The impact of nitric oxide (NO) was seen in the cell culture medium of PBMC containing diverse concentration (25, 50,100 and 200 µg/ml) of the solid powder obtained from the ethyl acetate fraction from the flower *Pedalium murex L* as appeared in Table 4. The NO production percentage of the solid fraction at different concentration (25 to200 µg/ml) ranged between 86.41±0.75% to 64.19±0.62% are depicted in Table 4. This study demonstrated that the solid ethyl acetate fraction from the flower *Pedalium murex L* much reduced the production of Nitric oxide (NO). The data demonstrate a significant decrease in the nitric oxide production in the solid fraction as compared to control group (Fig.4).

Hence, it's clear that the solid powder obtained from the ethyl acetate fraction of *Pedalium murex L* flowers has anti inflammatory activity in NO assay and showed a maximum reduced NO production 64.19±0.62% at 200µg/ml.

Table 1: DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay

Concentration (µg/ml)	% of Inhibition	
	Sample	Standard (Ascorbic acid)
10	76.43 ± 0.10*	78.98 ± 0.43
50	77.23 ± 0.44*	84.68 ± 0.04
100	79.49 ± 0.19*	86.10 ± 0.34
200	81.09 ± 0.06*	89.82 ± 0.82
250	82.41 ± 0.13*	92.15 ± 0.50
	IC_{50}< 10 µg/ml	IC_{50}< 10 µg/ml

Values are shown as for means ±SD of triplicate. *p<0.05 compared with standard (one-way ANOVA and t-test)

Table 2: Ferric Reducing Capacity (FRC) assay

Concentration (µg/ml)	% of Reduction	
	Sample	Standard (Ascorbic acid)
10	18.42± 0.90*	25.07±0.98
50	47.49±1.22*	55.20±1.11
100	65.73±0.95*	78.00±0.48
200	81.60±2.03*	92.57±1.02
250	88.40±1.06*	95.73±0.91
	IC $_{50}$=50.24± 2.2 **µg/ml**	IC $_{50}$=32.88±2.50 **µg/ml**

Values are shown as for means ±SD of triplicate. *p<0.05 compared with standard (one-way ANOVA and t-test)

Table 3: PBMCs with LPS of cell viability

Concentration (µg/ml)	Cell Viability (%)
Control	100
Positive control (LPS)	60.79 ± 0.74
25	75.43 ± 0.50*
50	86.44 ± 0.62*
100	99.38 ± 0.37*
200	99.50 ± 0.25*

Values are shown as for means ±SD of triplicate. *p<0.05 compared with control and positive control (one-way ANOVA and t-test).

Table 4: Nitric oxide (NO) inhibition assay

Concentration (µg/ml)	NO production (%)
Control (LPS)	100
25	86.41 ± 0.75*
50	80.75 ± 1.05*
100	71.23 ± 0.45*
200	64.19 ± 0.62*

Values are shown as for means ±SD of triplicate. *p<0.05 compared with control (one-way ANOVA and t-test)

