

ANALGESIC ACTIVITY OF *SOLENOSTEMMA ARGEL* BY MODULATING PAIN NOCICEPTION PATHWAY IN MICE

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ABSTRACT

The present study aims to investigate the centrally and peripherally acting analgesic activity of the aqueous extract of *Solenostemma argel* (AESA) in mice by using Eddy's Hot plate and acetic acid induced writhing model respectively. The extract was administered per orally at dose of 10 ml/kg and 20 ml/kg while diclofenac sodium was used as standard for both the models. In hot-plate model, the AESA exhibited significant analgesic activity by increasing the reaction time compared to distilled water treated group. Diclofenac Sodium considered as a mild analgesic and showed analgesic effect but not significantly in this study. In comparison with control group 20 ml/kg AESA has shown most significant ($P < 0.05$) anti-nociception effect during 60 min and 90 min observation, while 10 ml/kg AESA showed significant effect only

at 90 minutes. The AESA exhibited significant analgesic activity at both the doses against acetic acid induced writhing model ($P < 0.05$) by reducing the number of writhes, the percent inhibition was 43.27% & 21.04% respectively. The reference drug diclofenac sodium was found more potent (44.71%) than both the dose levels of plant extracts. Surprisingly 10 ml/kg dose of the plant extract has shown better protection than 20 ml/kg. The administration of acetic acid intraperitoneally activates both peripheral and central mechanisms of pain because of nociceptive mediators such as prostaglandins (PG) E_2 and I_2 at the site of noxious stimulation. The major constituents of the *Solenostemma argel* are tannins and saponines

which comprises up to 60% of its total content may be responsible for its analgesic activity. In conclusion, the aqueous extract of *Solenostemma argel* displayed analgesic activity.

KEYWORDS: *Solenostemma argel*, nociception, hot plate, acetic acid, phytochemistry.

INTRODUCTION

Pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage”.^[1] Pain is also known as protective response indicates body's uneasiness and disturbed physiology so in recent decades there is huge market for the analgesic drugs. Varieties of analgesic drugs are used to subside acute and chronic pains which are acting centrally and/or peripherally. Inmost instances, these analgesic drugs, particularly opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) can only relieve 50% of the pain in about 30% of patients.^[2] In addition, many of these drugs cause serious unwanted effects. Data suggests that opiates cause physical dependency, tolerance and addiction while NSAIDs usually cause gastrointestinal disturbances like ulcers and liver and renal failure.^[3] To date, pharmacological treatments do not appear to be effective in producing sustained analgesia therefore, future research is necessary to discover new drug therapies that can be used safer, potent and cheaper analgesic drugs. Currently dissatisfaction from the synthetic drugs research has diverted to discover drugs from the natural sources.

Ayurvedic system of medicines is one of the oldest systems of medicine having a history of more than 3000 years. Several prototype derived from these herbal medicines are in use for various kind of disease and disorders. It not only gives new molecule but also with newer mechanism of action, hence is called Gold mine.

For the current study we have selected a *Solenostemma argel* whole plant extract for its central and peripheral analgesic activity by using hot plate and acetic acid induced wreathing technique respectively.

Solenostemma argel (Apocynaceae) is a desert plant widely distributed in Egypt (WadiAllaqui) with the common name hargel^[4] and in Sudan which is its richest source.^[5] It is the most important one from the many Egyptian plants which are known to be of potential medicinal value in herbal medicine. *S. argel* is used for the treatment of diabetes and jaundice, purgative properties which may be due to the latex present in the stem parts.^[6] Also, an extract from the leaves of this plant showed fungitoxic activity.^[7] It is used for the

treatment of some diseases of liver and kidney and for allergies and as incense in the treatment of measles and anti-inflammatory activity. It is an effective remedy for bronchitis and is used to treat neuralgia and sciatica. Its leaves are used to treat gastro-intestinal disorders and to treat urinary tract infections and are effective as an anti-syphilitic if used for prolonged periods of 40-80 days.^[8-9] The native Sudanese have commonly used *S. argel* to suppress stomach pain, pains due to childbirth and loss of appetite. It proved that its crude aqueous extracts possessed larvicidal activity against mosquito larvae.^[10]

