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Evaluation of Analgesic Activity of Berberin in Mice

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ABSTRACT
In folk medicine, Chelidonium majus is employed as bile and liver disorders, for treatment of warts, corns, eczema and solid tumors for generations where the chief component berberine is known to exert pharmacological activities. However, no data on the role of berberine as potential analgesic is available in pharmaceutical databases. The present study was directed to evaluate the analgesic activity of berberine at 200 mg/kg and 400 mg/kg doses. A variety of tests including formalin-induced paw licking test, acetic acid induced writhing test, and tail immersion test were used to assess the analgesic activity in mice. In formalin-induced paw licking test, acetic acid induced writhing test, and xylene-induced ear edema test, the extracts exhibited significant inhibition ($P < 0.05$ versus control) of pain. From this study, it could be shown that berberine expressed noteworthy analgesic activity which supports its usage. It is possible to obtain analgesic agent from the plant source and serve as an alternative bio-resource in managing pain, rather than dependence over the marketed synthetic products. However, further quantifiable studies are now essential to categorize the particular mechanism which is responsible for the analgesic activity of berberine. At last but not least, to be a safe therapeutic agent, not only acute oral toxicological evaluation but also genotoxicity study of this plant should be conducted in future.
KEYWORDS: Berberine, Analgesic, Mice, Evaluation, Pharmacology, Animal models

I. INTRODUCTION

Inflammation and/or peripheral nerve injury can contribute to pathological pain, which is considered a result of the nervous system malfunction. Inflammatory reactions caused by tissue injury or microorganisms' activity lead to the release of damage- and pathogen-associated molecules that are recognized by the immune system components like macrophages and dendritic cells. Activated macrophages produce chemoattractant molecules directed by nuclear factor-κB (NF-κB) transcription that can further induce inflammatory enzymes [e.g., cyclooxygenase-2 (COX-2)] and pro-inflammatory cytokines, which are main players in pain and inflammation development [1].

Pain is a complex and common complaint that leads to frequent access of the US healthcare system. Chronic pain alone affects more Americans than diabetes, cancer, and heart disease combined, with an estimated annual cost of $600 billion. Pain is often under recognized leading to inadequate management and numerous patient safety concerns, particularly in special populations and minority groups. Untreated acute pain may lead to adverse sequelae. With the recent opioid epidemic and advances in pain research, there is a renewed emphasis on early multimodal pain management, non-pharmacologic options and non-opioid alternatives [2].

Pain

According to the International Association for the Study of Pain, neuropathic pain is explained as “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system”. In this definition, two terms are emphasized ‘disease’ that refers to all types of conditions such as inflammation and auto-immune disorders, and ‘dysfunction’ that can differentiate this pain from those originated from the nervous system **(Figure 1.1)**. Since natural products exert promising biological and pharmacological properties and are believed to possess less adverse effects compared to synthetic drugs, they have been widely studied against pain conditions, including neuropathic pain [3].

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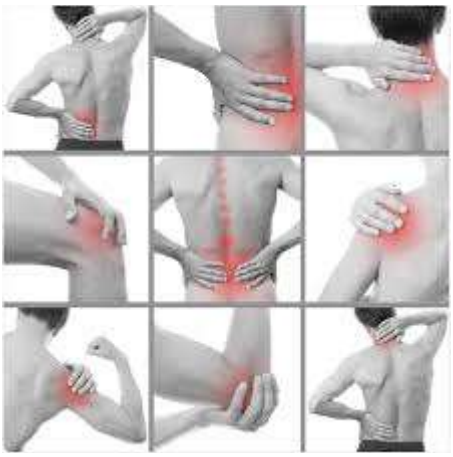

Figure 1.1. Types of pain.

Pain management is 'core-business' for the anaesthetist. Indeed, anaesthesia developed from the humanitarian desire to control pain during surgery by (pharmacologically) altering consciousness, initially with chloroform, nitrous oxide or ether and prior to the 19th century with opioids, alcohol and even asphyxiation. Involvement of anaesthetists in the management of acute post-surgical and post-trauma pain, labour pain, chronic and cancer pain soon followed. But what is this phenomenon called pain and how is it different to (and often confused with) nociception, which is defined as the "neural processes of encoding and processing a noxious (tissue-injuring) stimuli" [4].

