ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

In-vitro Anti-inflammatory activity of Liquorice (*Glycyrrhiza glabra*) using Aqueous Extract

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Article History:

Received on: 26.08.2019
Revised on: 05.11.2019
Accepted on: 16.11.2019

Keywords:

Glycyrrhiza glabra, Phytochemical screening, antioxidant assays, Anti-inflammatory assays

ABSTRACT

The *G.glabra* is otherwise called liquorice is a medicinal plant is used for various diseases like cold, cough, hypokalemia and muscle weakness, etc. The liquorice family belongs to the Fabaceae family of the *G.glabra*. Hence this study tells about the anti-inflammatory and antioxidants. The quantitative study of phytochemical analysis, antioxidant and Anti-inflammatory, cytotoxicity assay using a response from the root extract of *G.glabra* The results are showed above preliminary activity phytochemicals were present Alkaloids, Flavonoids, Coumarin, Saponins, Terpenoids, Steroids, Cardiac Glycosides. The antioxidant activity of aqueous extract of *G. glabra* were evaluated with the 2,2-diphenyl-1-picryl hydrazyl (DPPH), nitric oxide radical (NO), superoxide radical (SO), hydrogen peroxide radical (H_2O_2), and hydroxyl radical (HO) scavenging activity. This study shows about the activity of *Glycyrrhiza glabra* herbal medicinal plant aqueous extract using antioxidant assays, Anti-inflammatory assays. The *in vitro* MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide Cytotoxicity assay were studied GG(*G.glabra*) extract using the macrophages Raw 246.7 Cell line. The cell line assay were studied in two different activity. One is anti-inflammation studies, and another is cytotoxicty of GG aqueous extract. The anti-inflammation IC50 value is 143.65, and GG extract dry sample were against the IC50 value is 326.27. From above, the results were a potential activity of GG extract R-value 0f 0.991.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i1.1872

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INTRODUCTION

The reactive oxygen species (ROS) provided by the results of free radicals on molecular oxygen, which increases abnormally during inflammation (Gupta *et al.*, 2016a) and causes an imbalance between the molecules oxidization and antioxidant activity of the body.

Inflammation is known to be a severe response to any kind of injury by tissues. Although inflammation is termed to be a defence response of our body to allergies/injury to the tissue, it rises the blood circulation in the affected area. Inflammation may be acute or chronic. There are many medicines used for controlling inflammation; steroidal and non-steroidal are mostly used as anti-inflammatory drugs to treat acute inflammatory disorders, despite their secondary effects. Since the NSAIDs and immunosuppressant, relief the inflammatory disease (Shah *et al.*, 2018) for a long term period of treatment, associated with serious side effects ulcer,

gastro problems. This leads to the search of various other treatments using medicinal plants (Kaur *et al.*, 2013).

Based on this, the medicinal plants revealed to have the secondary metabolites, which shows effective treatment for inflammation. Among all these medicinal plants, Glycyrrhiza glabra are of interest. They are found to be the native of the Middle East, southern Europe, and parts of Asia such as India. The root Liquorice (Glycyrrhiza glabra) has found to have anti-inflammatory and immune-boosting properties. This is used to treat various disorders like inflammation, gastro problems, respiratory problems, and to treat cancer (Zadeh *et al.*, 2013). Several studies report that different parts of the various plant have biological effects. The main activity tells about the anti-inflammatory effect of the aqueous extract of Liquorice (Glycyrrhiza glabra) (Chauhan *et al.*, 2018).

MATERIALS AND METHODS

Collection of plant materials

The Liquorice was collected from the local market authenticated by Dr. K. G. Purushothaman, Associate professor, Department of biotechnology, Dr. M.G.R. Educational, and Research Institute, Chennai.

Preparation of the aqueous extract

The root of Liquorice was cleaned, shade dried at 40°C, and ground to a fine powder using a mechanical grinder. The 100 grams of powder was mixed with 1 litre of water and kept in a shaker for 6 hours at 30°C,50 rpm. After the 3 days of the rest period, the extract was filtered using a muslin cloth. The filtrate was then centrifuged at 1500 rpm for 15 minutes of supernatant collected. The yield obtained was measured.

