



Comparative Analgesic Activity of Selected Medicinal Plants from Pakistan

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Abstract: Pain is a natural self-protective system which is always unpleasant both mentally and physically with probable tissue harm. Analgesics are the medicinal agents which relieve the pain without losing consciousness. Medicinal plants are being widely used all over the world for human healthy life form. We selected ten Pakistani plants which play an important role as traditional medicine in the prevention and management of acute and chronic diseases. All plants were extracted with ethanol and have been investigated for analgesic effects using hotplate and writhing reflex tests in rats. Phytochemical screening revealed the presence of polyphenols, saponins, sugar, flavonoids, and alkaloid. In our research, the analgesic effect of selected medicinal plants shows significant effect like standard aspirin and diclophenic. The intake of these aforementioned phytochemical flavonoids and polyphenols are considered to be responsible for pain management because they may contain many powerful antioxidant and free radical scavenging capabilities which are the backbone of other bioactivities such as anti-inflammatory action, anticancer, anti-aging, and protective action for cardiovascular diseases, diabetes mellitus, obesity and neurodegenerative disorders. In future, these plants may be utilized as a health benefit in view of their potential research in the prevention and management of pain.

Keywords: Analgesics, Medicinal plants, Non-steroidal anti-inflammatory (NSAIDs), Pain, Phytochemicals, Side effects.

1. INTRODUCTION

1.1. Pain & its Types

Pain is described as a condition of discomfort and anxiety. It is an indication of some trauma, injury, diseased state or even emotional misery. Pain is a vital feature of the body's defense mechanisms & it provides an urgent warning to transmit instructions to the motor neurons of the central nervous system to reduce and prevent further physical damage. Pain functionally is of two broad types' i.e. acute pain and chronic pain. Acute pain is for the short span of time and its causes are easily recognizable. It is alertness to a sudden damage to a tissue or any

disease. It is quite rapid and sharp immediately followed by throbbing pain. Chronic pain is the pain which persist for far longer duration than the acute pain. Chronic pain can be mild or severe, continuous or intermittent and is usually difficult to subside than acute pain. Pain can also be divided into different groups on the basis of its source as well as associated neurons such as somatic pain, referred pain, visceral pain, neuropathic pain, cutaneous pain, phantom pain and central pain [1]. Pain is an immense problem as evaluations indicate that 20% of adults experience pain globally and further 10% are diagnosed every year which suffer chronic pain. Pain is considered as a medical problem but is given less attention by the stake

holders of public health. Pain is not having same prevalence in all countries although it can affect all populations. Pain is an unpleasant experience which can be faced by people of any sex, age group, race and geographical region. It can be recurrent, acute, chronic or combination of all.

1.2. Causes & Consequences

Major causes of pain are spinal injuries, arthritis (Rheumatoid and osteo both), different types of cancers and postoperative conditions. Several consequences of pain have also been identified which are troublesome such as disturbed concentration, inability to perform any physical tasks, anxiety and depressed personality, disturbed social behavior and suicidal views **Fig. 1** [2] shows some typical causes & consequences of chronic pain. Pain has gained great level of impact among general public because of its high rate of occurrence. Pain is a multifaceted experience which is complicated to determine and quantify. About 10% of the total world's population is a victim of chronic pain that means 60 million people around the globe [3-8]. Prevalence of chronic pain in different countries is shown in **Fig. 2**. The burden of pain can be realized by assessing its level of severity as well as

disabilities accompanied with pain. It is, therefore, justified to regard pain as public health priority as it is linked with social and economic aspects of life [9-10].

1.3. Pain Etiology

Pain receptors called nociceptors have free nerve endings. As soon as they are stimulated by chemical, thermal and mechanical means, they send impulses to central nervous system through sensory neurons and perception of pain is being occurred **Fig. 3** [11] shows various steps in the perception of pains. A large number of somatic and visceral pain receptors are activated by various stimulants and inflammatory mediators like bradykinin, prostaglandins, leukotrienes, serotonin, histamine, glutamate, substance p, nervous growth factor (NGF), adenosine and adenosine phosphate capsaicin and free radicals [12-15].

