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Evaluation of cardio protective, anti-inflammatory, analgesic and CNS depressant activity of *Grewia asiatica* L. fruit extract

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Abstract: *Grewia asiatica* L. is a potential medicinal plant used for various diseases in traditional medicine. Current study was aimed to evaluate the cardio protective, anti-inflammatory, analgesic and CNS depressant activities of *Grewia asiatica* L. fruit extract. In cardio protective activity myocardial injury was produced by injection of Isoproterenol (200 mg/kg, s.c), *G. asiatica* 250 and 500mg/kg treated groups significantly ($p<0.05$) decreased the level of serum AST, ALT, LDH and CKMB, hence produced cardio protective effect. In analgesic activities *G. asiatica* produced significant ($p<0.05$) analgesic effects in acetic acid induced writhing, formalin, paw pressure and tail immersion test. *G. asiatica* at 250 and 500mg/kg oral dose, significantly ($p<0.05$) reduced the rat paw edema in carrageen an induced rat paw edema test. *G. asiatica* extract also produced significant CNS depressant effects in open field, hole board and thiopental sodium induced sleeping time. Findings of the current study suggest that *G. asiatica* fruit extract showed potential pharmacological effects and can be utilized in alternative medicine.

Keywords: *Grewia asiatica*, Cardio protective, Anti-inflammatory, analgesic.

INTRODUCTION

WHO statistics show that more than 80% of the third world countries rely on herbal medicines, obtained from medicinal plants as a means of remedy for various ailments (Vaou *et al.,* 2021). Traditional herbal medicine also known as folk medicine serves as an important means for the development and discovery of valuable new medicines against bacterial, fungal and viral diseases to decrease the occurrence of resistance and adverse drug reactions (Nigussie *et al.,* 2021). Plant based remedies also make up a vital component of an healthcare system which inter and multicultural comprising traditional as biomedical health approaches, in minority and underserved communities (Chassagne *et al.,* 2021).

Many rural communities in Pakistan solely rely on plant based medicines and this is due to the inaccessibility of primary health care services, very high prices of medicines and other health related services such as diagnostic measures (Shaikh *et al.,* 2005). Due to the side effects of chemical molecules and synthetic drugs, dependence on herbal products is increasing day by day and new drugs are being introduced in the market over a regular course of time. However, there is a constant need of research to investigate the benefits of plants for numerous diseases (Kausar *et al.,* 2022). Pakistan is home to a wide range of higher plants which equals around 7500 species. Out of these 7500 plants, 600-700 have been established as therapeutic plants utilized by homeopath doctors and registered herbalists (Shah *et al.,* 2021). Balochistan is rich in various medicinal plants, which have been investigated and established as remedies for several diseases and disorders (Bibi *et al.,* 2014). The plant based drugs are more safe economical and easily available in local market, therefore the current work aimed to find out a alternative remedy for common disorders. The objective of the current study was carried out to evaluate the cardio protective, anti-inflammatory, analgesic and CNS depressant activity of *G. asiatica* L. Fruit extract.

Grewia asiatica L.

Grewia asiatica L. is a unique plant which grows wild as well as cultivated domestically. It produces an acidic, sour fruit used in South East Asia and its adjacent regions (Moosa *et al.,* 2019). Plant fruit is edible, delicious, and known, locally, as phalsa. The color of the fruit is purplish, and it is small and spherical in shape. From late April until early June, it becomes available in District Multan, Pakistan and considered as gift of Summer. The fruit is excessively used by common folks to make Falsa squash, extract its juice and convert it into carbonated drinks which are famous, all over Pakistan as healthiest

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beverages in the hot months of Summer. (Imran *et al.,* 2021).

Phytochemical evaluation of plant showed that their extract of petroleum ether consists of fats, glycosides and diterpenes; chloroform extract of the plant contains glycosides and alkaloids, whereas ethanolic extract of the plant contains sterols, flavonoids, tanins and saponins (Zia-Ul-Haq *et al.,* 2013). Whole plant is used as a sweet, cooling astringent, appetizer, demulcent, aphrodisiac and antithrust tonic. Fruits, when ripe, are sweet and acidic and have a cooling effect on body, and also used as a digestive (Tripathi *et al.,* 2010). Due to its phytochemical composition, it is used, especially in Asian countries for the treatment and prevention of metabolic syndromes and bodily disorders (Kehinde *et al.,* 2020).

