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SOME PHARMACOLOGICAL AND TOXICOLOGICAL ACTIVITIES OF *ANNONA SQUAMOSA LINN.* ETHANOLIC EXTRACT

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ABSTRACT

The present study was to investigate phytochemical, toxicological and some pharmacological activities include antipyretic, analgesic and anti-inflammatory activity of *Annona squamosa Linn*.70% leaves in doses of 250 mg/kg. b. wt. and 500 mg/kg b. wt. and seeds in doses of 25 mg/kg b. wt. and 50 mg/kg b. wt. The phytochemical screening was done for detection of alkaloids, tannins, saponnin, flavonoids, glycosides and resin. The toxicological pattern was studied by the determination of LD50 in mice by oral administration of upgrading doses of leaves and seeds extract. The oral anti-inflammatory (Formalin induced edema), antipyretic (Brewer's yeast induced hyperthermia) were carried out on rats and analgesic activity (writhing test and hot plate method) were carried out on mice. Phytochemical screening revealed the presence of alkaloids, flavonoids and glycosides in both leaves and seeds. Resin was absent in leaves, seeds were devoid of tannins and saponin. LD50 was found to be 5000 and 520 mg/kg b. wt. for leaves and seeds respectively. Leaves exhibited significant analgesic and anti-inflammatory activity in doses of 250 and 500 mg/kg b. wt. and only higher dosage level (500 mg/kg b. wt) induced significant antipyretic activity. Seeds only exhibited significant analgesic and anti-inflammatory activity in a dose of 50 mg/kg b. wt. In conclusion, *Annona squamosa* leaves and seeds extracts induced antipyretic, analgesic and anti-inflammatory actions and leaves extract is consider more safer as compared with On the other seed extract.

KEYWORDS: *Annona squamosa*, Phytochemical, Toxicological, LD50, Antipyretic, Analgesic, Anti-inflammatory.

INTRODUCTION

Today medicinal and aromatic plants are the most widely used form of medicine in the world as they contain active compounds as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds **(Okigo *et al.*, 2009)**. Many synthetic drugs have serious side effects. The analgesic and Anti-inflammatory drugs like paracetamol and aspirin have toxic effects to various organs of the body.

Therefore, the development of novel compounds having analgesic, antipyretic and anti-inflammatory activities with improved safety profiles remains a clinical need **(pasin *et al* ., 2010)**. *Annona squamosa* linn is cultivated today in almost all tropical and subtropical countries **(yung *et al* ., 2004)**. The genus name *Annona* is from the Latin word Anon meaning yearly produce, contains approximately **2300** known species **(Audrey and Bisman 2004)**. Species name *squamosa* refers to the knobbly appearance of the fruit **(Vyas *et al* ., 2012)**. Different parts of *Annona squamosa* reported to possess several pharmacological activities such as analgesic, anti-inflammatory, anti-microbial, hepatoprotective activity and **(Gajalakshmi *et al.*, 2011)** And **(Neha, and Barve 2011)**. Wound healing **(Ponrasu, and suguna, 2014)** and insecticidal activity **(Khalequzzaman, and Sultana, 2006)**. For establishing and confirming of therapeutic role of *Annona squamosa Linn.* in modern medicine, experimental studies are required. The aim of the present study is to explore and confirm phytochemical, toxicological and some pharmacological activities including analgesic, antipyretic and Anti-inflammatory activities of *Annona squamosa Linn.* Leaves and seeds 70% ethanolic extract.

MATERIALS AND METHODS

Plant

***Annona squamosa Linn* leaves and seeds:** The tested plant leaves and seeds in this study were collected from Al-Orman garden which is located in Giza Egypt and identified by Department of Botany Faculty of Science Cairo University. Leaves and seeds were air dried, pulverized and stored in tightly closed glass containers.

Preparation of leaves extract: In two glass jars dried *Annona squamosa* leaves and seeds powder were separately extracted by percolation several times till exhaustion **(Egyptian Pharmacopeia 1984)** using 70% ethanolic solution then filtered, most of the solvent was evaporated using rotatory evaporator apparatus attached with vacuum pump & low temperature 50˚c.

ANIMALS

Mature mice of both sexes and weighing (20-25gm) were used for studying the acute toxicity, LD50 and analgesic activity. Mature albino rats of both sexes and weighing (200-250 gm) were used to reveal antipyretic and anti-inflammatory activity.