1.4. Analgesia

Analgesia means removal of pain without the loss of consciousness. Generally the condition of pain is treated with several over the counter (OTC) analgesic drugs. These are extensively researched

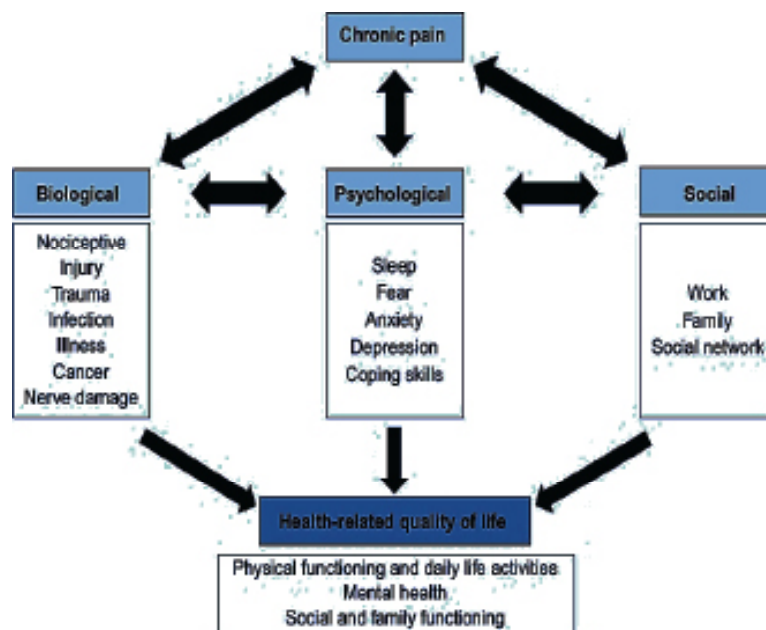


Fig. 1. Causes and Consequences of Chronic pain

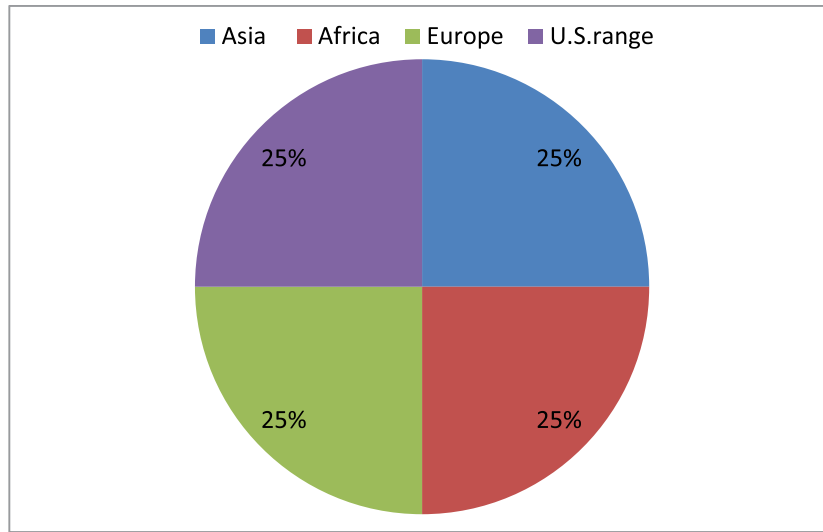


Fig. 2. Epidemiology of pain around the Globe in primary care setting

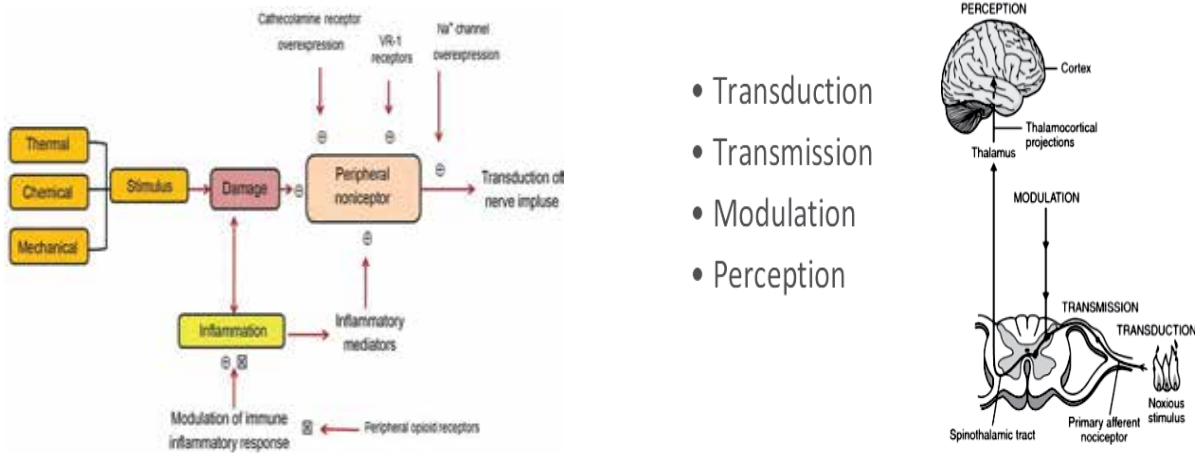


Fig. 3. Steps in perception of pain

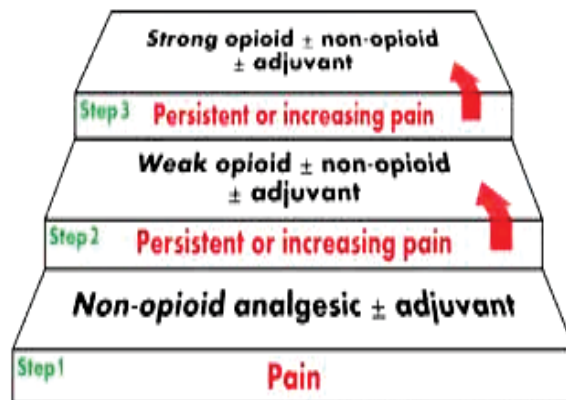


Fig. 4. WHO Treatment Ladder of pain

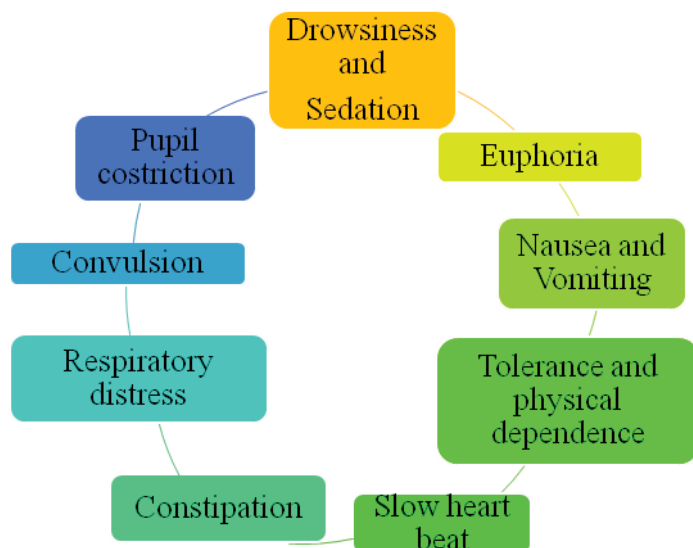


Fig. 5. Common side effects of Opioid analgesics

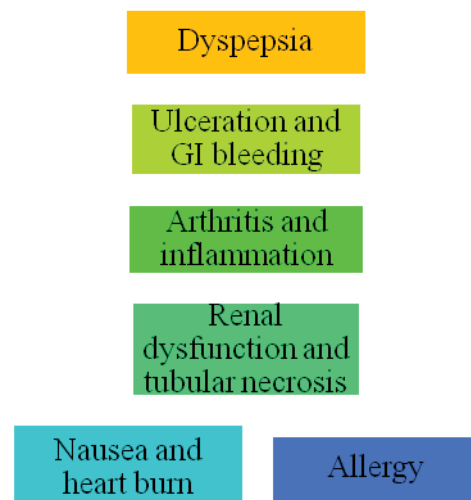


Fig. 6. Common side effects of NSAIDs

but are bound to have several side effects. Approximately 70% of the western population is habitually using analgesics to diminish headache, to treat dysphoric states of mood, to reduce sleep disturbances and for other pains and illnesses. Fig.4 shows the WHO pain ladder which starts with common OTC drugs at lower levels and reaches to strong opioids at higher levels [16]. However, long term or excessive use of analgesics is considered as abuse [17].