Studies reveals that *G. asiatica* has radio-protective potential and also contains neuro-protective characteristics against the radiation (Ahaskar *et al*, 2007, Tripathi *et al.,* 2010), also have antipyretic (Akhtar *et al.,* 2016) antiemetic (Yaqeen *et al*, 2008) anti-diabetic (Tripathi *et al.,* 2010) and antioxidant activities (Shukla *et al.,* 2016).

MATERIALS AND METHODS

Plant sample

Fruits of the plant were locally purchased from the market of Quetta in June 2021. The fruit of the plant were identified and later on voucher specimen (FOP/DPC/2020/ NA-12) was stored at Herbarium at Department of Pharmacognosy, University of Balochistan. Fruit of the plant was soaked in solvent (methanol) for 15 days. A rotary evaporator (BUCHI, Switzerland**)** was used to obtain the crude extract (Marvi *et al.,* 2016) and solvent was evaporated under reduced pressure and temperature (40°C), Percentage yield was 10.26%. Then a water bath was used to dry the powder and labeled as GSE (*Grewia asiatica* extract), afterwards conserved in a silica filled activated desiccator till ready for use. To reconstitute the extract for use in the experiments, distilled water was used as a vehicle.

Animals

Male Swiss albino mice weighing about 20-25g and Male Wistar rats weighing about 230-250 were purchased from the Dow University, Karachi, animal house. All the experimental animals were kept at animal house of FOPHS, University of Balochistan, Quetta, for 7(seven) days before conducting the experiments. Cages of the size ($34 \times 47 \times 18\ cm^3$) were used to house the animals. The cages were lined with shavings of softwood as bedding for the experimental animals and kept at a 12 hour light-dark cycle (temperature 23±2ºC and humidity 50±5%) while giving them access to food and water. For animal studies approval was granted by Faculty of Pharmacy ethics committee (No: FOPHS/AE/Pharm- 14/2021, dated 16/09/2021).

Acute oral toxicity

Development and Economic Coordination guidelines (Test No. 425) were used to assess the safety of methanolic extract of the plant extract. The rats were divided into three groups of five each. Animals were weighed individually and pointed with a permanent black marker on their tails. To carry out the toxicity test, a single oral dose of 100, 300, 500 and 2000 mg/kg with methanolic extract of *G. asiatica* was administered. Afterwards, various health parameters such as diarrhea. salivation, lethargy, changes in body weight, unconsciousness, hair and skin color were observed (Wangusi *et al.,* 2021).

Cardio protective activity

In this test four 04 groups of rats were utilized (6 rats in each group), normal saline (vehicle, 2ml/kg/day, P.O.) was administered to Group I. Group II was Isoproterenol (200mg/kg, s.c) treated Group. *G. asticia* 250 and 500 mg/kg (P O) was administered to Group II and IV. Vehicle and plant extract was administered daily for 14 days. On 14^{th} and 15^{th} day, Isoproterenol (200 mg/kg, s.c) was administered to group II, III and IV to induce myocardial injury (Thakker *et al.,* 2010).

Biochemical analysis

Blood sample was collected on 16^{th} day, serum was separated for valuation of marker enzymes. The serum level of ALT, AST, LDH (Lactate dehydrogenase), CK (Creatine kinase) were measured by using standard kits (Thakker *et al.,* 2010, Zaijun Zhang *et al.,* 2009)

Analgesic activity

Writhing test

The mice received an injection (Intraperitoneal) of 1% v/v acetic acid solution (10ml/kg) after 30 mins of administration of the drug. Transparent cages were used to house the mice individually. For 20 mints, acid-induced writhes were counted. For counting purpose, a writhe was described by at least one hind limb stretching or simultaneous stretching of the abdomen (Wang *et al.,* 2021).