Phytochemical studies: Phytochemical screening of leaves and seeds was carried out to identify the constituents, using standard phytochemical methods as described by **Evans, (1996)** and **Sofowora, (1993)**.The screening involves detection of alkaloids, saponin, tannins, flavonoids, carbohydrate/glycosides and resin.

Toxicological study: A pilot study was performed to determine minimum lethal dose of *Annona squamosa Linn.* 70% ethanolic extract. LD50 of leaves and seeds were determined using method described by **Kerber, (1941)**.

For this purpose 5 groups of five mice each weighing 20-25gm were administrated orally in upgrading doses ranging from 1500 to 7500 mg/kg b. wt. and 100 to 900 mg/kg b. wt. for seeds. Another group was left as control and given diluent only. The toxic symptoms, mortality rate and post-mortem a finding in each group was recorded 24 hours post administration. LD50 of the tested extract was calculated according to the formula using method described by **Kerber, (1941)**.

Pharmacological studies

Antipyretic activity: The method described by **Alperman, (1972)** was used for studying the antipyretic effect of 70% ethanolic extract of *Annona squamosa* leaves and seeds. Thirty rats of both sex weighing 200-250 grams were divided into six groups of five rats in each. All rats were made hyperthermic by subcutaneous injection of brewer's yeast in a physiological saline in a dose of 1.5 g/kg b. wt. After 17 hours, the initial body temperature of each rat was measured rectally using a medical thermometer. The first group was kept as control; the second group was given metamezole sodium 50 mg/kg b. wt. as a standard antipyretic. The third and fourth groups were used to reveal the antipyretic effect of the tested *Annona squamosa* Leaves 70% ethanolic extract when given orally in a dose of 250 and 500 mg/kg body weights, respectively. The fifth and sixth groups were used to reveal the antipyretic effect of the tested seed extract when given orally in a dose of 25 and 50 mg/kg body weight. The body temperature of each rat was then recorded every hour for 3 successive hours.

Analgesic activity

A. Writhing test

This experiment was carried out as described by **Okun *et al*., (1963)**.Thirty mice of both sex weighing 20-25 g body weight each which previously showed positive writhing (stretch torsion to one side drawing up of hind limb, retraction of abdomen and opisthotonus, so that the belly of the mouse touch the floor) with glacial acetic acid were selected and divided into six groups. Mice of the first group were kept as control nontreated, those of the second group was orally administrated aspirin 100 mg/kg body weight as a standard group.

Mice of the third and fourth groups were orally administered *Annona squamosa Linn.* Leaves *70%* ethanolic extract in a dose of 250 and 500mg/Kg body weight. Mice of the fifth and Sixth groups were orally administered seeds*70%* ethanolic extract in a dose of 25 and 50mg/Kg body weight. After 30 minutes, each mouse was intraperitoneally injected with 0.25 ml of 0.7% glacial acetic acid in distilled water, and the mice were then placed in transparent boxes for observation. The numbers of writhes were observed after 1, 2, 3, 4 and 5 hours post administration. Number of writhes for each animal in all groups were recorded and the analgesic potency of the tested extract was determined as protection % against writhing according to the following formula

$$\% \text{ of protection} = \frac{\text{Control mean - treated mean} \times 100}{\text{Control mean}}$$

B. Hot plate test

The experiment was carried out as described by **Janssen and Jageneau (1957)** and modified by **Jacob and Bosovski (1961)** using hot plate apparatus, thermostatically controlled at 56 ± 0.5˚c. Thirty mice were divided into 6 groups, 5 animals each. Reaction time was measured prior to extract (min 0) and after the drug treatment. Group I was kept as normal control.The 70 % ethanolic extract of *Annona squamosa* leaves was administrated orally to mice of groups II and III in doses of 250 and 500 mg/kg, respectively. Seed extract was given in doses of 25 and 50 mg/kg b. wt. to group IV and V. Mice of group VI (standard) were treated orally with acetylsalicylic acid at a dose rate of 100 mg/kg b. wt. The reaction time was measured at 15 min and repeated at 30, 60, and 90 min post-administration.