Phytochemical screening

The aqueous extract was subjected to phytochemical analysis in order to find out the presence of phytochemical constituents (Sharma and Pandey, 2013) like alkaloids, anthraquinone, flavanoids, phenols, coumarin, saponins, tannins, terpenoids, steroids, cardiac glycosides.Determination of antioxidant assays

Determination of antioxidant assays

The aqueous extract were used to determine the DPPH radical scavenging activity, superoxide radical scavenging activity, nitric oxide activity. Different concentrations of aqueous extract was used to determine the assay.

DPPH radical scavenging activity

To 3.0 ml of methanolic solution of DPPH (0.1mM), different concentrations of aqueous extract was added (Gupta *et al.*, 2016b). DPPH alone serves as a control. The reaction mixture was incubated for 30 mins at 37°C, and the absorbance was measured at 517 nm using UV - visible spectrophotometer. The percentage of inhibition was calculated using the formula,

$$\%\ inhbition = ((Abs\ control - Abs\ sample) / Abs\ control) x100$$

Superoxide radical scavenging activity

To different concentrations of extract, 1.0 ml of 0.12 M sodium carbonate, 0.4 ml of 25μM NBT, and 0.2 ml of 0.1mM EDTA were added, and the reaction was initiated by adding 0.4 ml of 1.0mM hydroxylamine hydrochloride and incubated for 20 minutes (Franceschelli *et al.*, 2011). Further, the absorbance was measured at 560 nm using a spectrophotometer. The percentage of inhibition was calculated.

Nitric oxide scavenging activity

The reaction mixture (3 ml) containing 10 mM sodium nitropruside in phosphate buffere saline, and different concentration of extracts was incubated at 25°C for 150 minutes, after the incubation period 1.5 ml of the reaction was removed, followed by 1.5 ml of the Griess reagent was added (Kowti *et al.*, 2010). The absorbance of the chromophore formed was measured at 546 nm. The percentage of inhibition of was calculated.

HRBC membrane stabilization test

10 ml of blood was collected and centrifuged at 3000 rpm for 10 minutes. The volume was measured, and it is reconstituted to 10% v/v suspension with normal saline. The reaction mixture consisting of 1 ml of aqueous extract and 1 ml of 10% RBC were incubated in a water bath at 56 °C for 30 minutes (Kumar *et al.*, 2013). Normal saline, along with 10% RBC, is used as control. After the incubation period, the tubes were cooled using running tap water. The reaction was then centrifuged at 2500 rpm for 5 mins, and the supernatants were collected. The absorbance was measured at 560 nm. Aspirin was used as a standard. Triplicates were obtained, and the percentage of inhibition was calculated as follows,

$$\%\ inhibition = (Abs\ of\ control - Abs\ of\ sample / Abs\ of\ control) x100$$

Anti-inflammatory assays

Inhibition of albumin denaturation

Based on the protocol with minor modifications, the method was conducted. The reaction mixture consists of different concentrations of aqueous extract and a 1% aqueous solution of bovine albumin fractions. The pH was adjusted, and the reaction mixture was incubated at 37°C for 20 minutes, followed by heating at 57°C for 30 minutes. After cooling, the turbidity was measured using a spectrophotometer at 660 nm. Aspirin is used as a standard drug. The Inhibition percentage was calculated using the triplicates obtained.

Proteinase inhibitory activity

Based on protocol . 2 ml reaction mixture containing 0.06 mg of trypsin, 1ml of 20 mM tris HCl buffer of pH 7.4, and 1 ml different concentration of the extract was incubated at 37 °C for 5 minutes. 1 ml of 0.8% (w/v) casein was added and incubated further for 20 minutes, followed by the addition of 2 ml of 70% perchloric acid to terminate the reaction. The reaction mixture was centrifuged at 3000 rpm for 10 minutes. The absorbance was read at 210 nm. Buffer was used as blank. The triplicates were obtained, and the inhibition percentage was calculated.

