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EVALUATION OF ANTIOXIDANT, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF TRADITIONAL POLYHERBAL COMBINATION USED IN WESTERN NEPAL

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ABSTRACT

Objectives: The present study was aimed for scientific evaluation of traditional polyherbal combination that has been practicing by the local traditional practitioner of western Nepal using different parts of five different medicinal plants which are *Terminalia chebula*, *Terminalia bellerica*, *Ziziphus mauritiana*, *Mimosa rubicaulis* and *Bergenia ciliate* primarily for the acceleration of bone fracture repair. **Methods**: The polyherbal formulation using five different medicinal plants was formulated and extracted using water and ethanol respectively. The preliminary phytochemical analysis was performed. The antioxidant activity was evaluated using DPPH assay method. Similarly, for anti-inflammatory activity, carrageenan induced paw edema test and for analgesic activity hot plate test and tail immersion test were performed. **Results**: The phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, saponins, phenols, terpenoids and carbohydrates. The aqueous extract showed high phenol content i.e. 259.96±2.21 mg GAE/g dry weight of extract also the higher antioxidant activity with IC50 value 4.64 µg/ml followed by the ethanolic extract with IC50 value 4.93 µg/ml and was comparable with the standard ascorbic acid IC50 value 4.16 µg/ml. Carrageenan induced paw edema test showed significant inhibition of paw edema at a dose 200 mg/kg when compared to the control. Similarly, the maximum possible analgesic effect of extract was found as 76.61% and 74.82% at a dose 200 mg/kg by hot plate method and tail immersion method respectively. **Conclusion**: The traditional use of the combination has been supported scientifically. The study may be helpful for the discovery of new anti-inflammatory and analgesic drugs.

KEYWORDS: Antioxidant activity, Anti-inflammatory activity, Analgesic activity, Polyherbal combination, Medicinal plants.

1. INTRODUCTION

Reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, peroxyl radicals are natural byproducts of the normal metabolism of oxygen in living organisms. However, excessive production and accumulation of ROS can cause oxidative stress, a deleterious process that can damage all cell structures.[1]

This may result in the depletion of immune system, change in gene expression and induce abnormal proteins and contribute to atherosclerosis, arthritis, central nervous system injury, gastritis, cancer like disorders.

Moreover, various free radicals also play an important role in the pathogenesis of inflammation.[2]

Inflammation is a part of complex biological response of vascular tissue to harmful stimuli, such as pathogens, damaged cells or irritants, which is characterized by heat, pain, redness, swelling and loss of function.[3] It involves the metabolism of arachidonic acid, catalyzed by enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX).[4] Cyclooxygenase is the key enzyme in the synthesis of prostaglandins, prostacyclins and thromboxanes which are involved in inflammation, pain and platelet aggregation.[5]

According to the International Association for the Study of Pain (IASP), "Pain is an unpleasant sensory and emotional experience, associated with or described in terms of tissue lesion".[6] It is also a protective mechanism to which body respond to harmful stimuli which is important for avoiding injuries. Pain is frequently associated with inflammation.[7]

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage donating hydrogen atoms to the molecules. Some antioxidants even have a chelating effect on free radical production.[8] The human body itself has several mechanisms to counteract with free radicals like several enzymes which act as antioxidant, such as superoxide dismutase, catalase and/or compounds like ascorbic acid, tocopherol and glutathione provide shield against the damaging action of oxidative stress.[9] There are also some synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ) but their use have been

limited due to their possible carcinogenic effect.[10] Similarly, nonsteroidal anti-inflammatory drugs (NSAIDs) are the choice of drug for acute inflammatory disorders and pain that acts by suppressing the formation of prostaglandins, by inhibiting the activity of enzyme cyclooxygenase (COX-1 and COX-2).[11] Whereas, opioids act by binding to specific opioid receptor (mu and kappa) and are very effective in the treatment of severe pain.[12] But these drugs are found to show several untoward effects like gastrointestinal ulceration and bleeding, hepato-renal dysfunction and organ failure, skin reactions, respiratory depression and possibly drug dependence (with opiods). Therefore, development of newer and more substantial drug with lesser side effects is needed.[13]

