Global Journal of Pharmacology 13 (2): 40-46, 2019
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2019.40.46

Acute Toxicity Study and Anti-Nociceptive Activity of the Aqueous Extract of a Recipe from Fruit Pulps of *Adansonia digitata, Hyphaene thebaica, Ziziphus mauritiana* and *Z. Spina-christi* in Rats and Mice

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Abstract: The fruit pulps of *Adansonia digitata, Hyphaene thebaica, Z. mauritiana* and *Ziziphus spina-christi* is used traditionally for the analgesic activities in Northeastern Nigeria. There are reports on the activities of the individual plants but not in a recipe. This research was conducted to investigate the acute toxicity and anti-nociceptive activities of the recipe from the fruit pulps of these plants. Equal amounts of the fruit pulps were used to prepare aqueous extract of the recipe. Standard methods for phytochemical analysis were used and OECD method was used to determine the LD_{50} value. Acetic acid induced writhing, formalin test and analgesy meter were used to study the anti-nociceptive activities in rats and mice. The phytochemical analysis of the recipe revealed the presence of saponins glycosides and terpenoids. The oral and intraperitoneal LD_{50} values was >5000 mg/kg in rats and >2000 mg/kg in mice, respectively. There was significant ($p<0.05$) anti-nociceptive activity at the doses of 200, 400 and 800 mg/kg, which was greater than diclofenac (positive control) in mice using acetic acid induced writhing method. The anti-nociceptive activity of the recipe was comparable to diclofenac using formalin test and analgesy meter methods. The aqueous extract of the recipe of the fruit pulps was safe and had anti-nociceptive activities. This has supported the folkloric use of the fruit pulps in controlling pain.

Key words: Fruit Pulps · Recipe · Aqueous Extract · Anti-nociception · *In vivo*

INTRODUCTION

Pain and its treatment is a major concern in the management of any disease condition. Analgesics are almost often included in every prescription to address this problem. Many drugs are available for this purpose but mostly associated with side effects. These include the opioid analgesics and non-opioid analgesics (the non steroidal anti-inflammatory drugs). Opioids are natural or synthetic compounds that include the opiates (morphine, codeine), semi synthetic opioids (apomorphine and etorphine), synthetic opioids (pentazocine and fentanyl) and endogenous opioids (enkephalins and endorphins). They act through their receptors in the central nervous system to increase threshold response to pain [1].

The non-steroidal anti-inflammatory drugs (NSAIDs) are a group of chemically dissimilar agents that differ in their anti-pyretic, analgesic and anti-inflammatory activities and are used for treatment of moderate pain, fever and/or inflammation. They are drugs (such as aspirin and diclofenac) that inhibit one or more steps in the metabolism of arachidonic acid such as prostaglandins

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and leukotrienes. They inhibit the cyclooxygenase (COX) enzyme I or II, or both during the synthesis of the eicosanoids [2-4]. Natural and synthetic steroidal anti-inflammatory agents (glucocorticoids) include hydrocortisone and dexamethasone. They produce anti-inflammatory activities by inhibiting the syntheses of prostaglandins and leukotrienes, probably by inhibiting phosphalipase A_2 [4].

The NSAID may induce ulceration of the gastro-intestinal tracts while opioid analgesics can cause respiratory depression, constipation and dependence [1]. Thus, the need to discover newer agents cannot be over emphasized. Plants are major targets for the isolation and identification of new potential pharmacologic agents including anti-infective and anti-nociceptive or analgesic agents [5]. They are the major source of therapeutic agents. Plants such as *Adansonia digitata, Hyphaene thebaica, Ziziphus mauritiana* and *Z. spina-christi* are common in the North Eastern part of Nigeria and have been consumed as fruit without reported adverse effects. These plants are utilized in folkloric medicine in the management of pain, inflammation and fever. There are scientific reports to support the analgesics properties of the individual plants [6, 7, 8] but no report about their use as a recipe has been documented in the literature. There may be potentiation leading to better efficacy when more than one plants are used in the form of a recipe. Therefore, this work was designed to investigate the acute toxicity and anti-nociceptive activity of a recipe from fruit pulps of *Adansonia digitata, Hyphaene thebaica, Ziziphus mauritiana* and *Z. spina-christi.*

