

## The Evaluation of Analgesic activity of Solanum Nigrum

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### Abstract

The present study was undertaken to evaluate the analgesic activities of water extract of leaves and methanolic extract of seeds of Solanum nigrum Linn in experimental animal models. The study was carried out using Swiss albino rats (210- 290g) of female sex. The analgesic activity of Solanum nigrum was assessed by using hot plate and tail flick method. For the determination of analgesic effect, doses of each extract of Solanum nigrum used in the present study were 500 mg/kg body weight (BW). Hot plate test showed a significant increase in the mean reaction time to heat stimuli in hot plate method at 500 mg/kg BW doses throughout the observation period in 1/2 hour, 1 hour and 2 hours after treatment, which was comparable to the standard Ibuprofen and control group. The present study suggests that each extract of Solanum nigrum has significant analgesic and activities.

### Introduction

Many of the currently used medicines originate from natural products, especially plants. Drugs and plants are closely related to each other through the use of traditional medicines or ethnomedicines that are mainly prepared from plants. Plants and herbs are the sources of not only crude drugs, but also bioactive compounds that could lead to novel drug structures.<sup>[17]</sup> Because medicinal plants and herbs have been used since ancient times for relieving pain caused by disease, injury and surgery,

some of them contributed to the development of modern anesthesia. For discovering drug candidates, plants of interest are screened for the presence of bioactive components, phytochemicals responsible for the bioactivity are isolated, their molecular structures are identified, and then the original structures of phytochemicals may be semi-synthetically modified to enhance the activity or reduce the toxicity.<sup>[17]</sup> Solanums comprise a very important group of medicinal plants having

multifarious uses. These plants belong to the family Solanaceae and genus Solanum. throughout the world, with different species prevalent in different areas. <sup>[1]</sup> It is also known as black nightshade. <sup>[11]</sup> Black nightshade can be a serious agricultural weed when it competes with crops. <sup>[11]</sup> It has been reported as a weed in 61 countries and 37 crops. <sup>[11]</sup> Black nightshade contains toxic glycoalkaloids in the plant. <sup>[15]</sup> the highest concentration is in the green

## Materials and Methods

### Collection of Plant Materials

The plant material (leaves and seeds) of Solanum nigrum (SN) were collected locally from Surman, April 2018 Libya and got identified by Department of Botany, University of Zawia. The plant materials were subjected to shed drying and grind by

### Preparation of Extracts

After washing the leaves, seeds drying at the room temperature, and powdered, it's extracted by three solvents; water, Ethanol, and methanol solvents, by mixing 10mg leaves and 20mg seeds with 100ml of the solvents. Everyone of this mixture was left

### Concentration of the extract

The extractions from the water extract from the SN leaves, and methanolic extract from SN seeds was firstly concentrated by rotary evaporator at 30<sup>0</sup> to 40<sup>0</sup>C (90 round per minute).

### Preliminary phytochemical analysis

<sup>[4]</sup> The plants are present immature berries. <sup>[15]</sup> all kinds of animals can be poisoned after ingesting nightshade including cattle, sheep, poultry, and swine. <sup>[15]</sup> children have been poisoned and have died after ingesting unripe berries. <sup>[15]</sup> the ripe berries cause reduced symptoms of mild abdominal pains, vomiting, and diarrhea. <sup>[15]</sup>

an electric grinder to turn it into a fine powder and then the powder was saved in a sterilized and tightly packed glass container which was store it in a cool place away from light until use.

for 48 hours with shaking at room temperature and the liquid was collected. Finally, the last residue of the extract was pressed out of the SN particles and extract was filtered.

And then empty the concentrated extraction in dark bottle, and reserve it in freezer.