Medicinal plants are believed to be important resource of novel chemical substances with potential remedial effects hence, are used as the primary source for the treatment of various ailments.[14] Plant polyphenols are reported to exhibit antioxidant activity and capacity to alleviate oxidative stress induced tissue damage which is associated with a number of chronic disease.[15] It has been reported that the antioxidant activity of herbal extracts showed the protective role in the management of inflammatory related diseases.[16] The rural population of the country is more disposed to the traditional ways of the treatment because of their easy availability and cheaper cost. Inflammatory and infectious diseases are among those treated using traditional medicine. Plants used traditionally as a medicine, but very few species have been investigated scientifically for their medicinal properties.[17] As like combination of various parts of five medicinal plants which are *Terminalia chebula, Terminalia bellerica, Ziziphus mauritiana, Mimosa rubicaulis* and *Bergenia ciliate* has been found to be used by people of western part of Nepal to accelerate bone fracture repair. Currently, there is no scientific evidence available for the study of such combination in the management of pain and inflammation. Thus, the present study is undertaken to evaluate the antioxidant, anti-inflammatory and analgesic activity of the combination in experimental animal model.

2. MATERIALS AND METHODS

2.1 Plant materials

2.1.1 Collection and identification of crude drugs

The stem bark of *Terminalia chebula, Terminalia bellerica*, root bark of *Mimosa rubicaulis, Ziziphus mauritiana*, and rhizome of *Bergenia ciliate* were collected from the Shuklagandaki area of Tanahun district of Nepal, under the supervision of local traditional practitioner. The collected plant samples were identified by comparing the morphological characteristics with

literatures and were confirmed by botanist. The plant samples were then preserved in Pharmacognosy Laboratory of School of Health and Allied Sciences, Pokhara University, Nepal.

2.1.2 Formulation of polyherbal combination

All the plant samples were dedusted, thoroughly cleaned and then cut into small pieces and left for shade drying. The total samples required were calculated as 36 g of *T. chebula*, 36 g of *T. bellerica*, 18 g of *Z. mauritiana*, 8 g of *M. rubicaulis* and 2 g of *B. ciliate* (as calculated on the basis of ethnomedicinal uses). All the samples were weighed accurately and mixed properly.

2.1.3 Extraction

The extraction of polyherbal combination was performed by the method of double maceration with water and ethanol respectively (solvent: crude sample = 8:1 or 800 ml: 100 g) and thus obtained extracts were filtered with filter paper. The filtrates obtained were then mixed properly and concentrated in rotary evaporator and samples were kept in refrigerator for preservation.

2.2 Animals

Wistar albino rats (n=30) weighing 150-250 g were purchased from science House, Pokhara, Nepal.

There were equal numbers of male and female rats. All the rats were kept in polypropylene cages at room temperature of 25±5ºC. Animals were kept for acclimatization in laboratory of Pokhara University for four weeks.

2.3 Ethical considerations

An ethical approval was obtained from Institutional Review Committee (IRC), Pokhara University Research Center (PURC) before all the animal experiment (Ref.No.107/074/75). All the research works involving use of animals was performed following IRC rules and regulations. The research activities were conducted in responsible without any kind of misconduct.

2.4 Phytochemical screening

The phytochemical screening tests of aqueous and ethanolic extracts were performed for the detection of phytoconstituents like alkaloids (Wagner's test, Mayer's test and Hager's test),

glycosides (Modified borntager's test), flavonoids (Alkaline reagent test and Molisch's reagent test), carbohydrates (Benedict's reagent test and Fehling's reagent test), saponins (foam test), tannins (Gelatin test), terpenoids (Salkowski's test) and phenols (Ferric chloride test).[18][19]

2.5 Determination of total phenol content

Folin ciocalteu method was used for determination of total phenol content.[20] In brief, 1 ml of sample was mixed with 5 ml of distilled water and 1 ml of folin reagent. After standing for five minutes, 1 ml of 10% sodium carbonate was added and stirred. The mixture was incubated for one hour at room temperature and the absorbance was measured at 725 nm against a blank. The total phenol content expressed as milligrams of gallic acid equivalent per gram dry extract weight using the calibration curve of gallic acid (50 mg/L-500 mg/L) standards.