From the previous phytochemical studies, it was found that its leaves were characterized by high carbohydrates, low crude fiber, protein, crude oil, ash and high potassium, calcium, magnesium, sodium and low copper, ferrous, manganese, lead and contained phytic acid and tannin.^[11] Kamel (2003) proved that it contained acylated phenolic glycosides, pregnene glycoside (solenoside A), kaempferol 3-O-glucoside and 3-O-rutinoside (R). Also it was found that its aerial parts contained two monoterpeneglucosides, a pregnaneglucoside, benzyl alcohol O- β -apiofuranosyl (1 \rightarrow 6) β -glucopyranoside, 2-phenyl-ethyl O- α -arabinopyranosyl (1 \rightarrow 6) β -glucopyranoside, astragalin and kaempferol 3-O-neohesperidose.^[12]

Based on these phytochemical observations and traditional claim we have undertaken this study to substantiate its traditional state scientifically.

MATERIAL AND METHODS

Plant Materials

The leaves of *Solenostemma argel* used in this study were collected from Sudan and authenticated by department of natural products and alternative medicine, faculty of pharmacy, Northern Border University, KSA.

Preparation of Extract: Ten gram of the dry powder was macerated in 100 ml distilled water for one hour. The mixture boiled for 20 minutes in water bath. After cooling the supernatant (decoction) was collected and filtered. The volume was adjusted to 50 ml for less concentrated solution and administered to mice *p.o.* in a volume of 10 ml/kg or 20 ml/kg.

Animals

Mice of either sex (25–30 g) obtained from the animal house at the faculty of pharmacy, Northern Border University were used for this study. The animals were housed in a standard controlled animal care facility, in cages (five mice/cage). The animals were maintained under

standard nutritional and environmental conditions throughout the experiment. The animals were fasted overnight and water *ad libitum*. The animals were divided into 4 groups, each group containing 5 animals treatment schedule is mentioned in table 1:

Table 1: Treatment schedule animal groups for Hot plate method.

Animal Group	Treatment
1	DW, 10 ml/kg, <i>p.o.</i>
2	Diclofenac sodium 10 mg/kg, <i>p.o.</i>
3	AESA, 10 ml/kg, <i>p.o.</i>
4	AESA, 20 ml/kg, <i>p.o.</i>

DW: Distilled water; AESA: Aqueous Extract *Solenostemma argel*

Acute Toxicity Study

Acute toxicity studies were performed according to OECD-423 guidelines category IV substance (acute toxic class method). Albino mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water only. The AESA was administered orally with an initial dose of 1000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose (OECD-423).^[13] (For results refer table 4)

Preliminary Phytochemical analysis

Preliminary phytochemical study was performed on Aqueous Extract *Solenostemma argel* (AESA) as per the method prescribed by Kokate, 1994^[14] (For results refer table 3)

Assessment of Analgesic activity

Hot plate method

The parameter evaluated for was the latency time for paw licking and jumping response after exposure on surface of hot plate. In this test, animals were individually placed on a hot-plate (Ugo Basil Hot-Plate) with the temperature adjusted to 55 ± 0.1 °C.

Reaction time recorded when animals licked their fore or jumped at time 0, 30, 60 and 90 minutes after oral administration of the samples. The cut-off time was 15 seconds in order to avoid damage to the paw.

Acetic acid-induced writhing test

The animals were divided into 4 groups with 5 albino mice in each group. Treatment schedule is mentioned in table 2:

Table 2: Treatment schedule animal groups for Acetic acid induced writhing method

Animal Group	Treatment
1	Distilled water (10 ml/kg, <i>p.o.</i>) + Acetic acid 1.0 % v/v (0.1 ml/10 g)
2	Diclofenac sodium 10 mg/kg, <i>p.o.</i> + Acetic acid 1.0 % v/v (0.1 ml/10 g)
3	AESA 10 ml/kg, <i>p.o.</i> + Acetic acid 1.0 % v/v (0.1 ml/10 g)
4	AESA 20 ml/kg, <i>p.o.</i> + Acetic acid (3% Sol. 30 mg/kg, <i>i.p.</i>)

DW: Distilled water; AESA: Aqueous Extract *Solenostemma argel*

Test samples and distilled water administered orally 30 minutes prior to *intraperitoneal* (*i.p.*) administration of 1.0 % v/v acetic acid (0.1 ml/10 g). Diclofenac sodium administered orally 15 minutes prior to acetic acid. The number of writhes was recorded for 15 minutes commencing just 5 minutes after *i.p.* administration of acetic acid. The percentage protection was calculated as follows.

$$X1-X2/X1 \times 100$$

X1=No. of writhing in control group

X2= No. of writhing in treated group

STATISTICAL ANALYSIS

The results were expressed as mean \pm SEM and statistical comparisons were made by conducting one way ANOVA ($P < 0.05$).