Statistical analysis

The triplicates obtained were subjected to statistical analysis, and the standard deviation was obtained.

RESULTS AND DISCUSSION

Reactive oxygen species (ROS) is commonly produced in tissues and linked to diseases like inflammation, diabetes, etc. Anti-inflammatory drugs have adverse effects. Such are prevented using plant-based medicines. The extract, which was prepared, produced a yield of 15 grams after the extraction process, which was used for future studies.

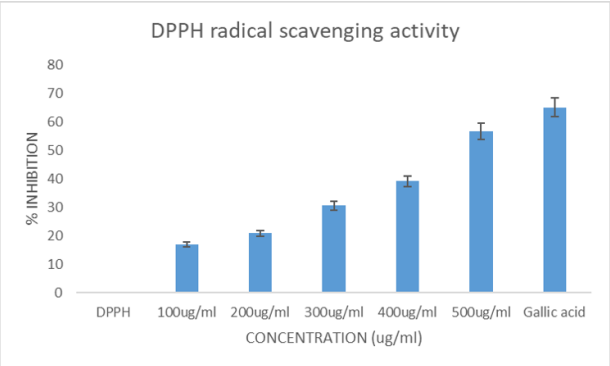

Figure 1: DPPH scavenging activity of aqueous extract of root *G.glabra*. Each values is expressed as means ± standard deviation. Concentration (μg/ml)taken on x-axis and % inhibition taken on y-axis

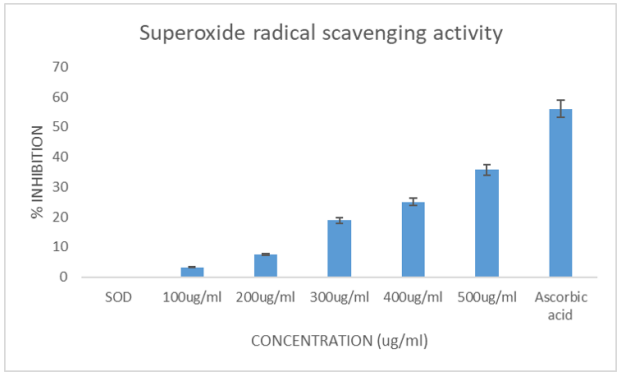

Figure 2: Superoxide radical scavenging activity of aqueous extract of root *G.glabra*. Each values is expressed as means ± standard deviation. Concentration (μg/ml)taken on x-axis and % inhibition taken on y-axis

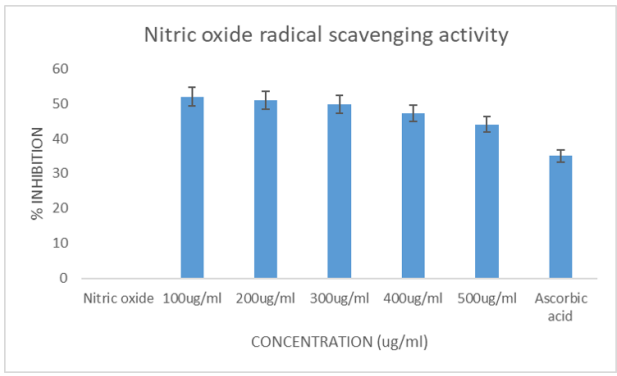

Figure 3: Nitric oxide scavenging activity of aqueous extract of root *G.glabra*. Each values is expressed as means ± standard deviation. Concentration (μg/ml)taken on x-axis and % inhibition taken on y-axis

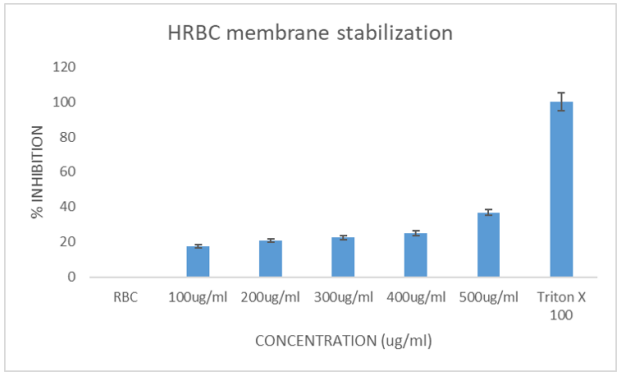

Figure 4: HRBC membrane stabilization assay of aqueous extract of root *G.glabra*. Each values is expressed as means ± standard deviation. Concentration (μg/ml)taken on x-axies and % inhibition taken on y-axis