MATERIALS AND METHODS

Sample Collection and Extraction: The fruit pulps of *A. digitata, H. thebaica, Z. mauritiana* and *Z. spina-christi* were collected from Gamboru market, Maiduguri, Borno State and Geidam, Yobe State. The dried fruit pulps were pulverized using wooden mortar and pestle. Equal amounts of each powdered sample were combined together in a container and shaken to form uniform mixture. Maceration method was used to obtain aqueous extract as described previously [8, 10]. The powdered plant mixture was soaked in distilled water at ratio of 1:10 for 3 days with occasional shaking by mechanical means. The resultant mixture/solution was filtered using Whatman filter paper (No. 1) and the filtrate was concentrated to dryness *in-vacuo* at 40°C using rotary evaporator.

Experimental Animals: Albino rats and mice were purchased at the Animal House of Department of Biochemistry and Department of Pharmacology and Toxicology, University of Maiduguri, Borno State. They were kept in plastic cages at the Veterinary Pharmacology Laboratory within the University for a minimum period of one week before the commencement of the experiments. They were fed with grower's mash (Vital Feeds Nig. Ltd., Jos, Nigeria), water provided *ad libitum*. Ethical clearance was obtained from the Research and Ethics Committee, Faculty of Pharmacy, University of Maiduguri with approval number FP/022018/TETFP03 and animals handled in compliance with the international guiding principles for biochemical research involving animals [11].

Phytochemical Analysis: The phytochemical analyses were carried out according to standard methods [9, 12, 13]. Phytochemicals analysed include flavonoids, cardiac glycosides, terpenoids, alkaloids, carbohydrates, saponins glycosides and anthraquinones.

Acute Toxicity Study: The median lethal dose (LD_{50}) was carried out in rats (oral) and mice (intraperitoneal) based on the modified Organization for Economic Co-operation and Development (OECD) guideline [14]. Three rats and six mice were used. One rat in the first phase and two rats in the second phase were all dosed with 5000 mg/kg. One mouse in the first phase and two mice in the second phase were also dosed with 5000 mg/kg. In the third phase, three mice were dosed with 2000 mg/kg.

Acetic Acid-Induced Writhing Test: Twenty-five adult mice of both sexes were randomly separated into five groups of five mice. Those in group A received distilled water (0.1 ml) to serve as negative control group while those in groups B, C and D received 200, 400 and 800 mg/kg body weight (BW), respectively, of the aqueous extract and those in group E received diclofenac (Clofenac SR) 25 mg/kg BW to serve as the positive control group. The route of administration was intraperitoneal route. Thirty minutes later, 0.1 ml of 1% acetic acid solution was injected intraperitoneally to all groups to induce writhing. Writhing response was observed [15]. The number of writhes was counted from five minutes after acetic acid administration for ten minutes. A reduction in the number of writhing as compared with the negative control group was considered as evidence of analgesia. The percentage protection was obtained using the formula below [16].

$$\% \ inhibition = \frac{W_C - W_T}{W_C} \times 100$$

where:
W_C = Mean number of writhes in control group
W_T = Mean number of writhes in test group

Formalin Test: Twenty-five rats of both sexes were randomly separated into five groups of five mice. Treatment was the same as for the thermal nociception test above using the two extracts. After 30 minutes each rat in all groups was injected 0.8 µl of 1% formalin at the plantar surface of the left hind paw and immediately placed in a transparent plastic chamber [17]. The mouse was observed for the first five minutes and then from 20 to 30 minutes after formalin injection. Time spent in licking the injected paw was recorded in seconds during these periods.