1.4.1. Side Effects of Synthetic Analgesics

Any injury or trauma leads to pain accompanied with swelling, burning sensation and erythema. Opioids and Non-steroidal anti-inflammatory drug (NSAIDs) are the drugs of choice to subside pain in joint and spine associated pains. They are known to have many undesirable side effects. Fig. 5 and 6 represent the common side effects of opioids and NSAIDs analgesic [18-19].

1.4.2. Medicinal Plants as Analgesics

Natural resources specifically plants are continuing their role in drug development, in order to discover the safe, effective, and reasonable treatment for the rising spectrum of human ailments. People experiencing different types of disorders and related pains desire to find drugs with lesser side effects [20]. Various medicinal plants have been advised for prevention and cure of certain pain related conditions. Drugs of herbal origin have received considerable attention of researchers because of

possessing low or no side effects [21-22].

Therefore, ten Pakistani medicinal plants have been selected for their phytochemical screening and analgesic activities by tail flick and writhing reflex method. Their name, family, parts used and tradition uses are summarized in Table 1 [23-33].

2. MATERIALS AND METHODS

2.1 Collection of plants

The rhizome, fruits and seeds of plants were procured from local market of Karachi, Pakistan. Leaves of plants were collected from the garden of University of Karachi. The identification of leaves of plant species was done by Botany department of University of Karachi, Pakistan.

2.2 Extraction

For extraction, leaves stem, and fruits of the selected plants were cut into thin slices and dried at room temperature. The dry plant materials were ground into powder and percolated in ethanol (Merck, Germany) in a separate container for one week at room temperature. The ethanol extract was evaporated via rotary evaporator at 40°C under reduced pressure.