2.6 Determination of antioxidant activity

2.6.1 DPPH radical scavenging assay

DPPH free radical assay was performed for the measurement of antioxidant activity[21]. In brief, 2 ml of different concentration of the extract solution (100 µg/ml, 10µg/ml and 1 µg/ml) were mixed with 2 ml of DPPH solution (60 µM). The mixtures were allowed to stand in dark for 30 minutes. Finally, the absorbance of each test samples was measured at 517 nm by using UV spectrophotometer UV (1800).

Then the test was repeated using lower concentrations (10 µg/ml, 7.5 µg/ml, 5 µg/ml, 2.5 µg/ml and 1 µg/ml). Radical scavenging activity of each sample was calculated by using following formula:

$$\text{Radical scavenging activity (\%)} = [(A0 — AS)/ A0] \times 100\ \%$$

Where, A0= Absorbance of control and AS= Absorbance of sample

Control is the test solution without sample. Ascorbic acid was taken as positive control. The antioxidant activity of each sample was expressed in terms of IC50 (concentration required to inhibit DPPH radical formation by 50 %), calculated from the graph after plotting percentage inhibition against extract concentration.

2.7 In vivo study design

2.7.1 Grouping of experimental animal

Rats were divided into five group (control group, standard group and three treatment groups) each comprising six rats. Control group was treated with distilled water. Standard group was treated with standard drug diclofenac 50 mg/kg. Three treatment group were treated with aqueous extract 50 mg/kg, 100 mg/kg and 200 mg/kg respectively.

2.7.2 Carrageenan induced paw edema test

The anti-inflammatory activity of test sample against acute inflammation was done by carrageenan induced rat paw edema method.[22] Briefly, animals were fasted overnight and received water *ad libitum* during the experiment. Acute inflammation was produced by sub-plantar injection of 0.1 ml of freshly prepared 1 % carrageenan suspension in normal saline in the left hind paw of rats in each group. A mark was made just below the tibio-tarsal junction of each rat so that every time the paw could be dipped in the column of plethysmometer up to the mark to ensure a constant paw volume. Plethysmometer was calibrated by using the standard probe of 1 ml and 2 ml. Initial paw volume was measured at 0 hr. Then different groups of animals were treated with 0.5 ml of distilled water (control) or 50 mg/kg of reference drug (diclofenac) or the extracts (50, 100 and 200 mg/kg) orally. Increase in paw edema was measured as the difference between the paw volume at '0' hr and paw volume at the respective hour. The percentage inhibition (PI) of the rat paw edema was calculated after each hour of carrageenan injection up to five hours by the formula:

$$PI = \frac{(control\ group\ Ii - test\ group\ Ii)}{control\ group\ Ii} \times 100$$

The inflammation index (Ii) was calculated as difference between the final volumes of the carrageenan injected paw (Vt) and the initial volume of the same paw before injecting it (V0).

i.e. Inflammation index (Ii) = Vt – V0

2.7.3 Eddy's hot plate method

Evaluation of analgesic activity of the aqueous extract was carried out using hot plate method.[23] This method is one of the most commonly used methods for the evaluation of central analgesic activity where heat is used as a source of pain. Briefly, rats were placed on a hot plate maintained at 55±1◦C surrounded by a plexiglas cylinder (height: 26 cm; diameter: 19 cm). Only rats that showed initial response within 30 seconds were selected and used for the study. The reaction time (in seconds) or latency period was determined as the time taken

for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before (0 min) and at 30, 60, 120 and 180 min after the administration of the extract (50, 100 and 200 mg/kg) and reference drug (50 mg/kg) orally. The maximum reaction time was fixed at 30 sec to prevent any injury to the tissues of the paws. If the reading exceeds 30 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

$$\text{MPA} = \frac{\textit{reaction time for treatment} - \textit{reaction time for control}}{30\,\text{sec} - \textit{reaction time for control}} \times 100$$