Analgesy-Meter Test: Twenty-five rats of either sex were randomly divided into five groups of five rats and subjected to the modified Randall-Selitto [18, 19]. Administration was by oral route. Those in group A received distilled water (0.1 ml) to serve as negative control group while those in groups B, C and D received 200, 400 and 800 mg/kg body weight (BW), respectively, of the extract and those in group E received diclofenac 50 mg/kg BW to serve as the positive control group. The rat's left hind paw was placed between the plinth and plunger of the Analgesy-meter instrument (Ugo Basile, Italy, model 37215) and then pressure was exerted on the middle dorsum of the paw until signs of pain were noticed. Stimulus was terminated and force threshold readings in grams were taken as soon as nociceptive response was elicited. Readings were taken at pretreatment, 30 min and 60 min post treatment.

Statistical Analysis: Data generated during the study was expressed as mean ± standard error of the mean (S.E.M.) and analysed statistically by one-way analysis of variance (ANOVA) and Dunnett post test using Graphpad computer statistical software where P <0.05 was considered significantly different [20].

RESULTS

Phytochemical Screening: The extract yield was 17% dark brown and soluble in water. The phytochemical constituents are presented in Table 1. The results showed that aqueous extract of the recipe contained cardiac glycosides, saponins glycosides, terpenoids and carbohydrates, while flavonoids, alkaloids, soluble starch and anthraquinones were not detected in the extract.

Acute Toxicity Study: The results of the median lethal dose (LD_{50}) determination are presented in Table 2. Out of the three rats treated with the aqueous extract of the recipe at a single oral dose of 5000 mg/kg, non died within 24 h but later one died 5 days after treatment during the 14 days observation period. Depression was observed as clinical signs of toxicity. Hence the LD_{50} value was greater than 5000 mg/kg. In mice, two out of three died following intraperitoneal single dose administration of the extract at 5000 mg/kg. One out of the three mice died in the third phase treated at 2000 mg/kg. Signs observed were depression and rough hair coat. Thus the intraperitoneal LD_{50} value in mice was greater than 2000 mg/kg but less than 5000 mg/kg, based on the OECD guidelines.

Acetic Acid-Induced Writhing Test: The results for the effect of aqueous extract of the recipe on acetic acid induced writhing in mice are shown in Table 3. At the 200, 400 and 800 mg/kg aqueous extract doses, there was inhibtion against writhing at 100, 97.56 and 100% respectively compared to the standard drug (diclofenac 25 mg/kg) with percentage inhibition of 48.22%. The number of writhes in the treatment groups and positive control group were all significantly ($p<0.05$) lower than the negative control group (distilled water).

Formalin Test: The result of the formalin test method is presented in Table 4. The extract showed significant inhibition ($p<0.05$) in both first and second phases of the formalin test. In the first phase there was reduction from 193.20 sec in the control to 133.40 and 116.8 sec in treated groups with 400 and 800 mg/kg aqueous extract respectively, which were similar to the result obtained in the positive control group (132.40 sec). In the second phase there were also significant reduction ($p<0.05$) at the doses of 400 mg/kg (217 sec) and 800 mg/kg (192.40 sec) of the aqueous extract compared to the control (341.20 sec). The reaction time of the positive control was 213 sec ($p<0.05$) in the second phase which was also similar to the control. In both phases there was dose dependent increase in inhibition to formalin-induced pain.

Table 1: Qualitative phytochemical analysis of the aqueous extract of a recipe from fruit pulps of *A. digitata, H. thebaica, Z. mauritiana* and *Z. spina-christi*

Phytochemical constituent	Test	Results
Test for flavonoids	Shinoda's test	-
	Ferric chloride test	-
	Lead acetate test	+
	Sodium hydroxide test	+
Test for cardiac glycoside	Lieberman Burchard test	+
	Salkowski test	+
Test for terpenoid		+
Test for alkaloids	Dragendorff's reagent	-
	Mayer's reagent	-
Carbohydrate	Molisch's test	+
	Test for monosaccharides: Barfoed's	+
	Test for reducing sugar: Fehling's	+
	Test for Combined reducing sugar	+
	Test for Ketoses	+
Saponins glycoside	Frothing test	+
Test for soluble starch		-
Anthraquinones	Free anthraquinones	-
	Combined anthraquinones	-