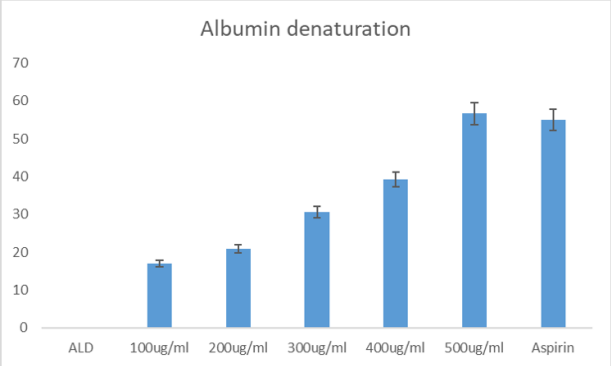

Figure 5: Inhibition albumin denaturation of aqueous extract of root *G.glabra.* Each values is expressed as means ± standard deviation. Concentration (μg/ml)taken on x-axies and % inhibition taken on y-axis

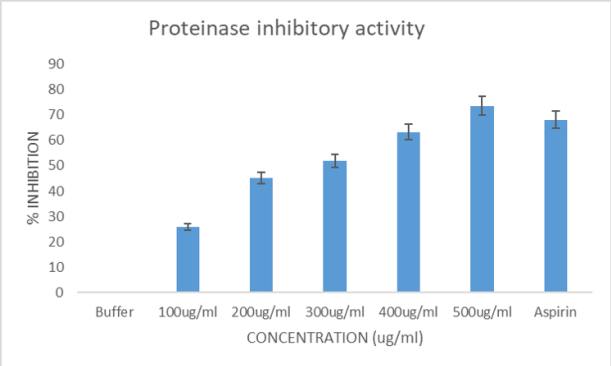

Figure 6: Proteinase inhibitory activity of aqueous extract of root *G.glabra.* Each values is expressed as means ± standard deviation. Concentration (μg/ml) taken on x-axies and % inhibition taken on y-axis