Anti-inflammatory effect

According to the method described by **Domenjoz *et al.*, (1955)** thirty rats of both sexes weighing 200-250 grams body weight were used. Inflammation was induced in the right hand paw of all rats by subcutaneous injection of 0.1 ml formalin 6% solution in normal saline. After four hours, the thickness of each rat paw was measured in mm using vernier caliber to detect the inflammatory process achieved by the formalin solution. Rats were then divided into six equal groups of five rats each. Rats of the first group were left as control with induced inflammation only. Those of the second group were orally administered diclofenac sodium (Voltarin®) in a dose of 30 mg /kg body weight as a standard.

Rats of the third and fourth groups were orally administrated ethanolic extract *Annona squamosa Linn.* Leaves in a dose 250 and 500 mg/kg body weight.Those of the fifth and sixth groups were orally administrated ethanolic seeds extract in a dose of 25 and 50 mg/kg body weight. Thirty minutes after drug or test compound administration, 0.1ml of 6% formalin solution in normal saline was injected subcutaneously in the right hand paw of all animals for induction of edema the thickness of each rat paw was measured in mm by vernier caliber after 1, 2, 3, and 4 hours post administration.

Statistical Analysis

Data were analyzed using commercial software statistical package for social science (SPSS version©16). Data were presented as mean ± standard error. The significance level was set as P value ≤ 0.05 significant.

RESULTS AND DISCUSSION

Phytochemical Screening

Preliminary phytochemical tests for detection of active principles in the *leaves and seeds* were carried out & recorded in table (1). The obtained results revealed the presence of alkaloids, flavonoids and glycosides in both leaves and seeds. Leaves were devoid of resin, while seeds contain considerable amount of resin .and devoid of tannins and saponins.

The obtained results correlates with that obtained by **Saleem *et al.*, (2012)** who found that the aqueous leaves extract contains alkaloids, flavonoids, saponins and tannins. **Yusha'u *et al.*, (2011)** who proved that the ethanolic leaves extract contains glycosides, tannin and saponin but diversely mentioned that flavonoids and alkaloids are absent. **Narasim *et al., (2015)*** and **Sharma *et al., (2009)*** who reported that the methanolic leaves extract contained glycosides,

saponins, tannins and flavonoids. The findings regarding seeds correlates with those obtained by **Silva *et al.*, (2016)** who found that the seeds extract contained alkaloids and flavonoids. **Panda,** and **kar, (2007)** who added that the methanolic extract of seeds contained flavonoids. **Bhardwaj *et al.*, (2014)** reported that methanolic extract contained alkaloids and flavonoids, in addition, **Sangala *et al.*,(2011)** and **Ghavan *et al.*, (2013)** found that the methanolic and ethanolic seed extract contain alkaloids, glycosides, flavonoids, saponins and tannins and these results were correlate with our obtained results except tannin and saponin that are absent in our investigation.

Table (1): Showing results of preliminary phytochemical tests on *Annona squamosa* leaves and seeds.

Test for	*Annona squamosa leaves*	*Annona squamosa seeds*
1-Alkaloid and / or nitrogenous bases	+	+
2- Flavonoids	+	+
3- carbohydrate and /or glycosides	+	+
4-Saponins	+	-
2-Tannins	+	-
6-Resins	-	+

(-) negative (+) positive

Toxicological Study

LD50 of the studied *Annona squamosa* leaves and seeds extract was found to be 5 g/Kg body weight and 520 mg/kg b. wt., respectively. This indicates for a great extent that leaves is nontoxic as LD50 value is very high while seeds considered being slightly toxic as the LD50 value is low. In this respect **Buck *et al.*, (1976)** reported that plants with LD50 less than 10 mg /Kg body weight are considered highly toxic and others with LD50 greater than 50 mg/Kg body weight are considered non toxic.

The obtained LD50 value concerning *Annona squamosa* leaves extract correlates with that obtained by **Onwusonye *et al.*, (2014)** who reported that oral LD50 of methanolic leaf extract was 5000 mg/kg b. wt. **Sharma and Goray (2009)** and **Madhu *et al.*, (2012)** found that the aqueous leaf extract Was safe at the dose level more than 2000mg/kg body weight of mice. **Richa Mishra *et al.*, (2012)** and **kumar *et al.*, (2015)** reported that ethanolic leaf extract showed LD50 > 2 g/kg body weight. However, **Masimba *et al.*, (2016)** reported that LD50 were >1.5

g/kg b. wt. for aqueous-ethanol fraction, Suggested that the aqueous-ethanolic extract was harmful if swallowed.The studied acute toxicity in our investigation concerning *Annona*

squamosa seeds extract agree with that obtained by **Aneela *et al.*, (2011)** who mentioned that the seed extract is toxic.