Various phytochemical analysis were carried out on SN extract to determine the presence of the phytoconstituents. Screening was performed for tannins, flavonoids, alkaloids, and saponins using the standard procedure.(REFF)

The color intensity or the precipitate formed was used an analytical response for these test. Tannins, Flavonoids, Saponins, Alkaloids

### **Thin-Layer Chromatography (TLC)**

According to preliminary phytochemical analysis results, the TLC profiling was performed for water extract of leaves, and methanolic extract of seeds. Prepared the mobile phase by adding the suitable solvents with different concentrations. The solvent and it is concentration we are used

is: Acetic acid: ethanol (1:3), Chloromethane: chloroform (9:1), Dichloromethane: chloroform: methanol (9:1:1), Chloroform: methanol (1:1), Chloroform: methanol (1:2), Chloroform: methanol: ethanol (1:1:1)

### **Experimental animals**

20 female Swiss albino, weighing between 214-283 were used for experiment were used in this study as test and the control group, the animals were obtained from local animal house National Medical Research Center, Alzawia, Libya. They were housed in standard environmental condition like, Foods were buy from the places of sale allocated to them, and water were available ad lib. The animals were kept in a room temperature varying between ( $20 \pm 25^{\circ}\text{C}$ ) and 12/12h light dark cycle. 3.5. Preparation of dosage of rats:

### **3.6. Pain state models using thermal stimuli**

Heat is a suitable stimulus for activating cutaneous receptors. <sup>[18]</sup> The source of nociceptive stimulation can be distant for its target (e.g., radiant heat from a lamp) in

To evaluate the analgesic activity of water extract of leaves and methanolic extract of seeds of solanum nigrum by hot plate and tail flick tests. the extract concentration used 500mg/kg, for preparation of this concentration to leaves extract 16000mg of extract where dissolve in 25ml distilled water, for preparation the same concentration to seeds extract 11000mg of extract where dissolve in 25ml, For the preparation of standard solution of 100mg/kg, 400mg of ibuprofen powder was dissolved in 4ml distilled water.

direct contact with the skin. <sup>[18]</sup> Radiant heat constitutes a relatively selective stimulus for nociceptors and has as advantage over the other modes of thermal

stimulation in that it produces no tactile

stimulus.<sup>[18]</sup>

### **Hot plate method**

The paws of rats is very sensitive to heat at temperatures, which are not damaging the skin. The responses are jumping, these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses. Procedure of method Several investigators have modified the method originally described by Woolfe and Mac Donald (1944). The following modification has been proven suitable. The hot plate, which is commercially available, consists

withdrawal of the paws and licking of the paws. The time until of electrically heated surface. The temperature is controlled for 55° to 56°C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stopwatch. The latency is recorded at 30, 60 and 120 minutes following oral administration of the standard or the test plants.

### **Tail flick test**

The tail flick test is used for the determination of analgesic activity to the same solution is applied to hot plate, and in the same groups arrangement. A cut-off

period of 10 sec was kept to avoid damage to the tail; the latency was recorded at 1/2hour, 1hour and 2 hours following oral administration of rats.

### **Effect of plant extract on serum biochemical parameters**

The biochemical analysis were done on serum after centrifugation of collected blood and the following parameters like aspartate transaminase (AST), alanine

transaminase (ALT), and total protein, All analyses were determined on using clinical chemistry analyzer (Vital Scientific, Netherlands).

### **Statistical analysis**

Statistical analysis was performed as mean of variance  $\pm$  SEM (n=5) followed by ANOVA test using Graph Pad Prism and for

multiple comparison test among the groups, Bonferroni test was performed. A robability level of  $p < 0.05$ . was accepted statistically.

## Result and Discussion

### Phytochemical screening

The aqueous and alcoholic extracts of the solanum nigrum (Leaves and seeds) showed a positive result for the presence of medicinally

active constituents. The Preliminary phytochemical screening of Solanum nigrum summarized in Table 1.