Table 1. Selected Pakistani Medicinal Plants

S. No.	Botanical name & parts used	Family	Chemical Constituents	Traditional medicinal uses
1.	<i>Zingibar officinale</i> Roscoe. Rhizhom	<i>Zingiberaceae</i>	sesquiterpenoids, with (-)-zingiberene, Sesquiterpene Lactones, carbohydrates, lipids, terpenes, and phenolic compound including gingerol, paradols, and shogaol	Acute and chronic cough, common cold, fever, allergic rhinitis, sinusitis, acute chronic bronchitis, respiratory troubles, pain, headache, backache
2.	<i>Cllistemon viminalis</i> L.Seeds	<i>Myrtaceae</i>	Phenolics, triterpenoids, flavonoids, saponins, steroids, alkaloids, tannin, carbohydrates, amino acids and proteins compounds	Gastroenteritis, diarrhea and skin infections
3.	<i>Citrullus lanatus</i> Fruit	<i>Cucurbitaceae</i>	Alkaloids, Amino acids, Saponins, Flavonoids, Glycosides, Steroids, Oils Tannins, Carbohydrates, Proteins and phenolics	diuretic and tonic. used in the treatment of the urinary passages, a good vermifuge and has hypotensive action.
4.	<i>Trachyspermum ammi</i> L.Seeds	<i>Apiaceae</i>	Carotenoids, Lutein and Zeaxanthin, p-cymene, γ -terpinene, α -pinene, β -pinene and α -terpinene	Paralysis, weakness of limbs, chest pain, liver disease, hiccups, kidney, spleen problem, carminative, diuretic and decreases the pain, in the acute phase of common cold or migraine
5.	<i>Adansonia digitata</i> L. Leaves	<i>Malvaceae</i>	Terpenoids, alkaloids, Flavonoids, Glycosides, Sterols, vitamins, Amino acids, minerals, carbohydrates, phenols, and lipids	Malaria, tuberculosis, fever, microbial infections, diarrhoea, anaemia, dysentery, toothache, immune stimulant
6.	<i>Murraya paniculata</i> L. Leaves	<i>Rutaceae</i>	Alkaloids, prenylated coumarins, polymethoxyflavones and flavonoids, sesquiterpenes (1- cadinene), a sesquiterpene alcohol and methyl anthranilate	Stimulant, Astringent, diarrhoea, dysentery, management of pain and inflammatory conditions. Diseases of teeth and gum, useful against rheumatism, useful against rheumatism, coughs and hysteria
7.	<i>Holoptelea integrifolia</i> (Roxb.) Planch Leaves	<i>Ulmaceae</i>	Terpenoids, sterols, saponins, tannins, proteins, flavonoids, phenols, cardiac glycosides, coumarins, quinines carbohydrates, and alkaloid	inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhea, and rheumatism
8.	<i>Nepeta adenophyta</i> Hedge Leaves	<i>Lamiaceae</i>	Diterpenes, sesquiterpenes, and triterpenes, flavonoids	Abdominal pain, kidney pain, menstrual pain, and headache and also to control bleeding disorders
9.	<i>Hibiscus schizopetalus</i> (Mast.) Hook Flower / leaves	<i>Malvaceae</i>	Alkaloids, steroids, anthocyanin and triterpenoids	Cold, Cough and to reduce fever analgesic and antipyretic diabetes, menstrual disorder and piles
10.	<i>Sesamum indicum</i> L. seeds	<i>Pedaliaceae</i>	50-60% oil, 18-25% protein, 13.5% carbohydrate and 5% ash	Anti oxidative, anticancer, anti hypersensitive and anti immunoregulatory actions

2.3 Preliminary Phytochemical Screening

The presence of secondary metabolites including polyphenols, alkaloids, saponins, sugar and flavonoids was analyzed in the following procedures described by Shareef et al., 2010 [34].

2.3.1 Analgesic Screening

2.3.1.1 Hot plate Test

Rats weighing 100-120 g of both sexes were taken for the mentioned study. The Ethanolic extracts (200-400 mg/kg) of the all extracts were administered to rats, which were divided in 13 groups (n=6 in a group). Aspirin and Diclofenac Sodium (5 mg/kg) were selected as reference drugs. Hot plate technique was employed and reactions were assessed one hour before treatment (control) and at different time interval treatment. The rats were placed on a Techno hot plate maintained at 56°C, and the time between placement of the rat on the platform and shaking or licking of the paws or jumping was recorded as the hot plate latency. Rats, which showed a pre-treatment time of greater than 15 seconds in hot plate test, were not included in the study. The pre- treatment time must not exceed 25 seconds in the test. To overcome chances of tissue damage it was ensured that [35] the percentage protection against thermal stimuli was calculated as follows:

$$\% \text{ Thermal stimuli latency} = \frac{[(\text{Treatment} - \text{Control}) / \text{Control}] \times 100}{}$$

2.3.1.2 Acetic acid induced writhing response

The rats of either sex, weighing 180-200 g were

selected for the study. The Ethanolic extracts (200-400 mg/kg) of the plant extracts were administered to rats, which were divided in 13 groups (n=6 in a group). Aspirin and Diclofenac Sodium (5 mg/kg) were used as reference drugs. The rats were treated with extracts of medicinal plants and reference drugs daily for a week. After 30 minutes of the final administration 0.6% acetic acid (0.1 ml/10 g) was injected intra peritoneal. After administration of acetic acid, the number of writhes was evaluated continuously for 20 minutes, by placing rats in transparent boxes. The control group only received saline. The number of writhes of the test group rats was compared with number of writhes of the control group and inhibition rate was determined as follows [35]:

$$\% \text{ Percent inhibition of writhes} = \frac{[(\text{Control mean} - \text{Test mean}) / \text{Control mean}] \times 100}{}$$