Formalin test

1 hour before the behavioral testing, mice were accustomed to the environment. Afterwards, the control group (Group I) was given saline 2ml/kg and Group II and Group III were given *G. asiatica* ethanolic extract 250 and 500mg/kg and finally, Group IV was administered diclofenac sodium 50mg/kg. Formalin of 0.3% (20µl) was given as intraplantar injection to the right hind paw of mice. After doing so, the animal behavior was analyzed for at least 45 mins to observed the total duration of each animal involved in licking, lifting, biting or shaking their

hind limb. Evaluation of the phase 1; acute phase, duration, 0-5 min and phase 2; inflammatory phase, duration 10-45 min was conducted (Liu *et al.,* 2021).

Paw edema and carrageenan-induced pain in mice

Carrageenan was administered intra-plantarly to induce an acute inflammatory response in mice right hind paw intra-peritoneal by administering 300µg/80µL in 0.9% saline vehicle 80µL, 30 minutes before experiment *G. asiatica* ethanolic extract 250 and 500mg/kg (Group II and III) were orally administered. Standard drug treated group was treated with Diclofenac sodium at 50mg/kg. At time intervals 0 and 1,2,3,2,5,24 and 48 hours after the treatment pain threshold was measured. The edema and the thickness of the paw was measured with a digital Vernier caliper and expressed in millimeters (Micheli *et al.,* 2021).

Paw pressure test

After pressure exertion, and consequently backing up the foot away after the extorted automated pressure on dorsal surface, degree of hyperplasia was determined by counting the latency in seconds. Mechanical force was exerted by using a calibrated cylindrical glass rod, weighing around 15 g. The weight was freed to move vertically after suspending it amongst 02 rings attach to a stand. 40 seconds was set as cutoff time (Micheli *et al.,* 2020).

Tail immersion test

A aquatic bath sustained at 50.0±1.0°C was used to immerse the tail of the mice, the distal part (2-3 cm). The time (seconds) consumed by mose to remove or deflect tail out of the water, was noted. Cut off time was 10 seconds in order to avoid any injury to the tissue. After 30 minutes of administration of *G. asiatica* (250 and 500 mg kg^{-1}, p.o.) and diclofenac sodium (50mg kg^{-1}, p.o.), latency of the withdrawal was taken. Antinociception was measured by observing the tail withdrawal latency score (Micheli *et al.,* 2020).

CNS depressant activities

Open field test

This test measures the various activities such as, stress, neophilia, anxiety and emotional behavior of animals. This test comprises of a square plateform measuring 45cm*45cm in width and breadth with 50cm walls. The floor of the box was partitioned into nine (9) equal squares which measured (15 cm*15 cm). A 60watt bright lamp was used to light the apparatus. Mice were maintained to kept at a corner. The numbers of squares were counted for 10 minutes. Afterwards, 70% ethanol was used to clean the open-field area (Ahmadu *et al.,* 2022)

Hole board test and exploratory activity in mice

By utilizing a wooden board, measuring 40cm × 40 cm with 16 equally spaced perforations, the exploratory behavior test was conducted. Each mouse observed whether or not dipping his head in the perforations by moving from one corner of the board to the other corner. It was taken a curiosity when mice poked their nostrils into the holes. It was considered as typical behavior of mice. The same method of cleaning of the board was applied which had been applied before i.e. 70% ethanol solution was used to clean the board. Time duration of 30 minutes after diazepam the head dips were recorded, while only for 10 minutes after (standard drug, *G. asiatica* (250 and 500 mg kg^{-1}, P.O.) by observing the mice on the board (Ahmadu *et al.,* 2022).

Sleeping time test by using thiopental sodium

In this test, rats (Swiss albino) were grouped into 04 groups (5 rats in each group). Vehicle (saline 10 ml Kg^{-1} per oral), was given to Group-I. *G. asiatica* 250 and 500 mg/kg P.O. was given to group II and III. The standard drug treated group was given diazepam at a dose of 1 mg/ kg by way of intraperitoneal rout. After 20 minutes, each animal was given TPS or Thiopental Sodium at the dose of 40 mg/kg body weight by way of peritoneal rout of administration. After the administration of TPS, sleep duration of the animals were observed (time between the loss and recovery of righting reflex) and latent period which is the time between the drug administration to loss of righting reflex (Hossain *et al.,* 2016).