RESULTS

Table 3: Results of Preliminary phytochemical screening of Aqueous Extract

Solenostemma argel (AESA)

Number	Phytoconstituent	Presence/Absence
1	Anthraquinones	-ve
2	Flavonoids	++ ve
3	Tannins	++ ve
4	Saponines	
5	Alkaloides	+ ve
6	Carbohydrates	+ ve

+ Indicates presence of constituents

- Indicates absence of constituents.

Table 4: Acute Toxicity Record Sheet

Sl. No	Code	Toxicity		Time of Death	Observations										
		On Set	Stop		Skin & Fur	Eyes	Resp	CNS	Tre	Con	Sali	Diah	Sleep	Let	Coma
1	AESA	X	x	x	x	x	x	x	x	x	x	x	x	x	x

Drug: Aqueous Extract *Solenostemma argel*

Dose: 2000mg/kg b.w.

Species: Swiss Albino mice

Sex: Female

Method: OECD-423

Duration: 24 hours

Place: Department of Pharmacology and Toxicology, Northern Border University, KSA

(*TRE-Tremor, CON-Convulsions, SALI- Salivation, Diah - Diarrhea, LET-Lethargy)
 x = Negative, ✓ = Positive AESA: Aqueous Extract *Solenostemma argel*.

Toxicity Study

As per the OECD-423 fixed dose procedure guidelines AESA at the dose of 2000mg/kg per orally has not shown any significant toxic effects in mice.

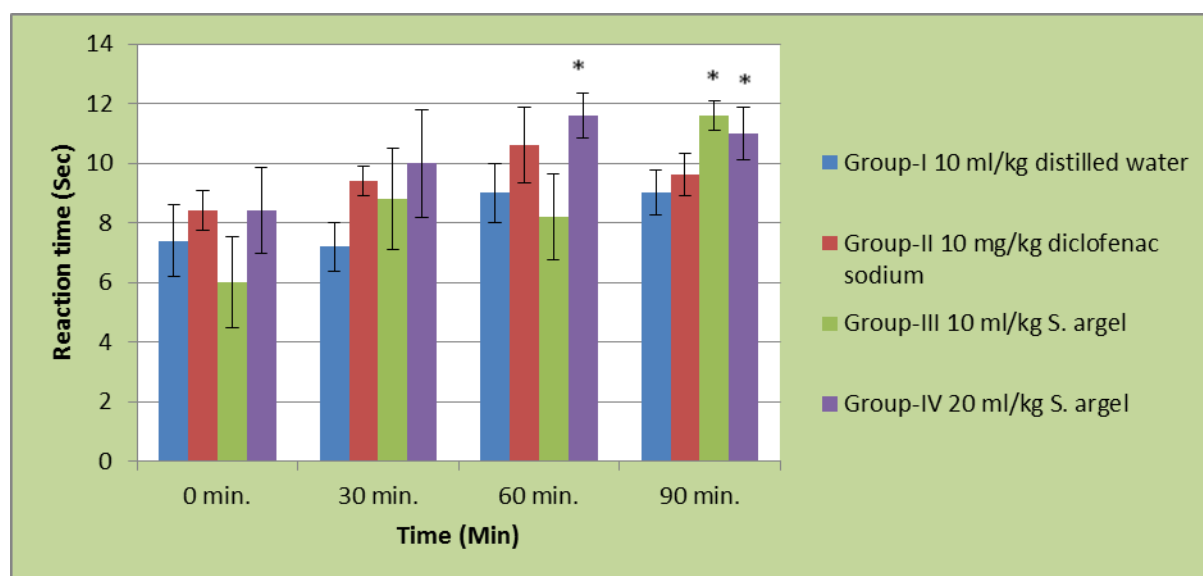


Fig 1: Effect of aqueous extract of *Solenostemma argel* on latency to hot plate test in mice

- 10 ml/kg *S. argel* caused significant ($P < 0.05$) Increase in mean latency after 90 min compared to time 0 (before administration).
- 20 ml/kg *S. argel* caused significant increase in mean latency after 60 min and 90 min compared to time 0 (before administration).

