Pakistan Journal of Pharmacology
Vol.38, No.1, January, 2021, pp.45-50

ORIGINAL ARTICLE

ANTI-INFLAMMATORY ACTIVITY OF ROSE WATER EXTRACTED FROM THE PETALS OF *ROSA DAMASCENE* MILL.

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ABSTRACT

Inflammation is the necessary system in which the body react to irritation, infection, or other tissue injuries. The explanation features being a pain, puffiness, reddishness and heat. *Rosa damascene Mill*, petal extract is a traditional medicine and reported to use as anti-oxidant, anti-inflammatory, analgesic and anti-tussive agents. In the present study twelve sample of rose water were evaluated as anti-inflammatory agent by using inhibition of albumin denatured method using diclofenic sodium as standard. The anti-inflammtory activity of rose water samples were observed at four dose interval (3, 5, 7 and 10ml) against standard diclofenic sodium dosese are (20, 40, 60, 80 and 100 µg/ml). Results indicate the concentration-dependent, anti-inflammatory activity. Effect of 10 ml sample was noted to be equivalent to 100µg/ml of diclofenac sodium. Anti-inflammatory activity of rose water is due to the presence of polyphenolic compounds and saponins that bind with surface action and produce an anti-inflammatory response.

Keywords: Anti-inflammatory, rose water, *Rosa damascene Mill.*, protein denature, albumin.

INTRODUCTION

The genus Rosa, belonging to the family Rosaceae, counting 200 type and more than 18,000 cultivars (Gudin, 2000). Rose used for decorative purposes, perfumery industries, medicinally, garden plants, slash flowers and internal plants. Rose essential oil has an extensive series of claim in many industries used for scenting and flavoring agents, direct consumption or making a range of foodstuff products. (Hassanein *et al*., 2010)

Among“200 species of rose the most important one is the *Rosa damascene Mill* which is also known as damask rose (Kaul *et al*., 2000) damask, rose of Castile, Gul-e-Mohammadi (Loghmani-Khouzani *et al*., 2007) and king of flowers (Mahboubi *et al*., 2016) (Nikbakht *et al*., 2004-2005). *Rosa damascene* is the hybrid species of *R.gallica* and *R.phoenicia*. Damask rose is grown as an ornamental plant both in houses and in gardens and has a good fragrance effect (Dolati *et al*., 2011). Beside this, it also possesess nutritional and industrial importance (Boskabady *et al*., 2011, Jabbarzadeh and Khoshkhui*et al*. 2005). This plant is called Damask rose because it was firstly brought to Europe from Damascus (Gudin *et al*., 2000) and mainly cultivated in Turkey, Bulgaria, Iran, India, Morocco, South France, China, South Italy, Libya, South Russia and the Ukraine” (Buttner *et al*., 2001).

MATERIALS AND METHODS

Plant material

Rosa damascena flower collected from the local market and identified. The sample is kept in the herbarium of the Department of

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Extraction of rose water

Rose water was prepared by the method described and reported earlier (Abidi *et al*., 2018) using the hydro distillation method in which 60g dried rose petal was distilled with 1L distilled water for 4hrs to yield 800ml of rose water that was kept in airtight jar and refrigerator at 4^{o}C.

Phytochemical analysis of rose water

The" phytochemical analysis of rose water was performed by earlier published paper and also for the functional groups were determined through FT-IR was mentioned (Abidi *et al*., 2018).

***In-Vitro* Anti-Inflammatory Activity**

In- vitro anti-inflammatory activity of twelve rose water samples was determined by inhibition of albumin denatured method (Mizushima and Kobayashi 1968) using diclofenic sodium (20,40,60,80 and 100 µg/ml) as a standard drug. Among twelve samples of rose water 11 were collected from the local market one was hydrodistilled in the lab. Anti-inflammatory activity of rose water samples was evaluate at 3, 5, 7 and 1ml volume.

Anti-Inflammatory Activity

Phosphate buffer saline (28 ml) of pH 6.4 was transferred to freshly prepared egg albumin (2ml) and 20 ml of different solution (standard diclofenac sodium 20,40,60,80 and 100 µg/ml and different rose water samples at 3 , 5, 7 and 10 ml volume respectively). All the solutions were incubated at 37±2^{o}C for 15 minutes, and it was then heated at 70^{o}C in a water bath for 15 minutes. The solution was allowed to cool at room temperature. The absorbance was then measured using UV-visible spectrophotometer at 660nm using the vehicle as blank. The percentage inhibition of protein denaturation was calculated from the control using below under formula (Ullah *et al*., 2014).

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

Whereas, Abs: absorbance the viscosity of the solution determined by using Ostwald viscometer.

STATISTICAL ANALYSIS

SPSS version 22 was used to calculate mean ± standard deviation. Graphs were plotted in Microsoft excel version 2010. The result of the experiment was expressed in mean ± SD.