STATISTICAL ANALYSIS

Data were evaluated by utilizing ANOVA (one-way analysis of variance) and Dunnett's multiple comparison test by using SPSS software (Version 22). P-value < 0.05 considered significant and p<0.001 considered highly significant (Khan *et al.,* 2022)

RESULTS

Acute toxicity test

G. asiatica crude extract at the dose of 100, 300, 500 and 2000 mg/kg did not produced any mortality or significant toxicity (table1).

Table 1: Effect of *G. asiatica* fruit extract in acute oral toxicity on mice

Treatment and Dose	Mortality %
G. asiatica 100mg/kg	Nil
G. asiatica 300mg/kg	Nil
G. asiatica 1000mg/kg	Nil
G. asiatica 2000mg/kg	Nil

Cardio protective activity

AST

The concentration of AST in control group was 53.22±0.37, for *G. asiatica* ISO treated group the

Table 2: Effect of *G. asiatica* fruit extract in cardio protective activity on rats

Group	Treatment and Dose	AST	ALT	LDH	CK
Group I	Control (saline) 5ml/kg	53.22±0.37	73.22±0.38	954.8±1.24	345.22± 0.38
Group II	Isoproterenol 200 mg/kg, s.c	107.51±0.40 [a]	141.50±0.84 [a]	1765.6±0.68 [a]	713.19±0.97 [a]
Group II	*ISO+G. asiatica* 500mg/kg	90.40±0.38 [b]	79.10±0.32 [b]	1054.65±1.15 [b]	411.31±0.16 [b]
Group III	*ISO+G. asiatica* 500mg/kg	71.37±0.23 [b]	61.94±0.40 [b]	997.26±0.58 [b]	408.15±1.01 [b]

Results represent Mean +SEM, n=5, [a]= p<0.05 when compared with control, [b]=p<0.05 when compared with ISO treated group

Table 3: Effect of *G. asiatica* fruit extract in acetic acid induced writhing test on mice

Group	Treatment and Dose	Number of writhes (Mean±SEM)
Group I	Control 5ml/kg	76.98±0.29
Group II	*G. asiatica* 250mg/kg	68.06±0.16*
Group III	*G. asiatica* 500mg/kg	45.69±0.38*
Group IV	Diclofenac Sodium 50mg/kg	38.38±0.15**

Table 4: Effect of *G. asiatica* fruit extract on pain in the formalin test

Group	Treatment and Dose	First Phase (Mean number of licking and biting)	Second Phase (Mean number of licking and biting)
Group I	Control 5ml/kg	68.8±0.27	85.87±0.32
Group II	*G. asiatica* 250mg/kg	57.92±0.24*	71.82±0.13*
Group III	*G. asiatica* 250mg/kg	48.07±0.37*	67.43±0.17*
Group IV	Diclofenac	41.43±0.45**	53.10±0.26**

Table 5: Effect of *G. asiatica* fruit extract in carrageen an induced rat paw edema

Group	Treatment and Dose	0 hour	1 hour	2 hour	3 hour	4 hour	5 hour	24 hour	48 hour
Group I	Control 5ml/kg	7.148 ±0.14	11.31 ±0.10	12.44 ±0.14	13.05 ±0.16	13.344 ±0.25	16.33 ±0.22	13.396 ±0.15	12.172 ±0.06
Group II	*G. asiatica* 250mg/kg	8.21 ±0.06	10.48 ±0.15*	11.582 ±0.18*	12.15 ±0.16*	11.55 ±0.16*	12.37 ±0.21*	11.236 ±0.14*	10.74 ±0.21*
Group III	*G. asiatica* 500mg/kg	8.248 ±0.07	10.96 ±0.26*	11.434 ±0.12*	12.6 ±0.14*	12.25 ±0.10*	12.6 ±0.14*	11.396 ±0.30*	10.16 ±0.19*
Group IV	Diclofenac sodium 50mg/kg	8.28 ±0.17	9.66 ±0.14**	10.42 ±0.13**	11.17 ±0.04**	11.42 ±0.19**	12.24 ±0.31**	11.38 ±0.32**	10.58 ±0.19**

concentration of AST was 107.51±0.40 in *G. asiatica* crude extract 250 mg/kg treated group the concentration AST was 90.40±0.38 and 500mg/kg concentration of AST was 71.37±0.23 (table 2).