Pharmacological Studies

Antipyretic effect: The antipyretic effect of 70% ethanolic extract of leaves and seeds were studied in hyperthermic rats using brewer's yeast and data were recorded in table (2).

The subcutaneous injection of brewer's yeast suspension markedly elevated rectal temperature after 17 hours of administration. Oral administration of *Annona squamosa* 70% ethanolic leaves extract induced significant antipyretic activity at a dose of 500 mg/kg b. wt. while the low dose 250 mg/kg failed to decrease the raised body temperature.The effect of the high dose 500 mg/kg b.wt., nearly similar to that of the standard metamezole sodium (50 mg/kg b. wt.) after 3 hours. Significance was indicated by lowering the body temperature were (37.76±0.41 and 37.98±0.06) after 2 hours of administration of standard drug and leave extract, respectively and at 3 hours (37.74±0.2 and 37.92±0.1) when compared to the control non-treated group. The 70% ethanolic seed extract failed to decrease the elevated body temperature of hyperthermic rats at 1, 2, and 3 hours post administration at a dose of (25 and 50 mg/kg b. wt.).

Table (2): Showing the antipyretic effect of 70% ethanolic extract of *Annona squamosa* linn leaves and seeds hyperthermic rats (Mean ± SE, N=5)

Treatment	Dose (mg/Kg b. wt.)	Rectal temperature (°C)			
		Before treatment	1 hour	2 hours	3 hours
Control	0	38.7±0.17^{a}	38.84±0.15^{c}	38.72±0.14^{c}	38.86±0.13^{b}
Metamezole sodium (standard)	50	38.74±0.12^{a}	37.62±0.11^{a}	37.76±0.41^{a}	37.74±0.2^{a}
Ethanolic extract of *Annona squamosal* leaves	250	38.8±0.14^{a}	38.81±0.15^{c}	38.73±0.1^{c}	38.6±0.14^{c}
	500	38.96±0.11^{a}	38.08±0.15^{b}	37.98±0.06^{b}	37.92±0.1^{a}
Ethanoic extract of *Annona squamosal* seeds	25	38.84±0.07^{a}	38.86±0.08^{c}	38.76±0.11^{c}	38.8±0.1^{c}
	50	38.92±0.13^{a}	38.9±0.06^{c}	38.82±0.06^{c}	38.9±0.11^{c}

Values represent the mean ± S.E. of five animals for each group, values in each column with different superscript letters (a,b,c) are significantly different at P<0.05.

These results correlates with that obtained by **kumar *et al.*, (2015)** who reported that *Annona squamosa* leaf extract showed significant decrease in rectal temperature. Hyperthermia occur As the infected or damaged tissue promotes the formation of pro-inflammatory mediators (cytokines like interleukin 1β, α, β and TNF-α) which increase the synthesis of PGE2 near

pre-optic hypothalamus area thereby triggering the hypothalamus to elevate the body temperature (**Spacer and Breder 1994)**. The antipyretic activity may be attributed to the presence of phytochemical constituents such as β-sitosterol triterpenes, flavonoids, saponins, glycosides, tannins and alkaloids **(patel *et al.*, 2008)**. The β-sitosterol reduces PG and leukotrienes synthesis and in turn shows anti-inflammatory and antipyretic activity by inhibiting the pro-inflammatory cytokines and TNF-α **(Gupta *et al.*,1996)** and **(Bouic** and **Lamprecht 1999).** The steroids, tannins, and flavonoids are predominant inhibitors of PG synthetase and cyclooxygenase or lipoxygenase, this mechanism helps in inhibition pyrexia **(narayana *et al.*, 2001)**.

Analgesic effect

a-Writhing test: The peripheral anti-nociceptive activity of the 70% ethanolic extracts of *Annona squamosa* leaves and seeds was evaluated using acetic acid induced writhing technique in mice and recorded in table (3). Standard group orally administrated acetylsalicylic acid (100 mg/kg b. wt.) showed 75% protection against writhing induced glacial acetic acid for 5 hours . Oral administration of the 70% ethanolic extracts of *Annona squamosa* leaves in a dose of (250 and 500 mg/kg b. wt.) exhibited significant analgesic activity with 56.4.7% and 70.7 % protection percentage for 5 hours. Seed extract in a dose of (25 and 50 mg/kg b. wt.) exhibited analgesic activity with 19.3% and 37.14% protection percentage for 5 hours. These results correlates with that previously obtained by **Richa Mishra *et al.*, (2012)** who reported that leaves ethanolic extract had a significant peripheral antinociceptive effect. The antinociceptive effect of leaves ethanolic extract could be attributed to the peripheral effects of tannins and flavonoids, including the inhibitition of cyclo-oxygenase in peripheral tissues **Deraedt *et al.*, (1980)**, thus interfering with the mechanism of transduction of primary afferent nociceptors.