+ = present; - = not detected

Table 2: Acute toxicity test of aqueous extract of a recipe from fruit pulps of *A. digitata, H. thebaica, Z. mauritiana* and *Z. spina-christi* in rats and mice

Phase	Dose (mg/kg)	No. of Rats	Death*	No. of Mice	Death**
Phase 1	5000	1	0	1	0
Phase 2	5000	2	1	2	2
Phase 3	2000	-	-	3	1

* Depression preceded death

** Depression and rough hair coat preceded death

Table 3: Effect of the aqueous extract of a recipe from fruit pulps of *A. digitata, H. thebaica, Z. mauritiana* and *Z. spina-christi* on acetic acid induced writhing in mice

Group	Dose (mg/kg)	No. writhes (mean±SEM)	% inhibition
Control (Dist. water)	0.1ml	57.40±4.70	-
Diclofenac	25	32.00±4.69*	48.22
Recipe (low dose)	200	0.00±0.00*	100.00
Recipe (medium dose)	400	1.40±1.40*	97.56
Recipe (high dose)	800	0.00±0.00*	100.00

* $p<0.05$ compared to the control, values are in mean±SEM

Table 4: Effect of the aqueous extract of a recipe from fruit pulps of *A. digitata, H. thebaica, Z. mauritiana* and *Z. spina-christi* on formalin test in rats

Group	Dose (mg/kg)	First phase	Second phase
Control (Dist. water)	0.1ml	193.20±12.60	341.20±32.19
Diclofenac	50	132.40±15.06*	213.00 ±11.42*
Recipe (low dose)	200	181.00±18.88	338.80±39.81
Recipe (medium dose)	400	133.40±4.16*	217.00±33.81*
Recipe (high dose)	800	116.80±22.96*	192.40±33.88*

* $p<0.05$ compared to the control, values are in mean±SEM

Table 5: Effect of aqueous extract of a recipe from fruit pulps of *A. digitata, H. thebaica, Z. mauritiana* and *Z. spina-christi* on analgesy-meter in rats

		Threshold (Mean±SEM x20 g)		
Group	Dose (mg/kg)	0 min	30 min	60 min
Control (Dist. water)	0.1ml	2.66±0.34	2.30±0.29	3.00±0.31
Diclofenac	50	2.88±0.61	6.32±0.68*	6.18±0.79*
Recipe (low dose)	200	2.58±0.37	3.74±0.22*	5.54±0.69*
Recipe (medium dose)	400	3.46±0.49	9.10±1.57*	8.76±1.30*
Recipe (high dose)	800	3.62±0.61	4.56±0.38	6.60±0.76*

* $p<0.05$ compared to the zero minute value within the same row, values are in mean±SEM

Analgesy-Meter Test: The result of the aqueous extract of the recipe using analgesy-meter test is shown in Table 5. The extract induced significant ($p<0.05$) increase in threshold to pain at all the doses when compared to the respective initial pretreatment readings. Diclofenac (positive control) increased the threshold to pain significantly ($p<0.05$) from 2.88±0.61 x20g to 6.32±0.68 x20g and 6.18±0.79 x20g thirty minutes and sixty minutes post administration, respectively. At 200 mg/kg, the threshold increased significantly ($p<0.05$) from 2.58±0.37 x20g before administration to 3.74±0.22x20g and 5.54±0.69 x20g within the same period of measurements, respectively. Highest activity was recorded at the dose of 400 mg/kg from pretreatment value of 3.46±0.49 x20g to significant ($p<0.05$) levels of 9.10±1.57 x20g and 8.76±1.30 x20g at thirty minutes and sixty minutes post administration, respectively.