- Control group (distilled water) and standard (Diclofenac sodium) caused no significant increase in mean latency.

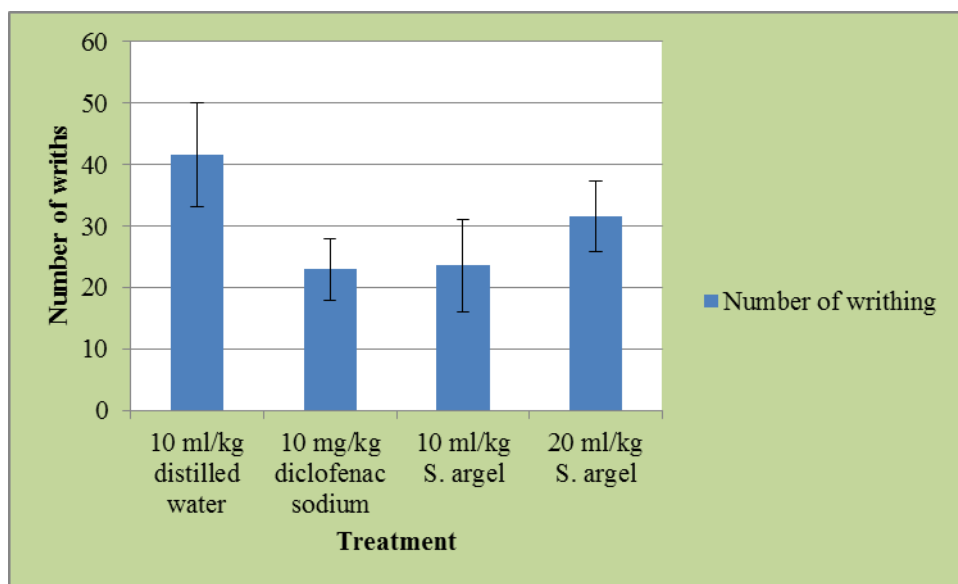


Fig 2: Effect of aqueous extract of *Solenostemma argel* on acetic acid induced writhing in mice

- No significant difference between groups.
- *S. argel* at 10 ml/kg showed inhibition percent approximately similar to Diclofenac sodium.

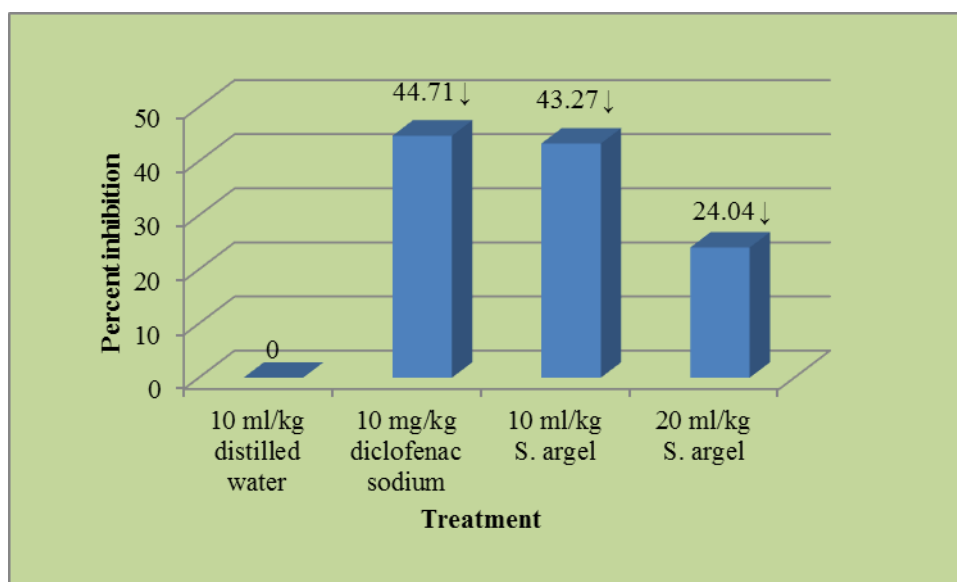


Fig 3: Effect of aqueous extract of *Solenostemma argel* on acetic acid induced writhing in mice.

- No significant difference between groups.