Origin of the word pain or analgesia

Exploring the origins of the English word 'pain', provides insights into its meaning and conceptualization in Western and other civilizations. The word 'pain' was probably used for the first time in the Middle Ages and is a derivation of old French 'peine' and the Latin 'poena' (as in 'subpoena') meaning 'punishment' or 'penalty' and the earlier Greek root 'poine' with essentially the same meaning. 'Poneros' is Greek for 'evil' or 'grievous'. 'Poena' was the spirit of punishment in Roman mythology and the servant of Invidia (Latin) or Nemesis, the Greek goddess of divine retribution. This etymology promotes the concept of pain as an evil, punitive experience, judgment or personal nemesis, perhaps reflecting the religious ('wrath of God') and cultural overtones of Europe in the Middle Ages. 'Algos' is Greek for pain and is again linked to sorrow or punishment; 'odyne' (Greek) is also used to describe pain but means 'to eat or consume' and 'nocere' (Latin) means to injure, damage or harm. The Latin word 'dolor' with derivatives still used in modern languages such as French and Spanish, means 'hurt' or 'ache' which is more descriptive of the sensory experience, although there is still linkage to 'emotional' words such as 'sadness', 'suffering' or 'anguish'. In some Asian languages such as Japanese or Bahasa, the word for pain is used interchangeably with 'disease', 'illness' or 'hurt' without reference to punishment or suffering. The concept of pain as an 'evil punishment' expressed in many languages, cultures and epochs, suggests that it is more than simply an unpleasant sensation or 'hurting'; it is a negative emotional experience linked to 'suffering' with social, spiritual and philosophical dimensions [5].

Definition

A sub-committee of the International Association for the Study of Pain (IASP) Task Force on Taxonomy headed by Professor Harold Merskey, 'crafted' the most commonly used definition of pain in 1979. A recent update of IASP pain terminology was remarkable in that, after due consideration and debate, it was decided not to modify the original definition at all after 30 years, despite major advances in pain-related fields as diverse as neuroscience and philosophy. However, this document is still subject to revision after a period of consultation [6].

Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." Breaking-down the components of this seemingly simple line of text is useful in gaining an understanding of the concepts of pain. "Pain is an unpleasant sensory and emotional experience." Pain has to be unpleasant, however similar unpleasant sensations such as dysesthesiae, itch or cold are not pain. Curiously, some patients with cortical injuries (such as after a stroke) clearly report 'pain' (as understood from their past experiences) but do not experience it as 'unpleasant'. This is pain asymbolia and causes a dilemma for the IASP definition. Pain is more than perception, 'sensory processing' or 'nociception'. To stress this point, consider that pain isn't even one of the five primary senses. Pain not only has 'sensory-discriminative', but also 'emotional-affective', 'cognitive-evaluative', 'motivational' and perhaps even spiritual dimensions. These 'higher dimensions' of pain are important in the expression of 'pain language' [7].

Personal experience and neurobiology demonstrates that pain is usually associated with tissue damage in the body. However by including

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the word 'potential', the definition avoids the obligation of 'tying' pain to tissue damage. This is a revolutionary change from the time-honoured Cartesian concept of pain as a (real-time) 'alarm system' for injury. Pain in the context of "potential tissue damage", reflects situations where damage has not actually occurred but may occur (so called 'tissue threat') for example, pressing hard on your thumbnail or briefly touching a hot plate, or perhaps in situations where pain is reported by persons who simply 'perceive' that their tissues are 'under threat'. In some cases this is conceptualised (rightly or wrongly) as 'psychogenic' or somatoform pain. A person can clearly experience pain in the absence of tissue damage with 'phantom pain' (where there's no tissue at all) being the classic example. 'Phantom phenomena' clearly demonstrate that 'experiences' such as pain, touch, and even our sense of 'self' can be 'generated' in the absence of real-time sensory inputs (such as nociception) from the physical body. The phenomenon of allodynia (pain due to a stimulus that is not normally painful) is another example of pain in the absence of tissue damage. Pain is a totally subjective experience of the sufferer's 'internal world' of the self, which is expressed to others in the 'outside world' (doctors, family or even insurance case managers) using the 'language of tissue damage' (pain narrative), either actual or threatened. Individuals learn the application of the word (pain) through their experiences related to injury in early life." An important note appended to the IASP definition of pain states that, "in the absence of tissue damage or any likely patho-physiological cause if they regard their experience as pain and if they report it in the same way as pain caused by tissue damage, it should be accepted as pain" [8].

