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Pharmacological and Toxicological Studies On *Calendula officinalis* flower extract

Thesis presented by

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Abstract

Phytochemical, toxicological and pharmacological studies were carried out on ***Calendula officinalis*** flower commonly used in folk medicine. Flowers different phytochemicals were analyzed. The ***Calendula officinalis*** flower 70 % ethanolic extract toxicological studies (Acute and chronic toxicity) were investigated. The oral anti-inflammatory, antipyretic, analgesic, anticonvulsant, tranquilizing and hypnotic activities of ***Calendula officinalis*** flower 70 % ethanolic extract were studied. Hepatoprotective, diuretic, healing, hormone like activity and effect of tested extract on male fertility were also investigated. Moreover ***Calendula officinalis*** 70 % ethanolic extract genotoxic, *in vitro* antibacterial and antispasmodic activities (on isolated rabbit's duodenum) were analyzed. Phytochemical analysis of ***Calendula officinalis*** flower revealed the presence of flavonoids, glycosides, tannin, saponins, resin and absence of alkaloids. ***Calendula officinalis*** LD_{50} was 2450 mg/Kg b.wt. Prolonged administration of ***Calendula officinalis*** 70 % ethanolic extract (12 weeks) produced significant increase in number of leucocytes, decrease in ALT and AST enzymes. Studied extract showed significant anti-inflammatory, antipyretic, analgesic, anticonvulsant, tranquilizing, hepatoprotective, diuretic, estrogen, progesterone, healing, antigenotoxic, antispasmodic and antibacterial activities.

Key Words: ***Calendula officinalis***, Estrogen, Diuretic, Healing, Genotoxic, antispasmodic.

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1. Introduction

The study of compounds of plant origin has generated great interest in the fields of food and medicine **(Barceló *et al.*, 1996), (Garci´a-Gasca *et al*., 1998)** and **(Kim *et al*., 1998)**. Natural products are gaining a revitalized attention in medical community and their therapeutic uses are gradually increasing as many synthetic drugs have revealed serious side effects. Therefore, a better strategy is to look for natural substances with useful pharmacological effect and less cytotoxicity.

However, enormous improvements have been observed in modern systems of medicine**.** Herbal drugs therapy is regarded as an important therapy for the treatment of wide range of diseases. Plants came in use in the modern medicine through their uses in folklore or traditional medicine. All cultures have folk medicine traditions including the use of herbs in treatment of various ailments. It is known that more than 400,000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine **(Odugbemi 2006)**.Indian herbal plants provide a rich source for healthcare to prevent different pathological states (**Khan *et al,* 2011)**. India is called the botanical garden of the world for its rich natural resources. Over 6,000 plants in India are used in traditional, folklore and herbal medicine (**Agrawal and Raju 2006).** The use of herbal infusions to cure different types of diseases is very common in Brazilian folk medicine and frequently replaces modern medicine (**Leffa *et al.* 2012).** The use of medicinal plants as antibacterial and

anti-inflammatory drugs in folk medicine is a common practice in Iran (**Hajhashemi *et al.,* 2002)**. In certain localities in Egypt folk medicine is the most widely used method of health care. Egypt has a very old and glorious history in using herbal medicine. May be Egypt was among the first countries in the world to use herbal medicine on a rather scientific bases **(Haggag 2004)**.

Medicinal plants were used from ancient times in the treatment of various human and animal disorders. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional medicine **(Singh *et al.* 2010).** Some plant decoctions are of great value in the treatment of diarrhea or gastrointestinal disorders, urinary tract infections, skin infections, infertility, wound and cutaneous abscesses **(Ergene *et al.,* 2006).**

In the last few years much attention was directed to the potential health promoting properties of phenolic phytochemicals **((Block 1992), (Block and Langseth 1994), (Kartal 2007))**. Herbal medicine is based on the fact that plants contain natural substances that can promote health and alleviate illness. Medicinal and aromatic plants are the most widely used form of medicine in the world today where medicinal and aromatic plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds **(Okigo *et al.,* 2009)**.

Today estimate that about 80% of people in developing countries still relays on traditional medicine based largely on species of plants

and animals for their primary health care according to WHO **(Verma and Singh 2008).** According to WHO about 25% of modern medicines are described from plants first used traditionally and others are synthetic analogues built on prototype compounds isolated from plants **(Pathak and Das 2013).** One of the major problems faced by the herbal drug industry is unavailability of rigid quality control profile for herbal materials and their formulations, so it became very important the study of pharmacological and toxicological effects of herbs used in folk medicine.

The aims of this study were to investigate phytochemical, toxicological and pharmacological aspects of ***Calendula officinalis*** one of the most important and widely used flower in folk medicine.

2. Review of literature

In this part phytochemical, toxicological and pharmacological aspects of ***Calendula officinalis*** flower were reviewed. The following literature represents researches carried out.