2.7.4 Tail immersion method

Tail immersion test was used to assess the analgesic activity of aqueous extract of polyherbal combination which involved immersing the extreme 5 cm of rat's tail in a water bath at a temperature of (55±1)◦C.[24] After immersing within a few minutes, the rat reacted by withdrawing the tail. The initial reaction time for withdrawing the tail was recorded by immersing the marked part of tail at hot water at 0 min and animals were pretreated with 0.5 ml of distilled water (control) or 50 mg/kg of reference drug (diclofenac) or the extracts (50, 100 and 200 mg/kg) orally. The reaction time was recorded in 0.01s units by a stopwatch at 30, 60, 120 and 180 min. The cut off time was 15 sec to avoid the damage to the rats. The maximum possible analgesia was measured by the formula as:

$$\text{MPA} = \frac{\textit{reaction time for the treatment} - \textit{reaction ime for control}}{15\,\textit{sec} - \textit{reaction time for control}} \times 100$$

2.8 Statistical analysis

The experimental data were expressed as mean ± standard deviation. Statistical analysis was performed using student's t-test. $P < 0.05$ was considered statistically significant. All the statistical methods were carried out through Microsoft Excel 2007.

3 RESULTS

3.1 Preliminary Phytochemical screening

Phytochemical analysis revealed the presence of several secondary metabolites such as flavonoids, terpenoids, carbohydrates, phenols, tannins, saponins and alkaloids in both the extracts.

3.2 Total phenol content

The calibration curve of gallic acid as standard at concentration 50 mg/L, 100 mg/L, 200 mg/L, 300 mg/L and 400 mg/L was found as shown in figure 1. On the basis of standard regression line for gallic acid (y = 0.0097x + 0.2507; R2 = 0.992), the equivalents of TPC were calculated as shown in table 1. The aqueous extract was found to possessed high concentration of phenol as compared to the ethanolic extract.

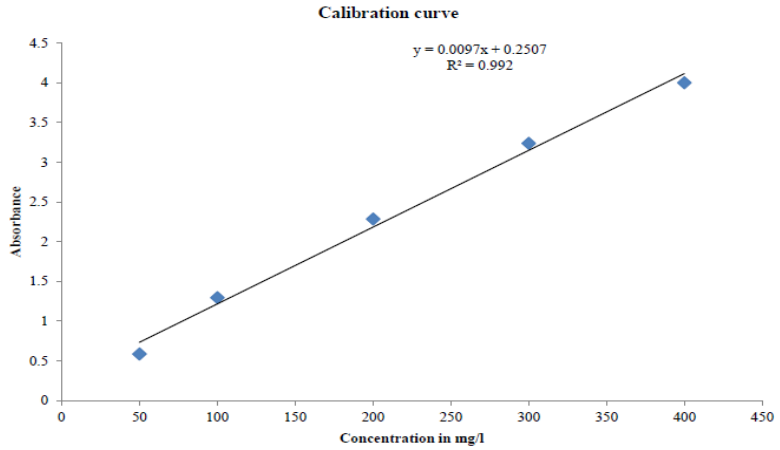

Figure 1: Calibration curve of gallic acid for calculation of total phenol.

Table 1: Total phenol content expressed as mg gallic acid equivalent per gram dry weight of extract.