In other words, pain is always what the sufferer says it is. There is no way that 'we' as external observers can really 'know' otherwise. "There is usually no way to distinguish their (the sufferer's) (pain) experience from that due to tissue damage, if we take the subjective report" (which we have to). Such license further 'unties' pain from the obligation of tissue damage. However it opens-up a potential dilemma with concepts such as 'psychogenic' or somatoform pain disorders. Is this 'real' pain according to the IASP definition? The answer is yes, given the sufferer experiences and reports they are in pain. However the validity of the definition clearly fails in factitious disorder or malingering, where the subject feigns pain (this may be considered 'acting') when there is no actual or even potential tissue damage. The IASP definition further explains that pain in the absence of tissue damage or any likely patho-physiological cause…usually happens for psychological reasons." Curiously, having just made the great leap forward of 'untying' pain from tissue damage (in the body) this statement simply serves to re-define pain as a problem of the mind instead (psychological 'damage'). A criticism and potential limitation of the IASP definition of pain is reliance on verbal reporting by the sufferer. This obviously 'excludes' non-verbal humans (e.g. infants, dementia) and animals. However, the definition does not technically preclude non-verbal humans or animals from experiencing the unpleasant sensory and emotional experience of pain. Verbal reports may be seen as an 'efferent' response to the internal (pain) experience. However other efferent responses, in particular pain behaviours (grimacing, groaning, rubbing an injured arm or running away) are not addressed in the IASP definition and yet in clinical practice and in everyday life, are keystones for identifying persons in pain, especially those who are non-verbal or non-lingual; persons who simply can't 'speak the (pain) language'. There may be a place for changing the definition of pain slightly from 'described' to 'expressed' (in terms of such damage) to encompass pain behaviors. Despite limitations, the IASP definition of pain remains essentially valid, widely applicable and clinically useful. Importantly, it unties pain from obligatory tissue injury and in so doing has ethical merit by promoting 'belief' of the sufferer's pain reports and alleviating the stigma of skepticism [9].

Nociception

Nociception is defined as "the neural processes of encoding and processing noxious stimuli." A noxious stimulus is "an actual or potential tissue-damaging event", usually in the form of physical (mechanical, thermal, electromagnetic) or chemical energy. It is interesting to note that not all noxious stimuli (e.g. X-rays) cause tissue damage and even if they do (for example, a slow growing liver or brain tumour) they don't always activate nociceptors and cause pain. A nociceptor is, "a sensory receptor that is capable of transducing and encoding noxious stimuli." In other words, nociceptors transform the 'energy of tissue damage' (mechanical, thermal or chemical) into electrical energy for neural transmission, just like the rods and cones of the eye convert the electromagnetic energy of light into electrical impulses. Nociceptive 'traffic' ascends from the tissues via nociceptive neurons, the dorsal horn and various spinal cord tracts to the brainstem,

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midbrain, thalamus and various cortical regions and is modulated by descending inhibitory and facilitatory pathways. Technically speaking there are no 'pain' pathways but rather nociceptive pathways for transmission. In other words, the spinothalamic tract does not actually transmit 'pain'. Neuro-physiological processes that 'amplify' nociception produce sensitization, which may be defined as "increased nociceptive output for a given input." When these processes occur in central nervous system (CNS) (mainly in the dorsal horn) it is called central sensitization which is characterized by increased (nociceptive) responsiveness, decreased threshold for activation, increased spontaneous activity ('ectopy') and an expanded receptive field [10].

Explanatory notes accompanying the definition clearly highlights that pain and nociception are not the same thing; "pain is a subjective phenomenon whereas nociception is the object of sensory physiology." Nociception (due to tissue damage) is the sensory process that most commonly (but no exclusively) 'triggers' the multidimensional and conscious experience of pain (the classical 'pain-as-an-alarm' paradigm). However pain can clearly occur in the absence of nociception (tissue damage) (eg. phantom pain or allodynia) and nociception can occur without 'triggering' pain (nociception in tissues during surgery under local anaesthesia or whilst unconscious during general anaesthesia). Pain is an absolute function of consciousness whereas nociception is not. There is no 'pain centre' in the brain and strictly speaking, there are no 'pain pathways'. Pain does not cause changes in the nervous system, although various processes such as cortical changes on fMRI are associated with pain. To find a sensory metaphor, nociception is the comparable to the process of sound energy being converted into nerve impulses in the inner ear, which are transmitted to the auditory cortex. Hearing is the conscious experience of these auditory stimuli and pain is more like 'music', a complex sensory and emotional experience. Like pain, you can experience music in the absence of sensory (auditory) inputs [11].