	SN leaves				SN seeds			
	Tannins	Flavonoids	Alkaloids	saponins	Tannins	Flavonoids	alkaloids	Saponi
Water extract	+++	++	+++	++	++	+	-	-
Ethanol extract	-	+	++	-	+++	++	-	-
Methanol extract	-	+	++	+	+	++	+++	-

**Table (1): Preliminary phytochemical screening of leaves and seeds extract.**

**SN; Solanum nigrum**                      **(+++)** high                      **(+)** medium                      **(-)** negative result

In the methanolic and ethanolic leaves extract flavonoids, alkaloids were most commonly found in the tested plants. While Tanins is absent in leaves Alcoholic. On the other hand saponins was absent in seeds extracts While Alkaloids was absent in water and ethanol seeds extract, while alkaloids is present in methanolic extracts. These finding correlated well with several earlier publications [ndhumathi and Mohandav[9], Jasimet al.,[10]. Modilal et al.,[12] also reported the similar results]. Plants were rich in a wide variety of secondary metabolites such as alkaloids, tannins, flavonoids and saponins appears

pharmacological activities. These may have potential to be used as chemotherapeutic agents or serve as starting material in the developing of new antibiotics [41]. Medical use of alkaloid-containing plants has a long history. Many synthetic and semisynthetic drugs are structural modifications of the alkaloids, which were designed to enhance or change the primary effect of the drug and reduce unwanted side effects. For example, naloxone, an opioid receptor antagonist, is a derivative of the baine that is present in opium. Plant flavonoids represent an important group of secondary metabolites and around 6500 varieties of flavonoids

have been reported so far. These polyphenolic compounds are found in almost all plant species. Extensive biological roles of flavonoids have been reported which include antimicrobial, anti-hepatotoxic, therapeutic and other roles in nature. The presence of flavonoids in leaves enhances the physiological survival of plants by shielding them from parasitic

### **TLC crude extract of *Solanum nigrum***

Thin layer chromatography is simple, cost-effective and easy to operate technique in phytochemistry and biochemistry with numerous applications, which use in the development of new drugs and various types of formulations from medicinal plants. Further needed detailed documentation for the sustainable development in education and research. In

### **Analgesia potential activity**

In this study; Seeds and leaves extract showed significantly ( $p < 0.05$ ) prolonged the reaction time to thermal pain at 30minutis. The reaction time to pain in rats administered with 500mg/kg b.w of the extract (seeds  $12.64 \pm 2.5$  and leaves  $13.35 \pm 3.6$ ) were longer than in rats administered with Ibuprofen,  $8.76 \pm 3.1$  while the control has  $2.84 \pm 0.5$  at 2 hour.

diseases and UV radiations. Moreover, flavonoids also play role in photosensitisation, photosynthesis, respiration, sex-determination and morphogenesis. Plant flavonoids are generally found in low, variable concentrations and hence, it is challenging to find their consistent supply.

this study; three bands were observed for which  $R_f$  value (0.39, 0.36, 0.41) TLC of extracts from the plants used in this revealed the presence of these compounds by suitable mobile phase (Dichloromethan: Methanol) 9:1 Better resolution depending on solubility components in mobile phase and size compounds.

The hot-plate test of analgesia is considered selective for opioid-like receptors. Although the central and peripheral analgesics act by inhibiting the number of contractions provoked by chemical pain stimuli, only the central analgesics increase the time of response in the hot plate test (García, et al, 2004).

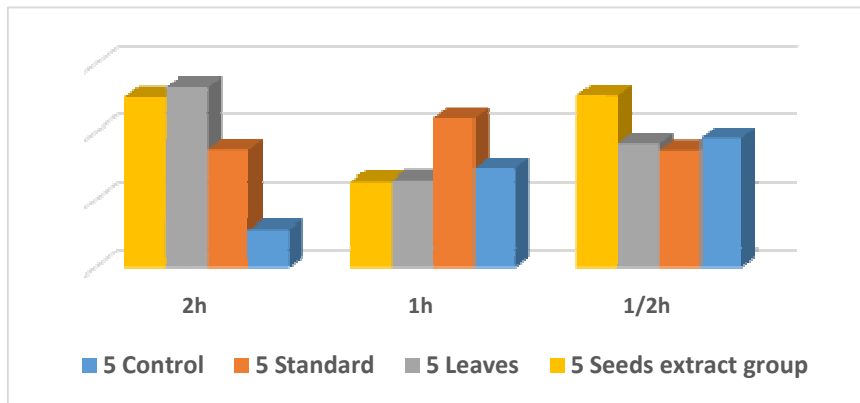


Fig (1): The effect of Seeds, Leaves and Standard on reaction (in seconds) to thermal pain induced in Hot plate method in rats