Sample	Total phenol content mg GAE/g dry weight of extract
Ethanolic Extract	199.85±1.70
Aqueous Extract	259.96±2.21

Note: Data expressed as mean±standard deviation (n=3)

3.3 ANTIOXIDANT ACTIVITY

3.3.1 DPPH scavenging assay

Radical scavenging activity of extracts along with the reference standard ascorbic acid has been estimated using DPPH radical scavenging assay. The lower value of IC50 indicates the higher antioxidant activity. The % DPPH radical scavenging activity of the extracts/ascorbic acid were found to be more than 50% at lower concentration 10 µg/ml represented by table 2. Thus, the % DPPH radical scavenging activity of extracts/ascorbic acid were also evaluated at lower concentrations (1 µg/ml, 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml and 10 µg/ml) for the calculation of IC50 value. It was found that the aqueous extract exhibited the higher antioxidant activity with the IC50 value 4.64 µg/ml as compared to the ethanolic extract

(IC50 value 4.93 µg/ml) that was comparable with the standard ascorbic acid (IC50 value 4.16 µg/ml) as represented by table 3.

Table 2: DPPH radical scavenging activity of extract and ascorbic acid at different concentrations.

		(%) DPPH Scavenging Activity	
Sample/Conc ⇨ ⇩	**1 µg/ml**	**10µg/ml**	**100 µg/ml**
Ascorbic Acid	26.48±1.25	92.22±0.28	96.57±0.58
Ethanolic Extract	23.80±1.25	82.22±1.00	93.33±0.28
Aqueous Extract	23.61±1.00	86.67±1.27	88.98±0.58

Note: Data expressed as mean±standard deviation (n=3).

Table 3: Percentage DPPH radical scavenging activity of extract and ascorbic acid (AA) at different concentrations.

	(%) DPPH Scavenging Activity					IC_{50} Value
Sample/Conc	1 µg/ml	2.5µg/ml	5µg/ml	7.5µg/ml	10µg/ml	
Ascorbic Acid	26.48±1.25	37.87±0.42	52.13±0.97	68.98±0.42	92.22±0.28	4.16
Ethanolic Extract	23.80±1.25	28.70±0.16	51.76±0.97	66.67±0.28	82.22±1.00	4.93
Aqueous Extract	23.61±1.00	38.05±0.56	61.94±0.56	68.52±0.85	86.67±1.27	4.64

Note: Data expressed as mean±standard deviation (n=3).

3.4 Antiinflammatory activity

3.4.1 Carrageenan induced paw edema test

The in vivo anti-inflammatory activity of aqueous extract was compared with control in hourly basis up to five hours after the induction of inflammation. The results of anti-inflammatory activity were expressed as mean±standard deviation of inflammation index as shown in table 4. Similarly, figure 2 illustrates the increase in percentage inhibitory activity of standard drug (diclofenac) and extract with time and dose. It was found that the test drug produced significant inhibition of paw edema. Antiinflammatory property of aqueous extract at different doses was significant even at the end of fifth hour after carrageenan injection. On the other hand, 50 mg/kg of standard drug (diclofenac) showed significant anti-inflammatory effect during all the observation period after carrageenan injection when compared to the control group.

Table 4: Inflammation index of rat paw edema.

Treatment Group	Inflammation Index				
	1 hr	2 hr	3 hr	4 hr	5 hr
Control	0.26±0.07	0.32±0.06	0.32±0.11	0.30±0.13	0.13±0.05
Diclofenac 50 mg/kg	0.13±0.04*	0.15±0.03*	0.14±0.05*	0.11±0.06*	0.07±0.03*
Aqueous extract 50 mg/kg	0.17±0.03*	0.20±0.04*	0.19±0.04*	0.12±0.07*	0.08±0.05
Aqueous extract 100 mg/kg	0.16±0.03*	0.19±0.03*	0.18±0.05*	0.12±0.04*	0.08±0.03*
Aqueous extract 200 mg/kg	0.16±0.03*	0.18±0.03*	0.17±0.04*	0.11±0.01*	0.08±0.02*

Note: Data expressed as mean±standard deviation (n=6). *p<0.05, when compared to control