2.4 Statistical Analysis

The results were presented as mean \pm SEM and calculate their percentages. One way Analysis of Variance (ANOVA) was used to considered the values of p <0.05 statistically significant.

3. RESULTS & DISCUSSION

Natural resources specifically plants are continuing their role in drug development, in order to discover the safe, effective, and reasonable treatment for the rising spectrum of human ailments. People experiencing different types of disorders and related pains desire to find drugs with lesser side effects [36]. Various medicinal plants have been

Table 2. Preliminary phytochemical analysis of selected medicinal plant species

S.No.	Plants name	Polyphenols	Reducing sugar	Saponins	Flavonoids	Alkaloids
1	<i>Zingiber officinale</i>	+	+	+	+	+
2	<i>Clistemon viminalis</i>	+	-	+	+	-
3	<i>Citrullus lanatus</i>	+	-	-	+	+
4	<i>Trachyspermum ammi</i>	+	+	+	+	+
6	<i>Adansonia digitata</i>	-	+	-	+	-
7	<i>Murraya paniculata</i>	-	+	+	+	+
8	<i>Holoptelea integrifolia</i>	+	+	+	+	+
9	<i>Nepeta adenophyta</i>	+	+	-	+	-
10	<i>Sesamum indicum</i>	+	+	+	+	+