DISCUSSION

The phytochemical analysis of the aqueous extract of the recipe from fruit pulps of *A. digitata, H. thebaica, Z. mauritiana* and *Z. spina-christi* revealed the presence of cardiac glycosides, saponins glycosides, terpenoids and carbohydrates. These fruit pulps are consumed locally not only because of their nutritional values but also they are important nutriceuticals. Nutriceuticals reduce symptoms of pain, fever and inflammation thereby improving the health conditions of patients. It has been reported the presence flavonoids, saponins, alkaloid, triterpenes, tannins, cumarin and glycoside in the fruit pulps of *A. digitata* [21]. In the fruit pulp of *Ziziphus spina-christi and Z. mauritiana*, flavonoids (e.g. quercetin and rutin), alkaloids, terpenes, tannins, saponins and sterols were isolated [22, 23]. While flavonoids have been reported to be present in most of the individual fruit pulps, these have not been detected in the recipe. Saponins and terpenoids which were present in the recipe and have been reported in the individual fruit pulps, constitute important secondary plant metabolites. Some of these secondary metabolites may have analgesic effects [24, 25, 26].

The acute toxicity studies showed that the extract of the recipe had oral median lethal dose (LD_{50}) value in rats greater than 5000 mg/kg and intraperitoneal LD_{50} value in mice greater than 2000 mg/kg. These imply the extract exhibit high safety in both rats and mice with no overt clinical signs of toxicity. According to the OECD guidelines [14], the extract is classified in category 5 on the Global Harmonized System (GHS) of classification and labeling. Also any substance with oral LD_{50} greater than 1000 mg/kg in rats is regarded as being of low toxicity or relatively safe [27]. Humans have been consuming the components of the recipe for thousands of years without reported toxic effects. The safety margin observed in this study supports the traditional use of the fruits as non-toxic substances for food and medicine.

The anti-nociceptive study of the extract of the recipe using two (chemical and mechanical) pain models revealed that the extract had significant anti-nociception in rats and mice. In the chemical pain model, acetic acid induced writhing and formalin test were used in mice and rats, respectively. The extract conferred 100, 97.56 100% protection against writhing at the dose of 200, 400 and 800 mg/kg, respectively. These were significantly ($p<0.05$) greater than the 48.22% protection conferred by the standard drug, diclofenac at 25 mg/kg. This model is used to evaluate anti-nociception and analgesia mediated locally by the inhibition of release of chemicals such as prostaglandins, histamine and serotonin [28]. The significant ($p<0.05$) graded inhibition observed in both first and second phases of formalin test indicates that the recipe has activity on both neurogenic and inflammatory process induced pain. This further confirmed the result of the acetic acid induced writhing because chemical mediators of acute inflammation and nociception are the same (prostaglandins, serotonin, histamine and bradykinin). Unlike the second phase, the first phase is transient caused by direct effect of formalin on sensory C-type fibres and the second phase is because of the release of chemical mediators [28, 29]. Centrally mediated anti-nociceptives inhibit both phases of formalin test (30), thus the recipe may also mediate its activity centrally.

In the mechanical pain model, analgesy meter method was used to evaluate centrally mediated anti-nociception. There was significant increase to threshold to pressure-induced pain at 30 minutes and 60 minutes in extract treated groups at 200 and 400 mg/kg and diclofenac 50 mg/kg in rats. This further signifies that the extract of the recipe possess centrally acting activity. Centrally acting analgesics elevate pain threshold towards heat and pressure [31]. Thus the recipe of the extract revealed both locally and centrally mediated anti-nociceptive activities in this study. These may be due to the presence of secondary metabolites such as saponins and terpenoids identified in the extracts. Previous work demonstrated that saponins can inhibit the release of histamine which is a local pain mediator [24, 26]. Ginkgolide B from *Ginkgo biloba* is a terpenoid known to have inhibitory effects on platelet-activating factor (PAF). PAF which stimulate conversion of phospholipids to arachidonic acid, influences biosynthesis of prostaglandins and other mediators of pain and inflammation [25].