Central Sensitization

Central Sensitization is defined as, "increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input." Clinically, central sensitization can only be inferred by the presence of hyperalgesia or allodynia. Hyperalgesia is a psychophysical term defined simply as "increased pain sensitivity" (a painful stimulus feels more painful than 'usual'). Allodynia, which used to be defined as, "pain due to a stimulus which does not normally provoke pain", is now defined specifically as, "pain in response to a non-nociceptive stimulus." The only stimulus which doesn't stimulate nociceptors (with certainty) is tangentially brushing the skin (with a camel hair brush or tissue). This only activates A-beta (touch) fibres that should not normally initiate nociception, except where central sensitization has occurred (where A-beta touch fibres have gained 'access' to the nociceptive system in the dorsal horn). When touch feels painful (like having a hot shower with sunburn), this is evidence that central sensitization has developed and is always associated with 'pathological' pain states. In other words, allodynia is the clinical sign for central sensitization. Hyperpathia is defined as a "painful syndrome characterized by an abnormally painful reaction to a stimulus, especially a repetitive stimulus (such 'poking' a painful region repetitively with a toothpick, at 3 Hz for 30 seconds) as well as an increased threshold." It may occur with allodynia, hyperesthesia, hyperalgesia, or dysesthesia and reflects the phenomenon of 'wind-up'. Wind-up is a specific experimental and clinical paradigm which demonstrates increased pain sensitivity with repetitive stimulation, usually over seconds-to-minutes; an amplifier effect. Wind-up is not the same as central sensitization and the terms should not be used interchangeably. Long-term potentiation is a nociceptive 'memory' or 'capacitor' effect (persisting output from nociceptive neurons in the CNS, in the absence of an afferent input) and is similar to the processes of laying down memory in the hippocampus. Processes of descending neuromodulation that inhibit or 'dampen down' (ascending) nociceptive traffic are collectively termed Diffuse Noxious Inhibitory Control (DNIC) [12].

Classification and taxonomy of pain

Functional classification

Physiological pain

'Adaptive pain' with a clearly protective (alarm) function, usually 'acute' and short-lived.

Pathological pain

'Maladaptive pain' with no beneficial role, usually (but not always) persistent or chronic, associated with hyperalgesia and often neuropathic in etiology [13].

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Etiological, Pathophysiological & Anatomical Classification

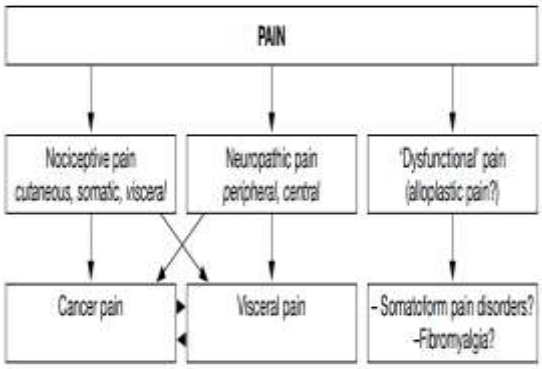

Figure 1.2. Classification of pain.

Nociceptive pain

Is "pain due to activation of nociceptors" in cutaneous, somatic or visceral structures and is the 'tissue injury pain' of the classical, physiological alarm system and is therefore usually 'adaptive' **(Figure 1.2)**.

Neuropathic pain

Is "pain arising as a direct consequence of a lesion or disease affecting the somatosensory nervous system" either in the periphery (e.g. painful diabetic neuropathy) or in the CNS (central pain) (e.g. post-stroke, MS or spinal cord injury). The definition was modified in 2008 to remove the term 'dysfunction' (of the nervous system) which was thought to be too broad and non-specific. Disorders such as fibromyalgia, with evidence of dysfunction in certain nervous system processes, were sometimes classified as neuropathic pain. Neuropathic pain is usually maladaptive, although one may consider that acute radicular leg pain due to a lumbar disc protrusion might force an individual to rest and therefore help to limit further 'damage'.