2.1. Plant description:

2.1.1. Taxonomic classification:

The genus ***Calendula*** was described previously related to family ***Asteraceae*** by **Linnaeus in 1753.**

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Asteridae*
Order	*Asterales*
Family	*Asteraceae*
Genus	*Calendula*
Species	*C. officinalis*

2.1.2. Botanical description: Studied plant has different names in different countries as mentioned by (**Kirtikar and Basu 1993)** and **(Franco 1996).**

Latin	***Calendula officinalis***
Hindi	Zergul
English	African marigold, Calendula, Common Marigold, Garden Marigold, Marigold, Pot Marigold
German	Butterblume
Chinese	Chin Chan Ts'ao
Romanian	Galbinele
Swedish	Ringblomma
Brazil	Margarida dourada, Malmequer and Maravilhas

2.1.3. Botanical morphology:

Calendula officinalis is an annual, seldom herb. It grows between 30 and 50 cm high, has about 20 cm long tap root and numerous thin, secondary roots. The stem is erect, angular, downy and branched from the base up or higher. The alternate leaves are almost spatulate at the base. On the tip of each stem, there is a 5 to 7 cm composite flower head, consisting of an epicalyx of numerous narrow-lanceolate sepals, which are densely covered on both sides with glandular hairs. The inner section of the flower head is made up of orange-yellow tubular florets. The disc florets are pseudohermaphrodites but the female is sterile. The zygomorphic ray florets at the edge are female, their stamens are completely absent, and their inferior ovaries are much more developed than those of the tubular florets. The fruit forms only in the female ray

flowers. The heterocarp achenes are sickle-shaped, curved and ringed **((Editorial Boards 2003), (Norman and Max 2001), (Ben-Erik and Michael 2004)).**

2.1.4. History and Traditional uses:

Calendula name is from the middle English calends derived from Latin *kalendae*, which means the day of the new moon. In the ancient Roman calendar it was the first day of the month. This name is derived from the ability of the herb to flower any month of the year in mild climates **(Talbert 2015).** European settlers brought ***Calendula*** with them when they migrated to the US and it was found to be used during the civil war as an aid to stop bleeding and promote healing of wounds. Ancient Egyptians valued it as a rejuvenating herb, Hindus used it to decorate temple altars, Persians and Greeks garnished and flavored food with the flower petals. The Germans added handfuls of the flowers to their soups and broths to add color and strength, and hence the nickname of "pot marigold". The Europeans have also long used it to flavor soups and stews and to color butter and cheese. ***Calendula*** has a long history of use as a minor medicinal plant used to treat a wide range of affections including fever, headaches, toothaches, stomachaches, irritated eyes, scorpion bites and as a general tonic **(Slaughter 2015)**.

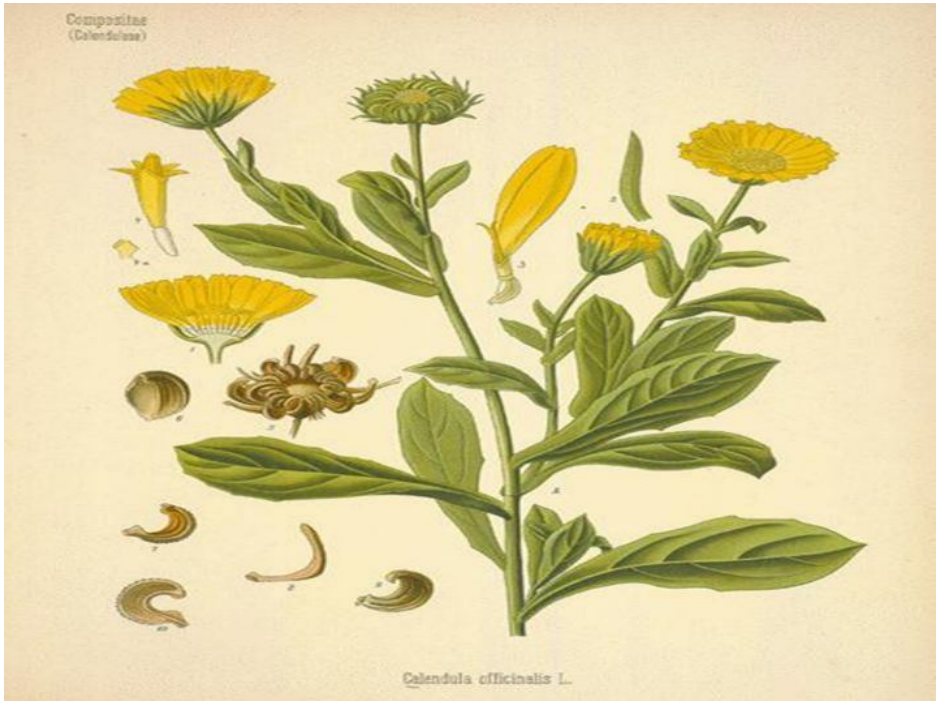

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Fig. (1): ***Calendula officinalis*** whole plant .

Fig. (2): ***Calendula officinalis*** flower.

2.2. Phytochemical literature:

Yoshikawa *et al.,* (2001) isolated some phytochemical ingredients from 1-butanol-soluble fraction of the ***Calendula officinalis*** flower including four new triterpene oligoglycosides, calendasaponins A, B, C, and D ,with eight known saponins, seven known flavonol glycosides and a known sesquiterpene glucoside. Their structures were elucidated on the basis of chemical and physicochemical evidence. They reported also that the principal saponin constituents and glycosides present in flowers exhibited potent inhibitory action represented by increase in serum glucose levels in rats, gastric emptying in mice and gastric lesions in rats.

Roopashree *et al.,* (2008) analyzed phytochemical ingredients of different extracts (petroleum ether, methanol, ethanol and aqueous extracts) of ***Calendula officinalis*** flowers. This investigation revealed presence of glycosides, saponins, triterpenes, diterpenes and flavonoids in its extract.

Muley *et al.,* (2009) investigated variety of phytochemicals such as terpenoids, flavonoids, coumarins, quinones, volatile oil, carotenoids, amino acids and lipids were present in ***Calendula officinalis*** flower

Chakraborthy (2010) screened different phytochemicals of petroleum ether, chloroform, methanol and aqueous extracts of ***Calendula officinalis*** leaf done by thin layer chromatography mean. Petroleum ether extract showed the presence of fatty acids. Chloroform extract showed the presence of triterpens and sterols. Flavonoids,