- *S. argel* at 10 ml/kg showed inhibition percent approximately similar to Diclofenac sodium.

DISCUSSION

Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness.^[15] Centrally acting analgesics act by raising the threshold for pain and also altering the physiological response to pain. On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain.^[16] The animal models employed for screening of analgesic activity in this study are pain-state models using thermal stimuli like hot plate method. This method is useful in illustrating centrally mediated anti-nociceptive responses which focus generally on changes above the spinal cord level^[17] and involves higher brain functions and is regarded a supra-spinally organized response.^[18]

In hot-plate model, the AESA exhibited significant analgesic activity by increasing the reaction time of the mice compared to control (distilled water treated mice). Diclofenac Sodium was used as standard drug which was considered as mild analgesic activity. In comparison with control 20 ml/kg AESA has shown most significant ($P < 0.05$) anti-nociception effect during 60 min and 90 min observation, while 10 ml/kg AESA showed significant effect only at 90 minutes. Diclofenac sodium showed analgesic effect but it is non-significantly.

The hot-plate method is based on the observation that morphine-like compounds (centrally acting) are selectively able to prolong the reaction time of typical paw licking/jumping effect in mice. This method is also useful in differentiating central opioid-like analgesics from peripheral analgesics.^[19] Analgesic drugs which are centrally acting elevate pain threshold of animals towards heat and pressure.^[20] Therefore, the analgesic effect of the extract on this pain-state model indicates that it might be centrally acting. With reference to the results (figure 1) reaction time, the analgesic effect of AESA (20 ml/kg) was evident after 60 min following *p.o.* administration and the extract has showed analgesia up to 90 min.

In the present study we have also investigated the effect of AESA against acetic acid induced writhing in mice.

From the figure 2 and 3 shows effect of AESA on acetic acid-induced writhing in mice. After oral administration of two different doses of AESA (10 ml/kg and 20 ml/kg) the percent inhibition was 43.27% & 21.04% respectively. The reference drug diclofenac sodium was found more potent (44.71%) than both the dose levels of plant extracts. Surprisingly 10 ml/kg dose of the plant extract has shown better protection than 20 ml/kg is may be because of the pharmacodynamic changes at receptor level or might be intra animal behavioral changes.

Writhing is an overt response to the intense pain induced by irritant principles via nociceptors characterized by episodes of retraction of abdomen and stretching of hind limbs. The signals transmitted to central nervous system in response to pain due to irritation, cause release of mediators such as prostaglandins which contributes to the increased sensitivity to nociceptors. The writhing response to acute nociception has been used to test the analgesic activity of drugs in rodents. Dilute acetic acid is the most frequently used irritant to induce writhing behavior. The administration of acetic acid intraperitoneally activates both peripheral and central mechanisms of nociception. It releases nociceptive mediators such as prostaglandins (PG) E₂ and I₂ at the site of noxious stimulation, the peritoneal cavity, and at central sites such as the dorsal horn of the spinal cord and some brain regions.^[21]

Previous preliminary phytochemical analysis has shown that aqueous extract of leaves of *Solenostemma argel* has shown the presence of many phytoconstituents like Acylated phenolic glycosides, namely argelin and argelosid, choline, flavonoids, monoterpene and pregame glucoside, sitosterol and a tri-terpenoid saponin.^[22]

Its other phytoconstituents are cyanogenic glycosides, saponines, tannins, coumarines, flavonides, phenilic acid and triterpienoid.^[22]

A number of alkaloids, flavanoids, steroids, and tannin isolated from medicinal plants have been reported to possess significant analgesic activity. The major constituent of the *Solenostemma argel* are tannins and saponines which comprises up to 60% of its total content.^[23]

Thus, analgesic activity observed with this extract might be attributed to the presence of this compound. Furthermore, there are reports on the role of tannin in analgesic activity. According to preliminary phytochemicals which were screened from *Scopariadulcis* L. including tannin might be responsible for the observed analgesic activity. Another research

suggested that the presence of tannin and flavonoid in extract of *Cassia auriculata* leaves seems to inhibit prostaglandin synthesis and exerts the anti-inflammatory and analgesic effects.^[24]

This preliminary study did not fully demonstrate the dose-dependent analgesic effect of the extract of the *Solenostemma argel* because different concentrations of the extract were not tested, which remains a limitation of the present study.

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