The individual fruit pulps have been studied for their pharmacological activities showing anti-nociceptive activities [6, 8]. The formulation of the extract in a recipe and its biological activities was tested for the first time in this study. The recipe represents a smaller fraction of each pulp but anti-nociceptive activities were observed. There may be synergism of activity and reduced toxic effect in a recipe compared to the use of individual plant.

CONCLUSION

This study showed that the aqueous extract of the recipe of the fruit pulps of *A. digitata, H. thebaica, Z. mauritiana* and *Z. spina-christi* was safe and had anti-nociceptive activities in laboratory animals which support the folkloric use.

ACKNOWLEDGMENTS

We acknowledge technical assistance from Mr. Yusuf Zongoma, Mrs. Lateefat Nana Giwa Imam, Mr. Abdullahi Usman and Mr. Michael Bitrus Bulama from the Veterinary Pharmacology Laboratory and Mr. Fine Akawu from Chemistry Research Laboratory, University of Maiduguri. This work was supported by Institution Based TETFund research grant of University of Maiduguri (TETFUND/ DESS/ UNIMAID/ MAIDUGURI/ RP/VOL.VI).

REFERENCES

1. Schumacher, M.A., A.I. Basbaum and W.L. Way, 2004. Opioid analgesis and antagonists. In: Basic and Clinical Pharmacology 9th ed. Ed., Katzung B.G. The Mr Graw -Hill companies Inc., pp: 512.
2. Boothe, D.M., 2001. The Analgesic, Antipyretic, Anti-inflammatory Drugs. In: Veterinary Pharmacology and Therapeutics 8th ed. Ed., Adams H.R. Ames, Iowa State University Press, U.S.A., pp: 434.
3. Wagner, W., P. Khanna and D.E. Furst, 2004. Non-steroidal anti-inflammatory drugs, disease-modifing antirheumatic drugs, nonopioid analgesics and drugs used in gout. In: : Basic and Clinical Pharmacology 9th ed. Ed., Katzung B.G. The Mr Graw -Hill companies Inc., pp: 577.
4. Burke, A., E. Smyth and G.A. Fitz Gerald, 2006. Analgesic-Antipyretic and Anti-inflammatory Agents; Pharmacotherapy of Gout. In: Goodman's and Gilman's The Pharmacological Basis of Therapeutics 11th ed. Eds., Brunton L.L., J.S. Laso and K. Parker. New York, U.S.A. McGraw-Hill Companies Inc., pp: 671-701.
5. Mahesh, B. and S. Satish, 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sc., 4(S): 839-843.
6. Ramadan, A., F.M. Harraz, S.A. El-Mougy, 1994. Antiinflammatory, analgesic and antipyretic effects of the fruit pulp of *Adansonia digitata*. Fitoterapia, 65: 418-422.
7. Adzu, B. and A.K. Haruna, 2007. Studies on the use of *Zizyphus spina-christi* against pain in rats and mice. Afr. J. Biotechnol., 6(11): 1317-1324.
8. Farag, M.A. and P.W. Pare, 2013. Phytochemical analysis and anti-inflammatory potential of *Hyphaene thebaica* L. Fruit. J. Food Sci., 78(10): 1503-1508.
9. Harborne, J.B., 1988. Phytochemical Methods: A Guide to Modern Techniques of plant Analysis. London Chapman and Hall, pp: 277.
10. Usman, H., F.I. Abdulrahman and A. Usman, 2009. Qualitative phytochemical screening and *in-vitro* antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). Afr. J. Trad. CAM, 6(3): 289-295.
11. CIOMS, 1985. Council for International Organization of Medical Sciences. 1211 Geneva, 27, Switzerland, c/o W.H.O.