Dysfunctional pain

Although not listed in the taxonomy, this term was suggested to classify pain that is neither nociceptive nor neuropathic in aetiology, with fibromyalgia as an example. Other terms including 'idiopathic' (unexplained) pain and perhaps (somatoform) pain disorders may fall under this category. The term alloplastic pain has been proposed as an alternative.

Cancer Pain

Is pain associated with a neoplastic process or its treatment (eg radiotherapy) which pathologically-speaking, may be nociceptive and/or neuropathic in nature.

Cutaneous Pain

Is pain associated with activation of nociceptors of the skin. Cutaneous pain is 'sharp', fast, well-localized and transmitted via (in evolutionary terms) neo-nociceptive pathways (eg spinothalamic tract) to the cortex. It is a fast, reactive system that responds to external (environmental) tissue threat and is of great survival benefit.

Visceral pain

Is pain associated with activation of nociceptors (kidney stones) or neuropathy (porphyria) in visceral organs. Visceral pain is usually poorly defined and localized (referred), often 'dull', 'aching' and diffuse and associated with considerable autonomic and emotional activation.

Somatic pain

Is pain associated with activation of nociceptors in muscle, tendon, ligament, bone or 'lining tissues' such as the peritoneum. The qualities of somatic (e.g. musculoskeletal) pain seem to share features of both cutaneous and visceral pain, which might reflect embryology (mesoderm) and function, in evolutionary terms [14].

Temporal classification

Acute pain

There is no IASP definition for acute pain, which has been defined as, "pain of recent onset and probable limited duration; it usually has an identifiable temporal and causal relationship to injury or disease."

Chronic (persistent) pain

Although quite remarkably, there is no IASP definition of 'chronic pain', it is commonly defined as, "pain lasting greater than 3 or 6 months duration, or pain that persists past the normal time of (tissue) healing. The latter definition does not reflect situations such as chronic inflammatory arthropathy (rheumatoid arthritis), neuropathic pain or hyperalgesia. Temporal definitions of pain are relatively artificial, with acute pain commonly considered as 'adaptive' or 'physiological' and associated with a proximate cause, and chronic

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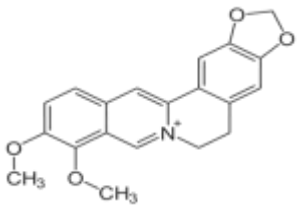
pain as 'maladaptive' often without a clear perpetuating pathology. There is considerable overlap between these terms and they likely exist on a temporal and patho-physiological continuum [15].

Disease-based classifications

ICD classifies pain purely as a symptom of various diseases states in organ systems. Where pain is not referable to an organ system, region or disease, it is defined as 'pain not elsewhere classified' which in turn may be acute, chronic, intractable or 'pain not otherwise unspecified'. The IASP has a coded 5 axis taxonomy for describing chronic pain disorders, based on body region, organ system, temporal characteristics, intensity and etiology.

Berberine



Berberine is an isoquinoline alkaloid that is present in various popular medicinal plants, most notably Hydrastis canadensis, Berberis vulgaris, Berberis aquifolium and Coptis chinensis. It has been used in different cuisines and as a dye because of its deep yellow and yellow florescent colour. Moreover, Berberine has shown various beneficial medicinal properties including anti-tumour, anti-hypertensive, anti-hyperlipidemic, cardioprotective, neuroprotective, anti-arthritic, antiinflammatory and anti-oxidant effects that could help in the treatment of diabetes, obesity and inflammation. Most of such pharmacological effects of Berberine were attributed to its anti-oxidant and antiinflammatory properties, as well as its modulatory effects on a spectrum of enzymes, receptors and cell signalling pathways. Clinically, the effects on Berberine - in combination with standard treatments - against Helicobacter pylori were assessed, and a meta-analysis showed that the beneficial effects of Berberine in this regard were accompanied by lower adverse effects. Also, Berberine in patients with irritable bowel syndrome was well-tolerated and it could reduce symptoms and improve quality of life. Randomized clinical trials on BER effects against hyperlipidemia/dyslipidemias are also reported [16].