RESULTS AND DISCUSSION

Inflammation is a characteristic biological process in reaction to tissue scratch, microbial pathogen infectivity and chemical irritation. This natural procedure additionally involves, innate the adaptive immune systems (Pan *et al*., 2010). At an wounded position, inflammation initiated by movement of immune cells from blood vessels and release of mediators followed by conscription of inflammatory cells and release of reactive oxygen species (ROS), reactive nitrogen species (RNS) and pro-inflammatory cytokines to get rid of foreign pathogens, resolving infection and repairing injured tissues (Libby, 2007; Medzhitov, 2008). Thus, the principal reason of inflammation is precious for a host's defense. In specific, common inflammation is speedy and self-limiting, but an abnormal affirmation and extended inflammation cause various chronic disorders.

The anti-inflammatory activity of rose water samples at four different dosage level (3, 5, 7 and 10ml) was determined by using inhibition of albumin denaturation against standard drug diclofenac sodium at five different concentrations (20, 40, 60, 80,100µg/ml). The results have been shown in the table 1 and represented in fig. 1. In the present study concentration-dependent, anti-inflammatory activity observed. Effect of 10 ml sample was noted to be equivalent to 100µg/ml of diclofenac sodium activity as

mention in fig. 2. The anti-inflammatory activity of standard drug diclofenac sodium was mentioned in table 2 while viscosities of samples were mention in table 3.

It is the well-recognized reality that chronic inflammation is related to a broad range of progressive diseases such as cancer, neurological disease, metabolic disorder and cardiovascular disease (Pan *et al*., 2009). Numerous studies propose the removal of chronic inflammation as a most important method to avoid various chronic diseases. Epidemiological studies provide persuasive proof that natural dietary compounds that humans consume as food have many biological activities. Among these natural bioactive compounds, flavonoids extensively documented for their biological and pharmacological effects, together with antiviral, anti-carcinogenic, antioxidant, antimicrobial, anti-inflammatory, anti-angiogenic and anti-thrombogenic properties (Libby, 2007; Pan *et al*., 2010).

Inflammation is a complicated process, driven by pre-existing conditions such as infection or injury or genetic changes. These conditions have resulted in triggering signaling cascades, activation of transcription factors, gene expression, increased of levels of inflammatory enzymes, and release of various oxidants and pro-inflammatory molecules (cytokines & chemokines) in immune or inflammatory cells (Medzhitov, 2008). In these conditions, excessive oxidants and inflammatory mediators have a harmful effect on normal tissue, including toxicity, loss of barrier function, abnormal cell proliferation, inhibit the normal function of tissues and organs, and at last leading to systemic disorders (Libby, 2007; Medzhitov, 2008). Over the past two decades, many studies disclosed that chronic inflammation is a serious element in many human diseases and situation, together with obesity, cardiovascular diseases (atherosclerosis, coronary diseases, cerebrovascular disorder, heart failure and cardiomyopathy), neurodegenerative diseases (Alzheimer & Parkinson), diabetes, aging, metabolic disorder and cancers.

Table 1: *In-Vitro* Anti Inflammatory Activity of Rose Water Samples

Sample●	01	02	03	04	05	06	07	08	09	10	11	12
Volume	% Inhibition of Protein Denaturation Mean ± S.D											
3ML(3.09g)	93.2± 0.01	92.06±0.05	95.1±0.01	80.7±0.15	21.4±0.34	50.5±1.43	38.2±0.27	28.8±0.06	52.0±0.06	23.7±2.60	6.73±2.81	71.6±0.24
5ML(5.21g	10.44±0.04	-6.5±0.49	62.70±0.28	40.54±0.88	29.33±0.31	57.5±0.10	14.8±0.88	91.0±0.14	95.2±0.01	84.4±1.98	50.6±1.16	40.0±0.08
7ML7.32g	77.09±0.05	77.1±0.01	93.9±0.05	66.2±0.05	83.42±0.05	81.4±0.01	64.4±0.02	88.1±0.06	60.7±0.14	71.6±0.06	64.6±0.11	51.1±1.15
10ML10.35g	99.22±0.32	69.65±0.08	81.22±0.03	57.82±0.01	95.16±0.34	85.6±1.66	82.8±0.06	96.8±0.08	98.6±0.06	99.5±0.04	99.0±0.08	68.1±0.08

●: Source & manufacturer's name are available upon request
●: Values are recorded as MEAN ± S.D

Table 2: *In-Vitro* Anti Inflammatory Activity of Diclofenic sodium

Standard (diclofanec sodium) µg/ml	% inhibition of protein denaturation Mean ± S.D
20	1.841 ± 0.184
40	50.47 ± 0.739
60	90.33 ± 0.05
80	94.36 ± 0.01
100	96.71 ± 0.09