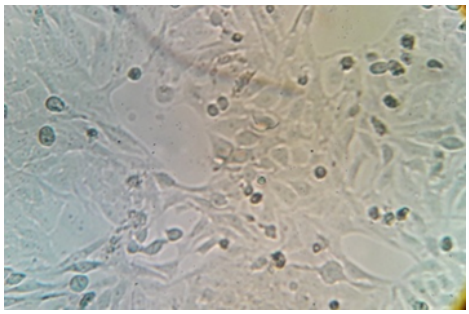

Figure 7: Control

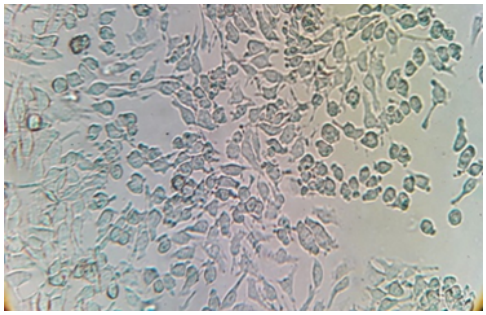

Figure 8: conc 200μg/ml

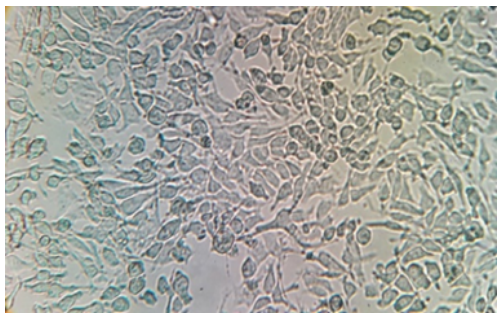

Figure 9: Conc.400 μg/ml

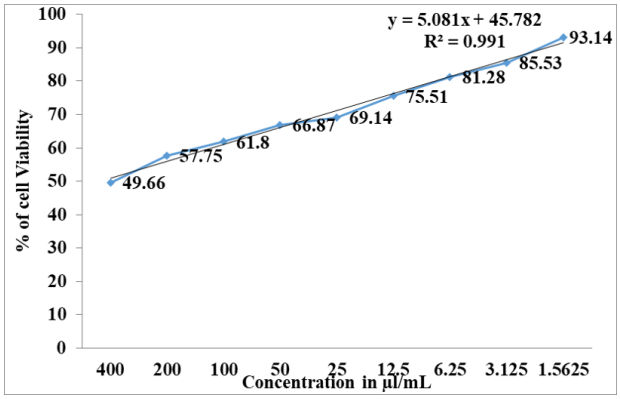

Figure 10: Cytotoxicity activity of Sample GG dry extract against the Raw 246.7 Cell lines

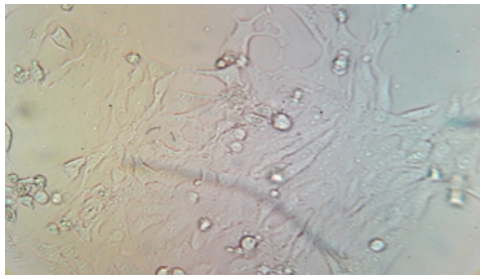

Figure 11: Control

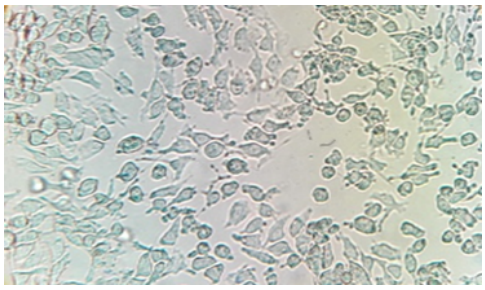

Figure 12: conc 100μg/ml

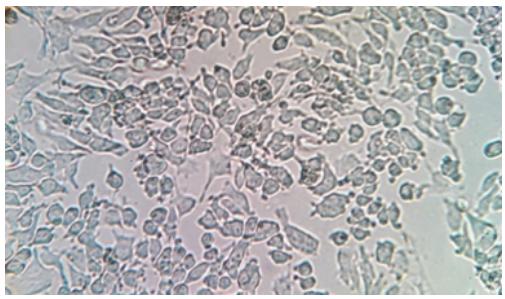

Figure 13: conc 200μg/ml

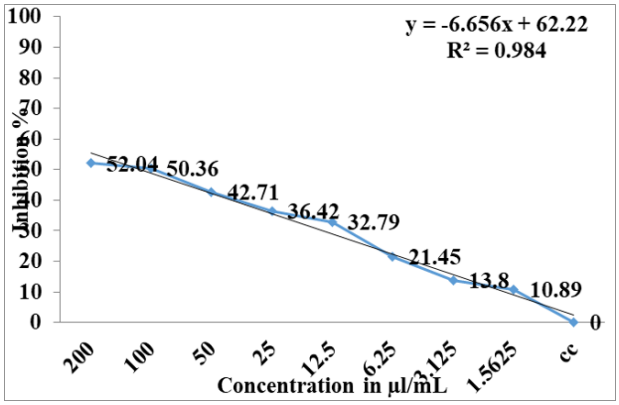

Figure 14: Anti-inflammation activity of Sample GG dry extract against the Raw 246.7 Cell lines

Table 1: A phytochemical analysis using an aqueous extract of *Glycyrrhiza glabra*

SL.No	Phytochemical Constituents	Results
1.	Alkaloids	Presence
2.	Anthraquinone	Absence
3.	Flavonoids	Presence
4.	Phenols	Absence
5.	Coumarin	Presence
6.	Saponins	Presence
7.	Tannins	Presence
8.	Terpenoids	Presence
9.	Steroids	Presence
10.	Cardiac Glycosides	Presence

Phytochemical screening

The phytochemical analysis revealed the presence and absence of phytochemical constituents, which was shown in Table 1.