Table (3): Showing the analgesic activity of *Annona squamosa* Linn. leaves and seeds 70% ethanolic extract in mice using writhing test (n=5).

Treatment	Dose (mg/Kg b. wt.)	Protection percentage against writhing after 5 hours				
		1 hour	2 hour	3 hour	4 hour	5 hour
Control	0	0	0	0	0	0
Acetylsalicylic acid (standard)	100	75	75	75	75	75
Ethanolic extract of *Annona squamosa* leaves	250	56.4	56.4	56.4	56.4	56.4
	500	70	70	70	70	70
Ethanolic extract of *Annona squamosa* seed	25	19.3	19.3	19.3	19.3	19.3
	50	37.14	37.14	37.14	37.14	37.14

b- Hot plate test

Results of central anti-nociceptive activity of the 70% ethanolic extracts of Annona *squamosa* leaves and seeds in mice are recorded in table (4). Data showed that leaves extract in a dose of 250 and 500 mg/kg b. wt., induced significant analgesic activity in a dose dependent manner since the tested dose relieved the pain in mice exposed to hot plate at different time intervals compared to the control (non treated group). Seeds in a dose of 25 mg/kg b. wt., showed no analgesic activity at the first hour while in a dose of 50mg/kg b. wt., produced analgesic activity compared to the control (non treated group) but less than the effect of the standard drug and leaves extract. Oral administration of the acetylsalicylic acid at a dose of 100 mg/kg b. wt. significantly increased the reaction time.

These obtained results correlates with that obtained by **Richa Mishra *et al.*, (2012)** who found that the ethanolic leaves extract exhibited significant increase in reaction time as compared to the standard drug Acetyl salicylic acid, in addition to **Dash *et al.*, (2001)** who reported that the aqueous extract induce significant central analgesic activity while he diversely mentioned that the ethanolic extracts of the plant had no significant analgesic effect. **Saluja,** and **Santan, (1994)** noted that methanolic seed extract showed significant analgesic activity. The central analgesic activity may be attributed to the presence of tannins and flavonoids which inhibit prostaglandin synthesis which play significant role in different phases of inflammatory reactions.

Table (4): Showing the effect of leaves and seeds 70% ethanolic extract on hot plate reaction time in mice n=5

Treatment	Dose (mg/Kg b.wt)	1 hour	2 hours	3 hours
Control	0	8.9 ± 0.5^{e}	8.1 ± 0.3^{f}	7.5 ± 0.4^{f}
Acetyl salicylic acid (standard)	100	20.4 ± 0.7^{a}	22.8 ± 0.5^{a}	23.2 ± 0.6^{a}
Ethanolic extract of *Annona squamosal* leaves	250	12.9 ± 0.4^{c}	14.8 ± 0.3^{c}	16.4 ± 0.3^{c}
	500	17.2 ± 0.3^{b}	18.8 ± 0.3^{b}	20.1 ± 0.3^{b}
Ethanolic extract of *Annona squamosal* seed	25	8.6 ± 0.4^{e}	10.5 ± 0.11^{e}	12.1 ± 0.2^{e}
	50	10.8 ± 0.21^{d}	12.9 ± 0.5^{d}	14.9 ± 0.3^{d}

Values represent the mean ± S.E. of five animals for each group, values in each column with different superscript letters (a,b,c,d,e,f) are significantly different at $P < 0.05$.