PLANT PROFILE

Chelidonium majus

Genus: Chelidonium
Species: majus
Family: Papaveraceae
Synonyms: Chelidonium cavaleriei, Chelidonium dahuricum
Common name: Greater celandine
Biological Source: It is a whole plant belonging to Chelidonium majus L. (Papaveraceae).
Geographical Source: It is also found in North Africa in Macaronesia, Algeria, and Morocco. In Western Asia, it is found in the Caucasus, Armenia, Azerbaijan, Georgia, Kazakhstan, Mongolia, Siberia, and Turkey.
Chemical constituents: Extracts of Chelidonium has been found to contain three types of benzyl isoquinoline alkaloids viz. protoberberine, protopine, benzophenanthredine. Sanuinarine and chelerythrine are the prominent compounds obtained from roots while coptisine, chelidonine and berberine are obtained from the aerial parts. Other constituents include malic, citric, gentisic, and hydrobenzoic acids. It also contains hydroxycinnamic acid derivatives, sparteine, saponin, carotenoids, chelidocystatin and flavonoids.

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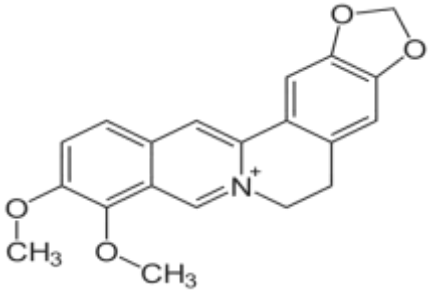

Berberine

Traditional Uses: In many European, Asian and African countries C. majus latex was used for bile and liver disorders, for treatment of warts, corns, eczema and solid tumors. It has traditionally being used to treat liver diseases, gastric ulcer, tuberculosis, skin eruptions and oral infections. In Chinese traditional medicine and in homeopathy C. majus is used to treat blockage of blood circulation, to relieve pain edema and jaundice.

Modern Uses: Hepatoprotective, Antimicrobial, Antiviral, Antiparasitic, Cardiovascular, Anti-inflammatory, Analgesic, Immunomodulatory, Choleretic, Anti-cancer, Cytotoxic, Reproductive systems, Antihyperglycemic, Hypoglycemic, Central Nervous System, Dysentry, Gastroenteritis, Periodontal, and Radioprotective effects [26].

Scientific Classification
Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Order: Ranunculales
Family: Papaveraceae
Tribe: Chelidonieae
Genus: Chelidonium
Species: majus

II. MATERIALS AND METHODS

Chemicals

The reagents, consumables, solvents, and chemicals for this study were purchased via a local distributor from HiMedia® India Pvt. Ltd., Mumbai. Double distilled water was obtained through Borosil® system.

Extract

S. A. Herbal Bioactive Ltd., Mumbai, Maharashtra provided standardized Chelidonium majus (containing raw 2.5% berberine) extract.

Berberine

Berberine HCl was commercially procured from Sigma Aldrich Ltd., Bangalore.

Animals

For the experiment, Swiss albino mice of either sex, 6-7 weeks of age, weighing between 25g and 30 g, were purchased. Throughout the experiment, animals stayed under standard environmental conditions (temperature: 27.0 ± 1.0°C, relative humidity: 55–65%, and 12 h light/12 h dark cycle) with one-week adaptation before experiment. They were housed in cages made of polypropylene and had free access to feed and water ad libitum. All protocols for animal experiment were approved by the Institutional Animal Ethical Committee.

Acute Toxicity Study

When a substance is exposed to several times in a short period of time, it is considered acute toxicity. The half-lethal dosage (LD50) of the experimental samples was determined in accordance with the recommendations of the Organization for Economic Cooperation and Development (OECD). Mice were divided into two groups: a control group and a test group, each with six animals, for this experiment. The experimental samples were delivered orally at different doses (100 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, and 4000 mg/kg body weight). Each group of animals was monitored for the next 5-6 hours for any indicators of toxicity such as death, diarrhoea, noise in the breathing, salivation, convulsions, damage, changes in locomotion, weakening and discharge from the eyes and ears. At the conclusion of each hour, these metrics were also checked. In addition, for the final evaluation, each group of animals was monitored for a period of two weeks [27].