DPPH (2,2-diphenyl-1-picryl hydrazyl)

DPPH is a widely used method which is used for screening anti-oxidant effect in plants. To reduce Diphenylpicryl hydrazine with plant extracts in a concentration-dependent manner. Among different concentrations of aqueous extract 100 micrograms per mililiter shows (Figure 1), the high activity and exhibits its inhibition percentage of about 46.37 %.

Superoxide radical scavenging activity

Superoxide most important biological radical. It can form hydroxyl radical as well as singlet oxygen, which contributes to the pathogenesis of diseases. Using NBT reduction, the extracts scavenging activity was studied (Figure 2). The scavenging activity of the aqueous extract is found to be 35.68% at 500μg/ml.

Nitric oxide scavenging activity

Nitric oxide is said to be a potential pleiotropic inhibitor; it is considered as a proinflammatory mediator which plays a role in the pathogenesis of inflammatory disorders. Scavengers of Nitric oxide compete with oxygen, which reduces nitric oxide production (Figure 3). The scavenging activity of extract showed 52% inhibition at 100μg/ml.

HRBC membrane stabilization assay

It is used to study the stabilization of the RBCs membrane. The plant extract inhibits the heat-induced haemolysis of RBCs, which showed the maximum activity of 36.88% at 500μg/ml (Figure 4). It is evident tells the extract stabilizes an RBC membrane and also protects the integrity of RBC.

Inhibition of albumin denaturation

Denature of proteins is the process where denature tertiary structure & secondary structure, which is the major for the cause of inflammation. Anti-inflammatory drugs shows dose-dependent ability to inhibit the protein denaturation. The ability of plant extracts to inhibit the protein denaturation for anti-inflammatory activity was studied. Based on the results, it was evidently proven the aqueous extract of different concentrations was more effective in inhibiting the protein denaturation. From this data, 500μg/ml showed the 56.59% of maximum inhibition, which was compared to Aspirin, standard, which exhibited 68% inhibition at 100μg/ml (Figure 5).

Proteinase inhibitory activity

As we known, neutrophils are the best source of serine proteinase, and they were localized at lysosomes. The different concentrations of the aqueous extract showed significant anti-proteinase activity. The maximum inhibition of 73.53% at 500μg/ml. Aspirin, the standard drug, showed the maximum inhibition of 55% at 100μg/ml (Figure 6).

Cytotoxicity and anti-inflammatory analysis using macrophages

The in-vitro cytotoxicity activity results of the GG dry extract sample against Raw 246.7 macrophages cells were triggered of cytotoxicity significantly with the increasing of sample concentration, and the results were obtained (Figures 7, 8 and 9). In these cell lines, the cytotoxicity effect was observed in tested sample concentrations in 48 hours, increased MTT over the tested cell lines. It was evident that the less cytotoxicity of the test sample showed no cell disintegration and migration after 48 h of treatment against the selected, tested cell lines. It was calculated that the IC50 of the test sample GGdry extract against Raw 246.7 macrophages cells was 326.274 μg/ml.

The anti-inflammation activity of Sample GG dry extract against the Raw 246.7 Cell lines

The macrophages cell line RAW 264.7 studies the active of the inflammatory against the chemical of nitric oxide produce from the macrophages cell line using the GG dry extract. The GG dry extract inhibits the active of the raw cell line with different conc. The 32.79 is the best active of mtt assay (Figures 11, 12, 13 and 14). Hence the value of 0.984 ± 0.51 for 100μg of active. The IC 50 value is 143.54 μg/ml.

CONCLUSIONS

Based on the results, it is concluded that the aqueous extract of Liquorice (*Glycyrrhiza glabra*) exhibits high radical scavenging activity. It is also evident that it showed significant results in ant-inflammatory activity against standards. Relate directly with its antioxidant and anti-inflammatory properties, and licorice extract cure the inflammation-related diseases and oxidative liver damage.

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