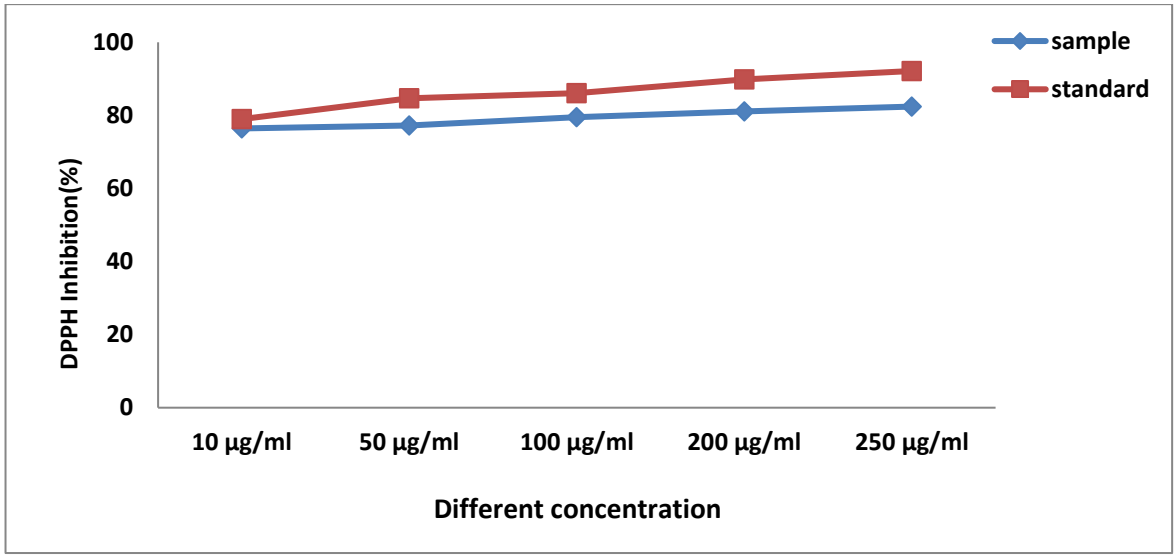

Fig. 1. DPPH inhibition (%) activity of the sample (solid powder of *Pedalium murex L* flower fraction) and standard (Ascorbic acid) at different concentration.

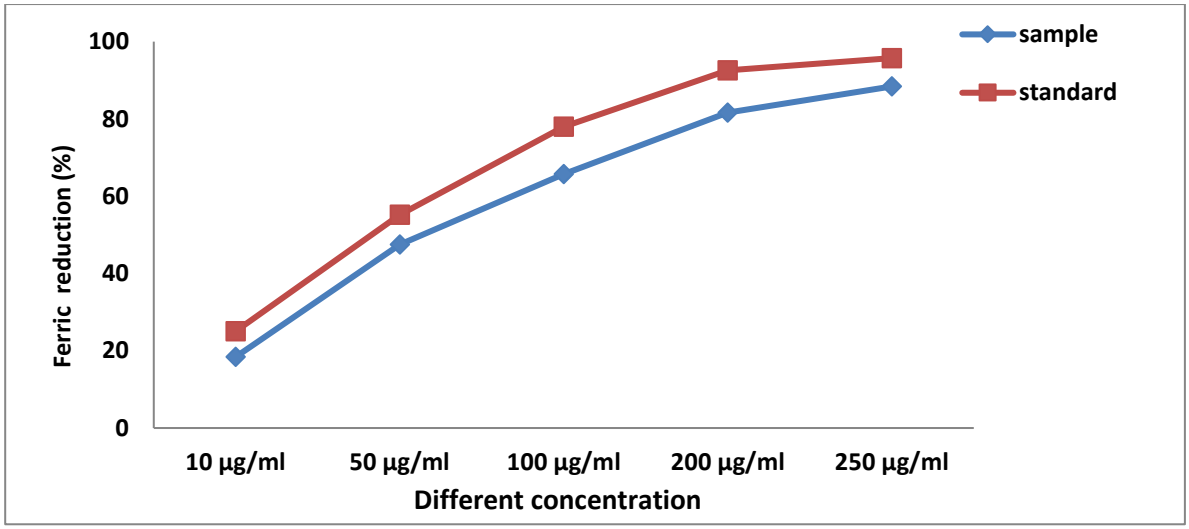

Fig. 2. Ferric reduction (%) of the sample (solid powder of *Pedalium murex L* flower fraction) and standard (Ascorbic acid) at different concentration.