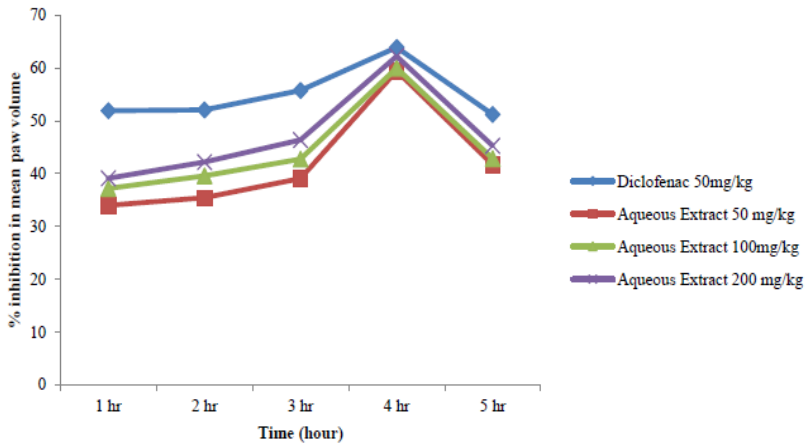

Figure 2: Percentage inhibition of rat paw edema after carrageenan injection upto several hours.

3.5 Analgesic activity

3.5.1 Hot plate test

It was found that the extract at different doses and standard drug (diclofenac) exhibited significant analgesic activity when compared to control. The standard and extract at three different doses showed significant analgesic activity after 30 minute of treatment as represented by table 5. Similarly, the maximum possible analgesia was found to be 76.61% for a dose 200 mg/kg followed by 74.55% for a dose 100 mg/kg and 60.60% for a dose 50 mg/kg whish was comparable with standard drug 50 mg/kg that showed 70.89% at 180 minute of treatment as shown in figure 3.

Table 5: Result of analgesic activity by hot plate method.

Treatment Group	Reaction Time in Seconds (Mean±SD)				
	0 min	30 min	60 min	120 min	180 min
Control	7.74±3.37	8.73±3.66	9.64±3.37	10.01±3.54	8.68±3.50
Diclofenac 50mg/kg	6.82±1.40	14.91±2.86*	19.70±5.81*	21.50±3.93*	23.80±1.97*
Aqueous extract 50 mg/kg	9.47±3.55	12.65±3.35	17.35±3.98*	19.98±5.35*	21.70±3.18*
Aqueous extract 100 mg /kg	9.26±4.01	12.99±2.58*	18.14±2.76*	22.22±3.24*	24.59±2.16*
Aqueous extract 200 mg/kg	8.52±3.10	13.54±2.87*	19.64±3.30*	23.15±1.66*	25.08±2.47*

*Note: Data expressed as mean±standard deviation (n=6). *p<0.05, when compared to control*

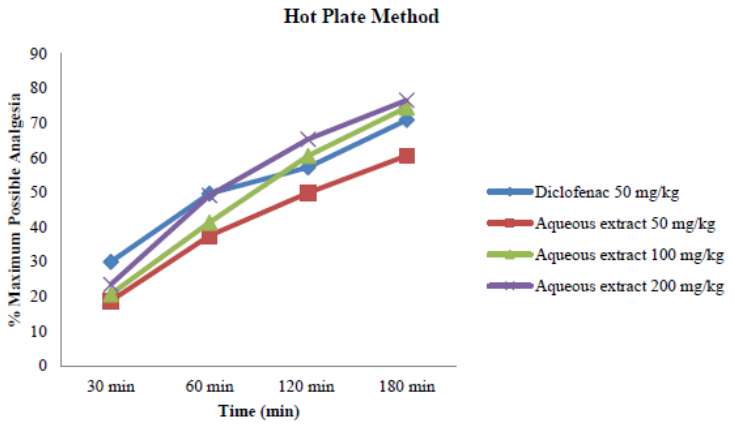

Figure 3: Maximum possible analgesia (%) of extract/diclofenac by hot plate method in rats.