ALT

The concentration of ALT in control group was 73.22±0.38, for ISO treated group concentration was 141.50±0.84 in *G. asiatica* crude extract 250 mg/kg treated group the ALT concentration were 79.10±0.32 and *G. asiatica* 500mg crude extract treated group of ALT concentration was 61.94±0.40 (table 2).

LDH

The concentration of LDH in control group was 954.8±1.24, for ISO treated group concentration was 1765.6±0.68 in *G. asiatica* crude extract 250 mg/kg treated group the LDH concentration was 1054.65±1.15 and 500mg/kg concentration of LDH was 997.26±0.58 (table 2).

CK

The concentration of CK in control group was 345.22± 0.38, for ISO treated group concentration was CK concentration was 713.19±0.97 in *G. asiatica* crude extract 250 mg/kg treated group the CK concentration was 411.31±0.16 and *G. asiatica* crude extract 500mg/kg concentration of CK activities were 408.15±1.01(table 2).

Analgesic activities

Acetic acid induced writhing test

For the control group the number of the writhes were 76.98±0.29, for *G. asiatica* extract 250mg /kg treated group sum of writhes were 68.06±0.16, in *G. asiatica* extract 500mg/kg treated group the sum of writhes were 45.69±0.38 and for Diclofenac 50mg/kg treated group number of writhes ware 38.38±0.15 (table 3).

Formalin test

For the control group the number of licking and biting were 68.8±0.27 in first phase and 85.87±0.32 were in 2nd

Table 6: Effect of *G. asiatica* fruit extract in paw pressure test

Group	Treatment and Dose	Withdrawal latency (Seconds)
Group I	Control 5ml/kg	37.90±0.34
Group II	*G. asiatica* 250mg/kg	29.39±0.04*
Group III	*G. asiatica* 500mg/kg	21.39±0.17*
Group IV	Diclofenac sodium 50mg/kg	14.99±0.22**

Table 7: Effect of *G. asiatica* fruit extract in tail immersion

Group	Treatment and Dose	Mean time (seconds) (Mean±SEM)
Group I	Control 5ml/kg	8.39±0.05
Group II	*G. asiatica* 250mg/kg	7.99±0.29*
Group III	*G. asiatica* 500mg/kg	7.528±0.21*
Group IV	Diclofenac sodium 50mg/kg	5.19±0.14**

Table 8: Effect of *G. asiatica* fruit extract in open field activity

Group	Treatment and Dose	Mean number of open field activities (Mean±SEM)
Group I	Control 5ml/kg	96.01±1.50
Group II	*G. asiatica* 250mg/kg	77.53±1.80*
Group III	*G. asiatica* 500mg/kg	63.84±1.50*
Group IV	Diazepam 2mg/kg	45.7±1.14**

Results represent Mean +SEM, n=5, *= $p<0.05$, **$p<0.01$

Table 9: Effect of *G. asiatica* fruit extract in hole board test

Group	Treatment and Dose	Mean Number hole crossed (Mean±SEM)
Group I	Control 5ml/kg	51.74±0.67
Group II	*G. asiatica* 250mg/kg	41.39±1.12*
Group III	*G. asiatica* 500mg/kg	33.60±0.69*
Group IV	Diazepam 2mg/kg	26.91±1.10**

Table 10: Effect of *G. asiatica* fruit extract in thiopental induced sleeping time test on mice

Group	Treatment and Dose	Onset of sleep	Duration of sleep
Group I	Control 5ml/kg	45.84±1.46	70.95±1.00
Group II	*G. asiatica* 250mg/kg	32.264±0.85*	137.27±1.22*
Group III	*G. asiatica* 500mg/kg	27.262±1.00*	144.92±2.22*
Group IV	Diazepam 2mg/kg	18.99±1.01**	161.25±2.49**

Results represent Mean +SEM, n=5, *= $p<0.05$, **$p<0.01$

phase, for *G. asiatica* crude extract 250 mg /kg number of licking and biting were 57.92±0.24 in first phase and, second phase 71.82±0.13, in *G. asiatica* crude extract 500 mg/kg number of licking and biting were 48.07±0.37 in first phase and in second phase 67.43±0.17 and in diclofenic 2mg/kg treated group number of licking and biting were 41.43±0.45 and in second phase 53.10±0.26 (table 4).