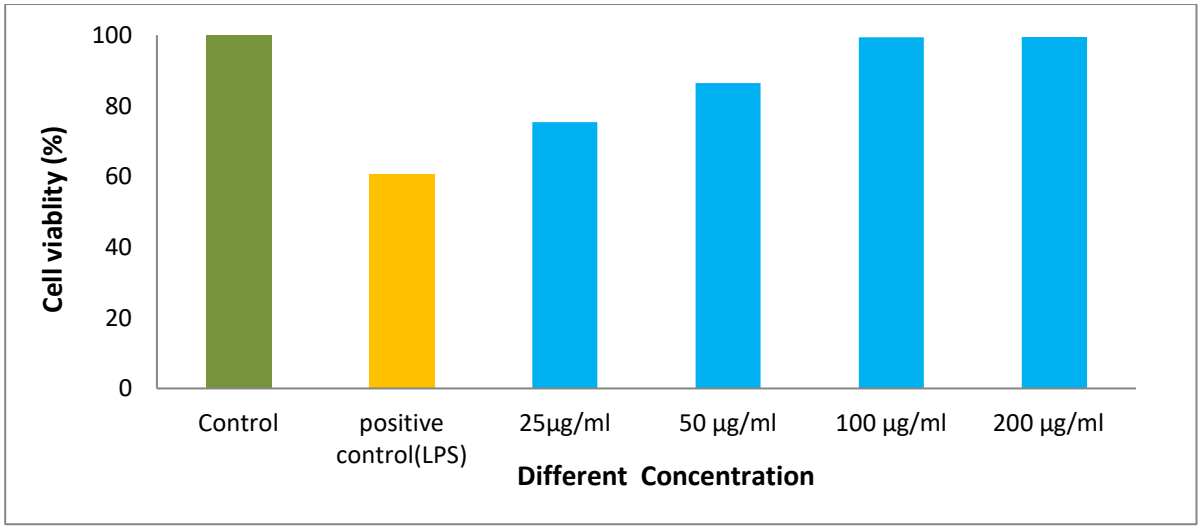

Fig. 3. The cell (PBMC) viability percentage of without LPS (control), with LPS (positive control) and the sold powder (*Pedalium murex L* flower fraction) at different concentration.

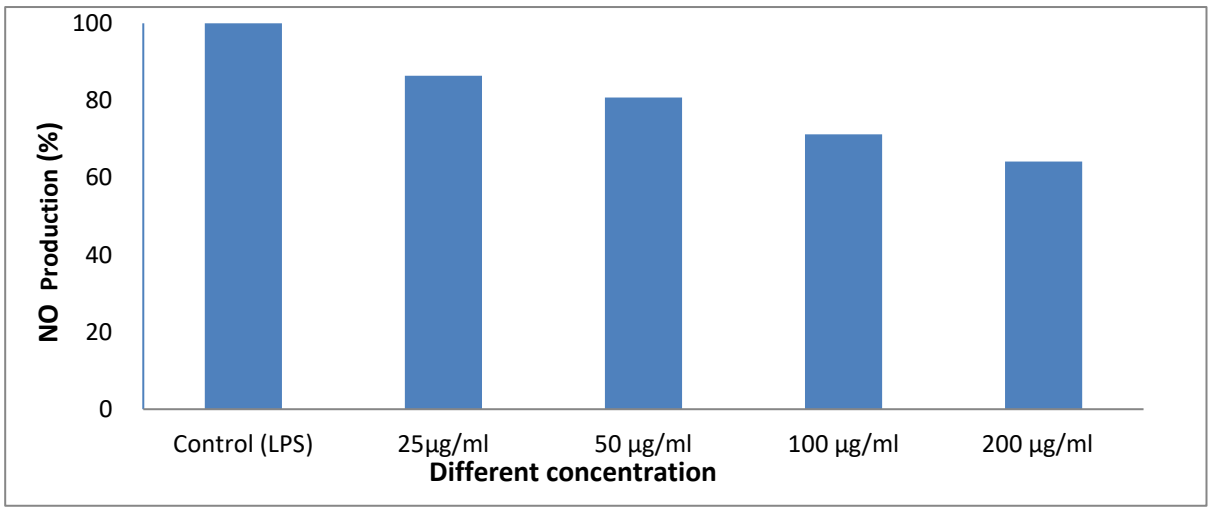

Fig. 4. The effect of solid powder (*Pedalium murex L* flower fraction) different concentration on NO production by human PBMC stimulated with LPS.

CONCLUSION

In conclusion, show contemplate uncovered the in-vitro antioxidant and anti-inflammatory action of the solid acquired from the ethyl acetate fraction of the flower *Pedalium murex L*. Moreover, work is required remembering the true objective to develop the character of the substance constituent responsible for antioxidant and anti-inflammatory activities. Studies are ahead of time in our examination office to outline the sub-nuclear structure of the compound. This contributes towards the progression of extreme antioxidant and anti-inflammatory pharmaceutical.

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