The analgesic property of the extract was also exhibited in the Tail flick test with the extract significantly ( $p < 0.05$ ) increasing the tolerance of the rats to pain, more than was observed for Ibuprofen. Tail flick test is a standard method for centrally acting analgesics (Ramabadrhan et al., 1989) indicative of morphine like effect (Domer, 1990) and NSAIDs which inhibit cyclooxygenase in peripheral tissues, thereby interfering with the

investigating nociception and analgesia, with the measurement of the response to a brief, noxious stimulus, which appears to be a spinal reflex, modulated by supraspinal inhibitory mechanism. The test is selective for mechanism of transduction in primary afferent nociceptors (Fields, 1987). This test also confirms that the mechanism of analgesia is both centrally and peripherally mediated.

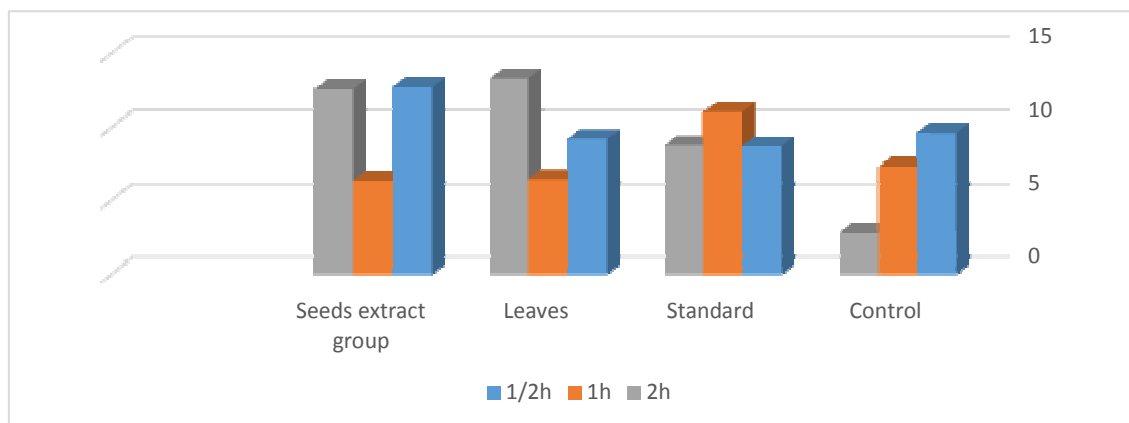


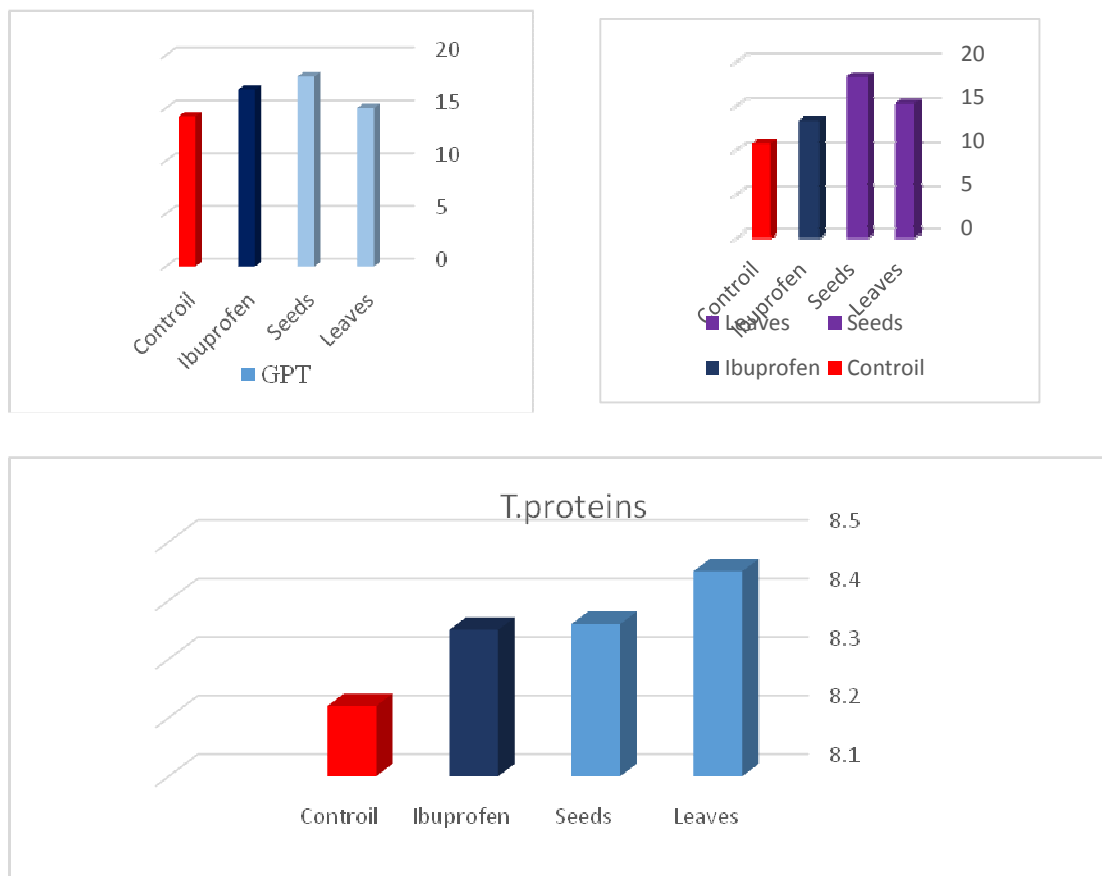
Fig (2): The effect of Seeds, Leaves and Standard on reaction (in seconds) to thermal pain induced tail flick method in rats

## Biochemical analysis results

There were changes in SGOT, SGPT and total protein content in groups on 2h of test group's treatment. It indicates there might be moderate increase in the catabolism of serum proteins.

The results of the various biochemical tests on the experimentally treated animals with

the plant extract (Seeds and Leaves and normal group are in figure (3) Oral administration of the plant treated extract at a dose of 500 mg/kg when compared to control group mice. There was a significant increase in (AST) and SGPT and Total protein.