(+): Presence (-): Absence

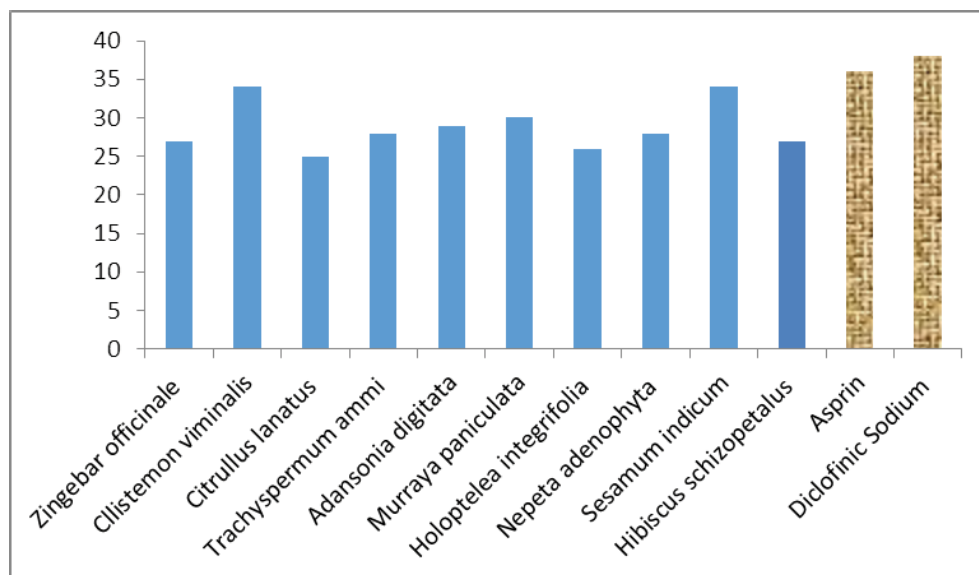


Fig. 7. % inhibition Writhing effect of medicinal plants

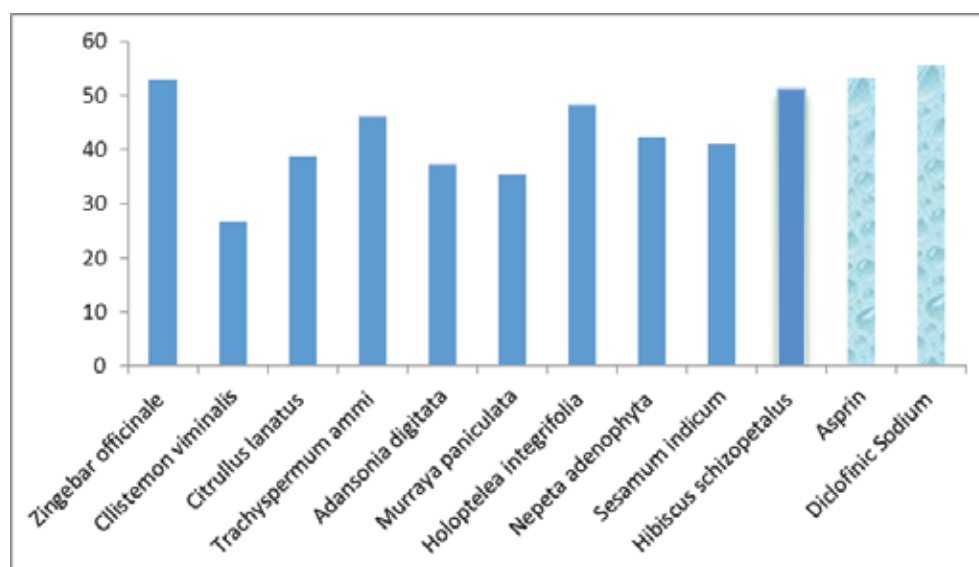


Fig. 8. % Protection in latency time of medicinal plants as compare to standards

advised for prevention and cure of pain and its related conditions because of possessing low or no side effects.

In the current research, we collected 10 Pakistani medicinal plants and performed their preliminary phytochemistry (Table 2) and analgesic activity by hot plate and writhing reflex methods. All selected Pakistani medicinal plants have showed dose dependent significant analgesic activity in both tests (Fig. 7 & 8). As in writhing reflex test, the number of writhes was decreased after administration of all selected plants. Moreover, in hot plate test, the

pain latency time was significantly increased in *Callistemon viminalis*, *Citrus lanatus*, *Zingibar officinale* extracts. Hot plate method is used generally for centrally acting analgesic [37], while peripherally acting drugs are ineffective in these tests but sensitive to acetic acid induced writhing test. It was observed that all of the medicinal plants significantly ($P < 0.05$) reduced the abdominal contractions induced by acetic acid even after 30 min of administration. Similarly, in the hot plate tests all of the plant extracts showed significant effect up to 120 minutes just as standard aspirin and diclofenic sodium drug.