Analgesic activity

Formalin-Induced Paw Licking Test

According to Owoyele et al., a formalin-induced paw licking test was conducted. 24 mice were chosen for this experiment and split into four groups of five each, with water available ad libitum for 16 hrs. Test groups received distilled water (10 mL/kg), diclofenac sodium (100 mg/kg), berberine at 200 mg/kg and 400 mg/kg, respectively, as a control group and a standard group. Oral gavage was used at every step of the procedure. Each mouse was injected with 20 L of formalin solution into the dorsal surface of the left hind paw one hour

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after treatment. It was deemed an acute phase when animals were examined for 5 mins following injection. A late phase was determined as 20 mins after injection, therefore they were re-monitored for 5 mins after that. The following formula was used to determine the percentage of inhibition of licking **(Figure 4.1)** [28].

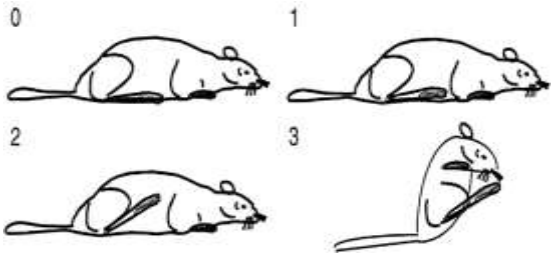

Figure 4.1. Illustration of Formalin-Induced Paw Licking Test.

cetic Acid Induced Writhing Test

According to Prabhu et al., this test was carried out. Previously, mice were pretreated with extracts and left unfed for 16 hours before the trial. The positive control was DS (100 mg/kg), whereas the normal control was distilled water. We used 10 mL/kg body weight for each mouse, and after 45 minutes of therapy, we gave them an injection of 0.7% (v/v) acetic acid intraperitoneally. After 15 mins of acetic acid treatment, each animal's writhing reactions were counted for a 5-minute interval **(Figure 4.2)**. The proportion of inhibition of writhing was calculated using the following formula [29].

Figure 4.2. Illustration of Acetic Acid Induced Writhing Test.

Tail Immersion Test

According to Adeyemi et al., this experiment was carried out. In this experiment, the central mechanism of pain or analgesia activity may be studied. Dipping the tail tip into 55 to 1°C hot water activates the thermal stimuli that cause a painful response. Mice were housed in groups of six and given the same care as detailed before. As a standard, we selected 10 mg/kg of tramadol hydrochloride as a reference medication. After one hour of treatment, the basal reaction time of each mouse was measured. After 30 minutes, 60 minutes, 90 minutes, and 120 minutes of treatment, the latency time was counted. In addition, the latency duration of each group was evaluated before 30 minutes of therapy. Experimentation was halted when the animal had a latency time of more than 15 seconds, which serves as a cut-off point **(Figure 4.3)** [30].

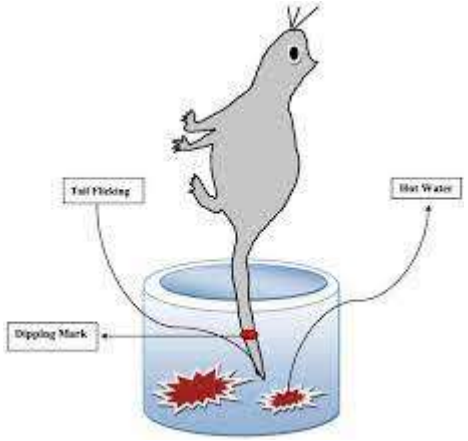

Figure 4.3. Illustration of Tail Immersion Test.

Statistical analysis

All data are given as the mean minus the standard deviation of the mean ± SEM. One-way ANOVA and Dunnett's-test were used to assess all of the data. An ANOVA with post hoc Tukey's HSD test was used to compare mean values across groups. Repeated measure ANOVA was used to examine the tail immersion test findings (RM-ANOVA). The significance level was considered significant at $P < 0.05$. SPSS software v.17 (IBM Corporation, New York, USA) was employed for study.

III. RESULTS AND DISCUSSION

Acute toxicity study

The acute toxicity investigation found no signs of toxicity or death up to the high dosage of Berberine or the control group. During the two-week monitoring period, there was no change in food consumption or other behaviors. There was no evidence of acute oral toxicity in the test groups.