Paw Edema and Carrageenan-Induced Pain in Mice

In this test *G. asiatica* crude extract 250 and 500mg produced significant ($p<0.05$) decrease of Paw Edema as compared with control and standard drug (table 5).

Paw pressure

For the control group the Paw pressure was 37.90±0.34, for *G. asiatica* crude extract 250 mg /kg treated group the Paw pressure was 29.39±0.04, in *G. asiatica* crude extract 500 mg/kg treated group Paw pressure was 21.39±0.17 and for Diclofenac 50mg/kg treated group mean Paw pressure was activities were 14.99±0.22 (table 6).

Tail flick

For the control group the tail flick time 8.39±0.05, for *G. asiatica* crude extract 250 mg /kg treated group tail flick time 7.99±0.29, in *G. asiatica* crude extract 500 mg/kg treated group the tail flick time was 7.528±0.21 and Diazepam 2mg/kg number of tail flick time was 5.19±0.14 (table 7).

CNS depressant activities

Open field

The open field activities were 96.01±1.50 for the control (non treated) group and for *G. asiatica* crude extract 250

mg /kg treated group were 77.53±1.80, in *G. asiatica* crude extract 500 mg/kg treated group the open field activities were 63.84±1.50 and Diazepam 2mg/kg number of open field activities were 45.7±1.14 (table 8).

Hole board

The number of activities were 51.74±0.67 for the control group and *G. asiatica* crude extract 250 mg /kg treated group the number of activities were 41.39±1.12, in *G. asiatica* crude extract 500 mg/kg treated group the number of activities were 33.60±0.69 and for Diazepam, 2mg/kg treated group, number of activities were 26.91±1.10 (table 9).

Sleeping time

For the control group the onset of sleeping was 45.84±1.46 and duration was 70.95±1.00 for *G. asiatica* crude extract 250mg/kg treated group the onset of sleeping was 32.26±0.85 and duration was 137.27±1.22, in *G. asiatica* crude extract 500 mg/kg treated group onset sleeping was 27.262±1.00 duration was 144.92±2.22 and for Diazepam 2mg/kg onset of sleeping was 18.99±1.01 and duration was 161.25±2.49 (table 10).

DISCUSSION

Over the past several years, the interest in medicinal plants has increased greatly and this is due to their disease curing and preventing potential. It is also equally important that the discovery of therapeutically significant substances should be supported by the Ethnopharmacological information of the plants from which these have been obtained (Tewari *et al.,* 2021). As for as medicinal importance of the plants is concerned, *G. asiatica* is an important plant of Pakistan in terms of its medical significance. We have performed tests to analyze the antidepressant, Cardio protective and anti-inflammatory activities and also the acute toxicity of *G. asiatica* ethanolic extract. It was observed that treatment with doses as greater as 2000 mg/kg , the methanolic extract of *G. asiatica* was found very safe and no mortality was observed. It was seen that *G. asiatica* contained all the essential elements such as carbohydrates, fatty acids, and proteins. Other active metabolites such as, steroids, tannins, flavonoids, triterpenoids, alkaloids, lactones, lignans, and flavones were also found in abundance in all plant parts (Paul *et al.,* 2015). Several studies have confirmed that fruit extract of *G. asiatica* contains constituents with analgesic effects (Akhtar *et al.,* 2016).

Findings of current study shows that administration of Isoproterenol (200 mg/kg, s.c) significantly increased the levels of ALT, AST, ALP, CK and LDH). Outcomes of current investigation were in agreement with previous work (Ayza *et al.,* 2020). Increase level of these enzymes in serum were related with damage of heart that results in heart failure, myocarditis and myocardial infarction (Sudharsan *et al.,* 2005). Studies show that *G. asiatica* contains phytoconstituents such as glycosides, flavonoids, anthraquinones, saponins alkloids and tannins (Zia-Ul-Haq, *et al.,* 2013). Therefore the cardioprotective activity of *G. asiatica* methanolic extract might be attributed to the these phytoconstituents found in the plant.