**Fig (3): Effect of Seeds, Leaves, Ibuprofen on Liver function**

Many studies have confirmed that elevated serum levels of hepatic enzymes, transaminases (SGPT and SGOT) and total protein are not a directly linked for liver injury but increase levels are responsible to cause inflammation, cellular leakage and damage of cell membrane to cells in the liver.<sup>[16]</sup> The main target organ for drug or bioactive active compound is liver where

exposed to the foreign substances being absorbed in intestines and metabolized to other compounds which may or may not be hepatotoxic to the rats.<sup>[17]</sup> Therefore, the increase in liver hepatic enzyme (SGPT and SGOT) and total protein after administration of the plant extract might be because of certain phytochemical compound that might have toxic potential



on liver with dose and result liver injury. Further specific assays of toxicity and more histological study could provide

## Conclusion

The present study concludes that the water extract of leaves and methanolic extract of seeds of *Solanum nigrum* containing the highest level of Alkaloids. Better resolution mobile phase of extract (1:1:1) from the chloroform, methanol and ethanol solvent. The plant under investigation possesses analgesic activities in rats at the doses 500 mg/kg, more active extract is water extract of leaves after 2 hour from

more information regarding to the toxic effect of the extract on liver.

administration of extract. Biochemical analysis results indicate present toxic effect of this plant extraction (seeds and leaves extract).

Further study is aimed at isolating and elucidating the chemical structure of the bioactive principles responsible for the anti-inflammatory and analgesic properties.

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