Values are recorded as MEAN ± S.D

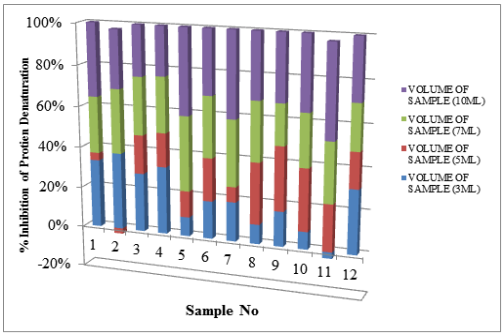

Fig. 1: *In-Vitro* Anti-Inflammatory Activity of Rose Water Samples

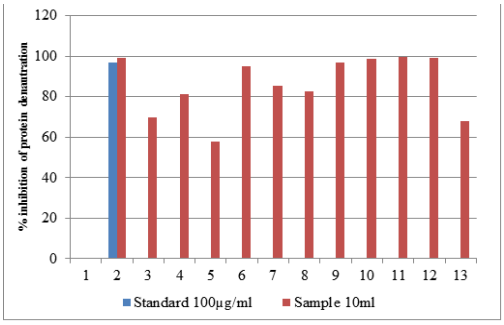

Fig. 2: *In-Vitro* Anti-Inflammatory Activity of 10ml Rose Water Samples Compared with 100µg/ml of Standard Drug (Diclofenac Sodium)

There are two isoforms of cyclo-oxygenase (COX) enzymes, i.e., COX-1 and COX-2 participatemeansto function in our body. The COX-1 enzyme is articulated in the majoritytissues and is accountable for maintenance roles for usual physiological functions. COX-2 induced by pro-inflammatory cytokines, growth factors, oncogenes, carcinogens, and tumor promoters and not visible in regular physiological conditions. COX-2 take part in a serious function in both inflammation and control of cell growth (Dannhardt and Kiefer, 2001). Thus, botanical COX-2 that inhibits the action or appearance of COX-2 might be an essential object for cancer chemoprevention or anti-inflammation (Ko *et al*., 2011). Nitric oxide synthase (NOS) is another key enzyme, which produces nitric oxide (NO) via oxidation of the terminal guanidine nitrogen atom of L-arginine. Nitric oxide has also been proposed to be avital mediator of tumor growth. Inducible isoforms of cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) are primarily accountable for the manufacture of large amounts of assorted mediators. These mediators such as cytokines, chemokines, prostaglandins (PGs) and nitric oxide (NO) are implicated in a variety of processes including inflammation and carcinogenesis (Ko *et al*., 2011; Yang *et al*., 2007). For that reason, selective inhibitors of COX-2 or NOS enzyme action may be an option to hold back of these genes and sluggish down the inflammation or carcinogenic process.

Protein denaturation is the famous reason for unusual inflammation-based diseases, due to protein denaturation its actual structure diminished and it will not perform its actual enzymatic and biological activity properly. (Ratnasurya *et al*., 2015).The viscosity of protein solution further supports the inhibition of protein denaturation.When protein is denatured, itsviscosity increased. The viscosity of protein solution will be affected by the external factor (pH, temperature, % of solvent and ionic strength) and the physicochemical nature of the protein. (Szymanska *et al*., 2008). Previously reported that on increasing the concentration of solution protein denaturation is also increasing. There are many factors which influence the viscosity ofprotein solution shape, concentration, size, molecular weight, intermolecular attraction, flexibility, charge and degree of hydration. Due to the large molecular size protein solution had higher viscosities.

Table 3: Viscosity Value of Rose Water Samples Used to Calculate Anti-inflammatory Activity

Sample●	01	02	03	04	05	06	07	08	09	10	11	12
Volume	Viscosity of Rose Water Samples											
3ML(3.09g)	0.181	0.406	0.407	0.4033	0.5680	0.4532	0.205	0.274	0.275	0.186	0.1800	0.473
5ML(5.21g	0.256	0.540	0.548	0.568	0.587	0.476	0.331	0.304	0.361	0.234	0.2128	0.541
7ML7.32g	0.366	0.555	0.558	0.578	0.560	0.566	0.519	0.448	0.573	0.517	0.2510	0.585
10ML10.35g	0.541	0.575	0.579	0.607	0.541	0.567	0.540	0.537	0.530	0.602	0.2599	0.627

In past several years, research on phytochemicals having anti-inflammatory property improved.Most of the secondary plant metabolites such as polyphenols had great medicinal importance, In the present study, the *in-vitro* anti-inflammatory activity of rose water samples is due to the presence of polyphenolic content such as flavonoids, tannins, and saponins. These compounds inhibit protein denaturation. Previously reported that many enzymes (cyclooxygenase-2, tyrosine kinase, and neutrophil degranulation) inhibit byflavonoids. These polyphenolic compounds established both anti-oxidant and anti-inflammatory activity (Shallangwa *et al*., 2015).

CONCLUSION

The anti-inflammatory activity of rose water is due to the presence of polyphenolic compounds and saponins that bind with surface action and produce an anti-inflammatory response. For the *In vivo* study of anti-inflammatory effect the main problem related to the approvals from the ethical committee (Shallangwa *et al*., 2015). Therefore, researchers move towards the *in-vitro* technique to study the inhibitory effect of the drug on protein denaturation that's why *in-vitro* experiments are the good approach towards the formation of topical medication. It is a simple and cheap method.

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