G. asiatica showed marked pain relieving effects in tail immersion, acetic acid induced pain method, and formalin test. Pain results from acute inflammatory processes which stimulate sensory neurons through mediators related to inflammation such as, prostacycline, prostaglandine, histamine, thromboxane, prostaglandins, substance P, bradykinins, and cytokines released after cell injury. This pain signal is then amplified and transmitted leading to clinical pain states and central sensitization (Kumatia *et al.,* 2021). The constriction of abdominal muscles, extension of the forelimbs, and elongation of the body occurs due to release of several endogenous inflammatory mediators which induce visceral pain. The gold standard for measurement of visceral pain is writhing test which is induced by acetic acid (Yimer *et al.,* 2020). Analgesic activity depends on the interruption of neuropeptides, inflammatory mediators, and or neurotransmitters used in the perception and transmission of pain, and Non-Steroidal Anti-Inflammatory (NSAIDS) inhibit various chemicals to achieve this goal. This includes, inhibition of mediators such as cyclooxygenase (COX) and therefore reduced release of prostaglandins (PGs) (Kumatia *et al.,* 2021, Tewari *et al.,* 2021).

It was revealed by tail immersion and formalin test that the plant extract possesses analgesic activity on both peripheral and central pain pathways. According to our study, it was found that the plant extract demonstrated, at different doses, pain suppression, and it was happened by modification of formaldehyde effect in both phases. Likewise, anti-inflamatory and analgesic activities were also reported (Qamar *et al.,* 2020, Akhtar *et al.,* 2016). The presence of plant constituents such as phenols and flavonoids may be deemed responsible for the anti-inflammatory and analgesic activities.

Various established models, such as hole board test, open field test and force swimming test were utilized to assess the CNS depressant activity of methanolic fruit extract of the *G. asiatica* on mice (Okokon *et al.,* 2018). The depressant activity of the mice was measured by the ability of the mice crossing fewer square blocks and holes in 30 mins while treated with the *G. asiatica* methanolic extract. It was considered as a lack of curiosity to the new environment and locomotion when in both tests, open field test and hole cross test the extract reduced activities (Moniruzzaman *et al.,* 2015, Islam *et al.,* 2015). All these results showed that the extract of *G. asiatica* reduced the activity in mice. The reduction in the level of these open

field and hole board test suggests the extract could depress the CNS and this could be related to a sedative effect (Sofidiya *et al.,* 2022).

Thiopental sodium, which is a barbiturate, is used to induce sleep in rodents and humans. Sedative and Hypnotic drug action was investigated in mice by sleeping time test induced by thiopental sodium (Hossain *et al.,* 2016). After oral administration of *G.asiatica* extract, same effects were seen with diazepam 20 min prior to the thiopental sodium sleep induction. It has been found that thiopental sodium induces hyperpolarization in postsynaptic neurons in CNS at the GABA receptor site by binding and stimulating these receptors (Hossain *et al.*, 2016). Diazepam, which is basically a benzodiazepine, is used as an anxiolytic and sleep inducer. It is a CNS depressant and used in treatment of insomnia. Benzodiazepines help Chloride ions to enter the cell and cause hyperpolarization of the cell which result in sleep hypnosis and sleep induction (Moniruzzaman *et al.*, 2015). These drugs reduce excitement of the CNS neurons and consequently, calm the recipient. Diazepam was used as a reference drug in our study. It was observed that diazepam induces barbiturate like sleep (Hossain *et al.*, 2016). Our results suggests that the depressent effect of *G. asiatica* were comparable to diazepam.

G. asiatica contains lactones, triterpenoids, flavonoids, lignans, phenols, and flavones (Paul *et al.,* 2015) therefore it is suggested that the CNS depressant activity may be due the presence of these compounds.

CONCLUSION

Beside uses as nutritional plant *G. asiatica* can also utilized as herbal remedy for numerous diseases. In present study *G. asiatica* fruit extract showed significant, cardio protective, anti-inflammatory, analgesic and CNS depressant activities. Further studies are needed on large number of animals with toxicological profile.

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