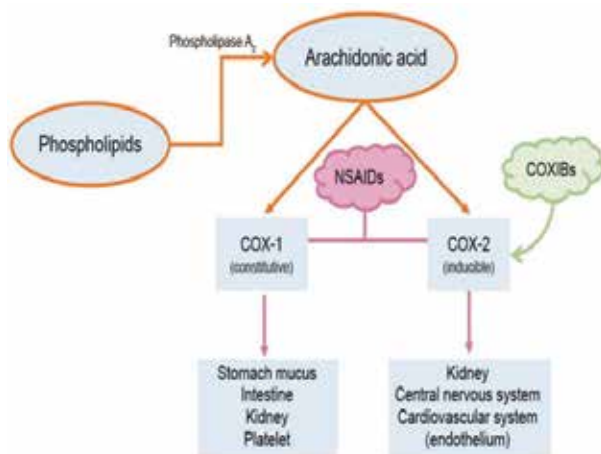


Fig. 9. Mechanism of action of NSAIDs

We found different phytochemicals such as flavonoids, phenols, alkaloids, tannins and saponins in all medicinal plants extracts. Six medicinal plants gave positive test for alkaloids as alkaloids basically affects the central nervous system, reduces pain perception and produces good analgesic activity in all the different models of analgesia as reported by different scientist [38-39]. Furthermore, the presence of flavonoids occurred in eight plant extracts. Flavonoids have analgesic activity that prevents oxidative cell stress [40-41]. Seven plants showed positive test against phenols. Phenols are bioactive polyphenols because these may also be helpful in prevention of oxidative stress diseases including cardiovascular disorder, cancer etc. and also used as antioxidant and analgesic [42-43]. Plant provides various promising medicinal agents as their phytochemical components, which may use to prevent various diseases. Flavonoid, poly phenols, saponins, alkaloids, terpenoids etc. are important phytochemical constituents that are used as antioxidant and work as analgesics and anti-inflammatory agents etc. These phytochemical constituents may play important role also in the formation of crude drugs that contribute for development of new drugs at pharmaceutical industries to cure pain and its complications.

Non-steroidal anti-inflammatory (NSAIDs) drugs are first choice of drugs for treatment of pain and inflammatory conditions (Fig. 9). Reported side effects of NSAIDs such as gastrointestinal bleeding and declined function of the immune system has shifted the attention of researchers to alternative

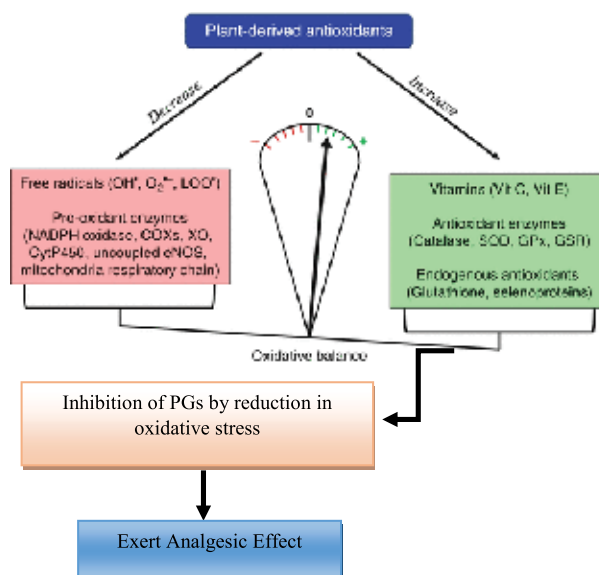


Fig. 10. oxidative balance of Analgesic from plants

pharmacotherapies which are rich in antioxidant agents [44-46]. The bioactive components such as phenolic compounds, flavonoids, saponins, and alkaloids of plants possess multiple therapeutic activities such as antioxidant, anti-inflammatory, antimicrobial and anticancer activities [47-48]. The mechanism followed for the analgesic activities via free radical scavenging or inhibition of major anti-inflammatory enzymes like cyclo-oxygenases (COX) and lipoxygenases (LOX) enzymes are shown in Fig. 10 [49-50]. Moreover, the possible antioxidant involved mechanisms is the major connection for their analgesic effects and our current findings demonstrated scientific rationale for the folk use of these selected medicinal plants as analgesic via antioxidant potential.

4. CONCLUSION

Pain is a neglected health issue affecting millions of people globally. Incidence of pain has been increased due to certain life threatening and degenerative disorders, such as Cancer, Arthritis, and Diabetes, or due to tissue damage. Chronic Pain could be a result of the imbalance between reactive oxygen species and naturally producing antioxidant supplementation is prescribed treatment. In conclusion, all the medicinal plant extracts demonstrated presence of different phytochemicals and exhibited significant analgesic activity by writhing reflex and hot plate test.

Analgesic activity may be linked with their antioxidant potential due to the presence of phenols and flavonoidal contents that boost physiological defense mechanism by decreasing free radicals and oxidative markers during injury through inhibition of prostaglandin pathway. However, more work is required in the isolation and characterization of the bioactive compound(s) and determination of Reactive Oxygen Species (ROS), Inducible Nitric Oxide (iNOS) and glutathione levels to determine the exact antioxidant mechanism.

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