3.5.2 Tail immersion method

The aqueous extract showed significant analgesic action at all three different doses when compared to control by tail immersion method. The analgesic activity was expressed as mean reaction time in seconds ± standard deviation (Table 6). The extract showed increase in the reaction time with the maximum possible analgesia 74.82% for a dose 200 mg/kg followed by 71.98% for a dose of 100 mg/kg and 60.50% for a dose of 50 mg/kg and was found to be comparable with standard diclofenac i.e. 69.79% after 180 min of drug administration (figure 4).

Table 6: Result of analgesic activity by tail immersion method.

Treatment Group	Reaction Time in Seconds (mean±SD)				
	0 min	30 min	60 min	120 min	180 min
Control	1.45±0.48	2.40±0.29	2.96±0.84	3.46±0.85	3.59±0.83
Diclofenac 50 mg/kg	3.06±0.79	6.09±0.54*	8.56±1.30*	9.41±0.70*	11.55±0.95*
Aqueous extract 50 mg/kg	3.68±0.89	4.96±0.38*	7.58±2.13*	8.80±1.39*	10.49±2.09*
Aqueous extract 100 mg /kg	2.79±0.40	5.25±0.65*	8.68±1.24*	9.94±1.38*	11.80±1.57*
Aqueous extract 200 mg/kg	3.40±0.98	5.48±0.62*	8.81±0.96*	10.25±0.47*	12.13±1.53*

*Note: Data expressed as mean±standard deviation (n=6). *p<0.05, when compared to control*

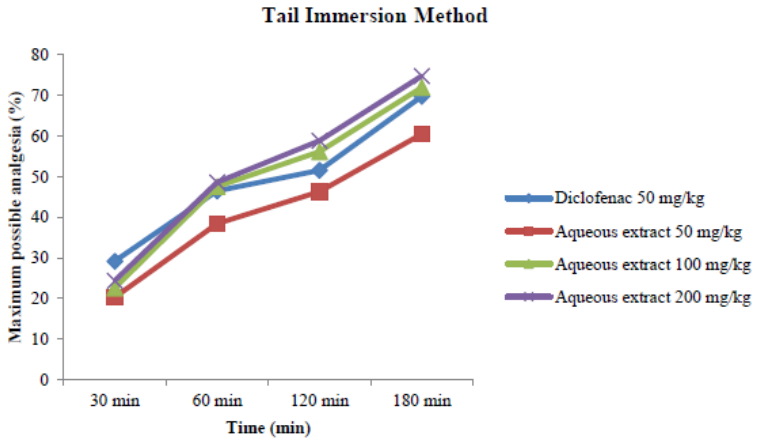

Figure 4: Maximum possible analgesia (%) of extract/diclofenac by tail immersion method.

4 DISCUSSION

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. In DPPH method, the antioxidants react with the stable free radical, 2,2-diphenyl-1- picrylhydrazyl (deep violet color) and convert it to 2,2-diphenyl-1-picrylhydrazine with discoloration which indicates the scavenging potential of sample antioxidant.[25] One of the study reported that hydrocarbon, cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxyaromatic compounds (hydroquinone, pyrogallol, etc) and

aromatic amines such as p-phenylene diamine, p-aminophenol etc., reduce and decolourise DPPH by their hydrogen free radical scavenging ability.[26]

In the present study, may be it appears that the compounds present in the extracts possess hydrogen donating capabilities to act as an antioxidant. The antioxidant potency order was similar to the phenolic contents of the extracts i.e. aqueous extract showed higher phenolic content and also the higher antioxidantactivity when compared with the ethanolic extract. Indeed, phytochemical analysis has revealed the presence of several secondary metabolites like tannins, saponins, carbohydrates, flavonoids, terpenoids, phenols and alkaloids. These compounds are good inhibitors of serotonin, histamine and leukocyte migration.[27] The antioxidant activity of the phenolic compounds is mainly due to their redox properties, which can play important role in absorbing and neutralizing free radicals by their hydrogen donating ability.[28] A study has reported that the phenolic contents of *T. chebula* are responsible for its antioxidant activity.[29] As their free radical scavenging abilities facilitated by their hydroxyl groups, the total phenolic concentration could be used as basis for rapid screening of antioxidant activity.[30] Therefore, these days crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly being used in the food industry for their antioxidative properties and health benefits.[31]