Anti-inflammatory effect

The anti-inflammatory effect of the 70% ethanolic extracts of leaves and seeds was studied using formalin induced edema in rat's paw and data were compared with that of control in table (5). Oral administration of 70% ethanolic extract of *Annona squamosa* leaves in a dose

of 250 and 500 mg/kg b.wt induced a significant decrease in inflamed rat paw thickness in a dose dependent manner when compared with control non treated group for 4 hours. Seeds extract in a dose of 25 mg/kg b. wt. and 50 mg/kg b. wt., also induced a significant decrease in inflamed paw thickness for 4 hours when compared with the control non treated group. These results matched with that previously obtained by **Richa Mishra *et al.*, (2012)** who reported that ethanolic leaf extract possessed marked anti-inflammatory activity by 49.3% and 61.73% inhibition of paw edema and with, **Sharma *et al.*, (2010)** and **Sanjiv (2011)**. Results correlated also with **(Dash *et al.*, 2001)** who reported that the aqueous extracts of *A.squamosa* exhibited anti-inflammatory properties but he diversely mentioned that the ethanolic leaf extracts of the plant had in significant anti-inflammatory effect. **Saluja,** and **Santan, (1994)** who reported that the ethanolic extract of *Annona squamosa* seed failed to inhibit paw edema.

Induction of edema in rat's paw by formalin is a biphasic response, in which the first phase is mediated by histamine, serotonin and kinins whereas the second phase is mediated by prostaglandins (cyclooxygenase product of arachidonic acid metabolism) and production of reactive oxygen species **(Chen, 1993)** and **(Panthong *et al.*, 2004)**. The anti-inflammatory effect of ethanolic leaf extract may be attributed to the inhibition of various chemical mediators of inflammation like histamine and 5-HT during the initial phase **(Harsh, 2000)** or inhibition of pro-inflammatory cytokines and Cox-2 synthesis and subsequent reduction in prostaglandin synthesis **(sharma *et al.*, 2010)** and may be attributed to inhibition of neutrophils infiltration and stabilizing lyzosomal enzymes which play key role in the development of inflammation **(Vijayalakshmi *et al.*, 1997)** and **(Abdul-shakoor *et al.*, 2007)** or due to inhibit liberation of the reactive oxygen species (second phase mediator) from phagocytes **(Cross *et al.*, 1987)** and **(Parke,** and **Sapota, 1996) .**

The link between both anti-nociceptive activity and moderate anti-inflammatory effect observed with the extract has been indicated in non-steroidal anti-inflammatory drugs (NSAIDs). It is a well-established fact that NSAIDs exert their analgesic and anti-inflammatory activity by the inhibition of cyclooxygenase activity **(Vane, 1971)**. Based on the pharmacological tests results, the *Annona squamosa* hydro alcoholic extract showed both anti-nociceptive and anti-inflammatory activities.

Table (5): Showing the anti-inflammatory effect of the 70% ethanolic extracts of *Annona squamosa* leaves *and* seeds in formalin induced edema in paw of rats.(n=5).

Treatment	Dose (mg/Kg b. wt.)	Mean of right paw thickness in mm				
		Pre treatment	1 hours	2 hours	3 hours	4 hours
Control	0	0.67± 0.01^{a}	0.68± 0.01^{e}	0.68± 0.01^{e}	0.68±0.01^{e}	0.67± 0.03^{e}
Acetyl salicylic acid (standard)	100	0.68± 0.01^{a}	0.53±0.02^{a}	0.50± 0.02^{a}	0.49± 0.01^{a}	0.49± 0.01^{a}
Ethanolic extract of *Annona squamosa* leaves	250	0.68±0.01^{a}	0.57±0.01^{c}	0.57±0.01^{c}	0.56±0.1^{c}	0.56±0.1^{c}
	500	0.68±0.01^{a}	0.55±0.01^{b}	0.55±0.01^{b}	0.53±0.01^{b}	0.53±0.01^{b}
Ethanolic extract of *Annona squamosa* seed	25	0.68±0.01^{a}	0.59±0.01^{d}	0.58±0.02^{d}	0.58±0.01^{d}	0.57±0.02^{d}
	50	0.68±0.01^{a}	0.59±0.01^{d}	0.57±0.2^{c}	0.56±0.01^{c}	0.56±0.01^{c}

Values represent the mean ± S.E. of five animals for each group, values in each column with different superscript letters (a,b,c,d,e) are significantly different at $P < 0.05$.

CONCLUSION

Annona squamosa leaves extract is considered to be safe as its LD50 is 5000 mg/kg body weight with many pharmacological activities include antipyretic, analgesic and anti-inflammatory actions that may be a result of its active biochemical ingredients alkaloids, flavonoids, glycosides, saponin and tannin. In addition, hand seed extract also induced a significant analgesic and anti-inflammatory effects with low safety compared with leaves.

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