12. Evans, W.C., 2002. Textbook of Pharmacognosy 15th ed. London, U.K., W.B. Saunders Co. Ltd., pp: 24-28.
13. Sofowora, E.A., 2008. Medicinal Plants and Traditional Medicine in Africa 3rd ed. Ibadan, Nigeria, Spectrum books Ltd., pp: 117-139, 195-248.
14. OECD, 2001. Organization for Economic Cooperation and Development. Harmonized Integrated Classification System for Human Health and Environment Hazards of Chemical Substances and Mixtures. EN V/JM/MONO.
15. Turner, R.A., 1965. Screening Methods in Pharmacology. Vol. 1 New York, U.S.A., Academy Press.
16. Hernandez-Perez, M., R.M. Rabanal, M. Camaandela-Torre and B. Rodriguez, 1995. Analgesic, anti-inflammatory, antipyretic and haematological effects of aethiopinone, an O-napthoquinone ditherpenoid from *Saliva aethiopis* roots and two hemisynthetic derivativrs. Plant Medica, 61: 505-509.
17. Hunskar, S. and K. Hole, 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain, 30: 103-114.
18. Randall, L.O. and J.J. Selitto, 1957. A method for measurement of analgesic activity on inflamed tissue. Achiev. Int. Pharm., 111: 409-419.
19. Adzu, B., B.A. Chindo, F.D. Tarfa, O.A. Salawu, O.J. Igoli, 2015. Isolation and analgesic property of lupeol from *Diospyros mespiliformis* stem bark. J. Med. Plants Res., 9(30): 813-819.
20. Graphpad, 2003. Graphpad InStat® version 3.00 for windows 95, Graphpad Software, San Diego California USA.
21. Abdelrahim, M.Y., B.M.A. Elamin, D.J. Khalil, S.M.A. El-Badwi, 2013. Antidiarrhoeal activity of ethanolic extract of *Adansoniadigitata* fruit pulp in rats. J. Phys. Pharam. Adv., 3(6): 172-178.
22. Pawlowska, A.M., F. Camangi, A. Bader and A. Braca, 2008. Flavonoids of *Zizyphus jujuba* L. and *Zizyphus spina-christi* (L.) willd (Rhamnaceae) fruits. Food Chem., 112(4): 858-862.
23. Mishra, T. and A. Bhatia, 2014. Antiplasmodial effects of the aqueous ethanolic seed extract of *Ziziphus mauritiana* against *Plasmodium berghei* in Swiss albino mice. Intl. J. Pharmacol. Res., 4(3): 111-116.
24. Rao, A.V. and D.M. Gurfinkel, 2000. Bioactivity of Saponins: Triterpenoids and Steroidal Glycosides. Canada, Freund Publishing House Ltd., pp: 211-235.
25. Samuelsson, G. and L. Bohlin, 2009. Drugs of Natural Origin A Treatise of Pharmacognosy 6th ed. Stockholm, Sweden, Swedish Pharmaceutical Press, pp: 426.
26. Mandade, R.J., V. Choudhuri, V. Mashirkar and D. Sakarkar, 2010. Analgesic and Anti-inflammatory activities of *Hedera helix* leaf extract. Pharmacie Globale (IJCP), 4(7): 1-3.
27. Clarke, C.G. and M.L. Clarke, 1977. Veterinary Toxicology 1st ed. London, Bailliere Tindall, pp: 10-20.
28. Di Rosa, M., J.P. Givoud and D.A. Wiloughby, 1971. Studies of the mediators of acute inflammatory responses induced in rats in different sites by carrageenan and turpentine. J. Pathol., 104: 15-29.
29. McNamara, C.R., J. Mandel-Brehm, D.M. Bautista, J. Siemens, K.L. Deranian, M. Zhao, N.J. Hayward, J.A. Chong, D. Julius, M.M. Moran and C.M. Fanger, 2007. Trpa1 mediates formalin-induced pain. PNAS, 104(33): 13525-13530.
30. Tjolsen, A., O.G. Berge, S. Hunskaar, J.H. Rosland and K. Hole, 1992. The formalin test: An evaluation of the method. Pain, 51(1): 5-17.
31. Singh, S. and D.K. Majumdar, 1995. Analgesic activity of *Ocimum sanctum* and its possible mechanism of action. Intl. J. Pharmacogn., 33: 188-192.