Carrageenan induced paw edema is an acute inflammation model widely used for assessing anti-inflammatory activity. When a tissue is injured, prostaglandin synthesis in that tissue increases that shows two major actions. First, they are mediator of inflammation which also sensitize pain receptors at the nerve endings, lowering their threshold of response to stimuli and second is they enhance the release of histamine, bradykinin etc.[32] The carrageenan is known for its classic biphasic effect. The first phase is mediated by the release of histamine and serotonin during the first hour and release of kinins up to 2.5 hours, while second phase is mediated by the release of prostaglandins from 2.5 to 6 hours.[33] In the present study, the extract showed the inhibition of edema at different hours that may suggest that the extract interferes with the action or release of serotonin or histamine, because these are the predominant mediators in the initial phase of carrageenan induced edema.[34] Also, this finding suggests a possible inhibition of cycloxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as diclofenac, whose mechanism of action is inhibition of the cyclooxygenase enzyme.[35] The observed anti-inflammatory effect of the extract may be also due to the presence of antioxidant

compound. It has been reported that the reactive oxygen species (ROS) generated during inflammation can activate phospholipase A2, which releases more arachidonic acid from the phospholipid membrane, which is subsequently transformed into prostaglandins and leukotrienes.[36]

The animal models used for screening of analgesic activity in this study are pain-state models using thermal stimuli which include hot plate method and tail immersion method. Both methods are useful in illustrating centrally mediated antinociceptive responses which focus generally on changes above the spinal cord level.[37] Prolongation of the reaction time indicates the involvement of supraspinal mechanisms.[38] Analgesic drugs which are centrally acting elevate pain threshold of animals toward heat and pressure.[39] Therefore, the analgesic effect of the extract on this pain-state model indicates that it might be centrally acting.

A number of alkaloids, flavonoids, steroids and tannin isolated from medicinal plants have been reported to possess significant analgesic activity.[40] *T. chebula* and *T. bellerica* is considered to be the rich source of tannin. Thus, analgesic activity observed with this extract may be due to presence of tannin. Furthermore, there are reports on the role of tannins in analgesic activity.[41] It is also reported that flavonoids such as rutin, quercetin and luteolin produced significant antinociceptive and anti-inflammatory activities.[42] Besides alkaloids are well known for their ability to inhibit pain perception.[43] Though it is not possible to identify the exact phytochemical, responsible for biological activities, it can be assumed that the effect is due to the phytochemicals present in the extracts examined by qualitative test.

The previous study has reported that this polyherbal combination was effective in acceleration of fracture repair by increasing the serum calcium level and serum phosphate level in rabbits which was also supported by x-ray analysis.[44] From the present study, it can be assume that the fracture healing property may be due to its antioxidant, anti-inflammatory and analgesic activity. Further study is required to identify the major compound present that is responsible for the biological activity and to elucidate the mechanisms involved.

5 CONCLUSION

In conclusion, the aqueous extract and ethanolic extract of traditional polyherbal combination revealed the presence of several secondary metabolites like tannins, carbohydrates, phenols terpenoids, alkaloids, flavonoids and saponins. Aqueous extract found to exert higher phenol content as well as antioxidant potency when compared to ethanolic extract. Due to higher

antioxidant potency, aqueous extract showed significant anti-inflammatory and analgesic activity when compared with the control. So that the formulation could be fruitful for new antioxidant, anti-inflammatory and analgesic drug development but further investigation is needed to study the detail mechanism of action.

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