Evaluation of analgesic activity

Formalin-Induced Paw Licking Test

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Table 5.1 shows the results of a Berberine paw licking test in mice that was induced with formalin. Licking was decreased in the early and late phases of the biphasic pain response, which were classified into neurogenic and inflammatory responses, respectively, by oral administration of 200 mg/kg and 400 mg/kg. Late phase ($P < 0.05$ vs. control) and acute phase ($P < 0.01$) inhibition were seen in all samples.

Table 5.1. Effect of berberine in formalin-induced paw licking test.

Group	Dose	Acute phase		Late phase	
		Licking time (s)	Inhibition (%)	Licking time (s)	Inhibition (%)
Control	10 mL/kg	73.39 ± 3.47	0.00 ± 0.00	48.67 ± 2.44	0.00 ± 0.00
Standard	100 mg/kg	47.55 ± 2.23	20.38 ± 2.29	11.17 ± 3.61	70.03 ± 2.64
Berberine	200 mg/kg	62.47 ± 2.65	15.78 ± 4.11	20.59 ± 3.16	36.62 ± 3.18
	400 mg/kg	52.14 ± 2.88	14.04 ± 2.93	12.42 ± 3.59	51.64 ± 3.33

Acetic Acid Induced Writhing Test

Table 5.2 summarizes the results of the acetic acid induced writhing test. As a positive control, 100 mg/kg of diclofenac was administered to rats, and an inhibition of acetic acid-induced writhing of 77.57% was observed. When taken orally, the test samples showed significant variations in the frequency of writhing and reduced pain perception. Tested sample demonstrated substantial writhing inhibition ($P < 0.05$ against control) at 400 mg/kg dose, with the maximum percentage of inhibition at this dosage level (46.75%).

Table 5.2. Effect of berberine in acetic acid induced writhing test.

Group	Dose	Writhing number	Inhibition (%)
Control	10 mL/kg	11.66 ± 2.82	0.00 ± 0.00
Standard	100 mg/kg	1.58 ± 0.94	77.57 ± 4.82
Berberine	200 mg/kg	11.72 ± 2.44	33.17 ± 7.29
	400 mg/kg	9.15 ± 2.51	46.75 ± 8.66

Tail Immersion Test

Table 5.3 shows the findings of Berberine's analgesic activity as assessed by tail immersion. A substantial delay ($P < 0.05$ against control) was seen at 30 minutes with 400 mg/kg, while no latency was observed with any of the other samples. It was shown that diclofenac (100 mg/kg) was efficacious and significant at 30 minutes, 60 minutes and 120 minutes ($P < 0.05$ against control).

Table 5.3. Analgesic effect of berberine in tail immersion test.

Group	Dose	Latency period (s)				
		0 min	30 min	60 min	90 min	120 min
Control	10 mL/kg	1.468 ± 0.34	1.42 ± 0.33	1.41 ± 0.89	1.74 ± 0.22	1.20 ± 0.29
Standard	100 mg/kg	1.702 ± 0.31	6.96 ± 0.41	6.68 ± 0.28	4.48 ± 0.33	4.07 ± 0.78
Berberine	200 mg/kg	1.518 ± 0.26	2.87 ± 0.63	3.74 ± 0.52	2.61 ± 0.82	2.06 ± 0.37
	400 mg/kg	1.654 ± 0.38	4.47 ± 0.55	3.75 ± 0.14	2.17 ± 0.21	2.64 ± 0.44

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IV. CONCLUSION

From the present study, it could be proposed that berberine demonstrated potent analgesic activity. Berberine reduced the number of abdominal writhing's significantly when compared to the reference drug (diclofenac). Therefore, it is possible to obtain analgesic agent from the plant source and serve as an alternative bio-resource in managing pain, rather than dependence over the marketed synthetic products. However, further quantifiable studies are now essential to categorize the particular mechanism which is responsible for the analgesic activity of berberine Therefore, it is possible to obtain analgesic agent from the plant source and serve as an alternative bio-resource in managing pain, rather than dependence over the marketed synthetic products. However, further quantifiable studies are now essential to categorize the particular mechanism which is responsible for the analgesic activity of berberine. At last but not least, to be a safe therapeutic agent, not only acute oral toxicological evaluation but also genotoxicity study of this plant should be conducted in future.

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