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Anticonvulsant activity of *Nigella sativa*, *Aegle marmelos* and *Benincasa hispida* in Pentylenetetrazole induced seizure in Swiss albino mice

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ABSTRACT

Aims and Objectives: To test anticonvulsant properties of three medicinal plants with pentylenetetrazole induced seizure test in Swiss albino mice. **Materials and Methods:** Aqueous extract of *Aegle marmelos* leaves (AmAE),Ethanolic extract of *Nigella sativa* seeds (NsEE) and crude extract of *Benincasa hispida* fruit juice (BhE)were prepared. Qualitative phytochemical analysis of the test extracts were done with Preliminary chemical reaction tests, and Liquid chromatography–mass spectrometry (LC/MS). Male Swiss albino mice, 3-4 months of age, weighing 25-30g were used in the study. Extracts and vehicle were administered orally 1 hour prior to inducing convulsions with Pentylenetetrazole (PTZ) induced seizure test [60mg/kg/i.p]. Onset of first jerky movement, onset of Straub's tail, onset of clonic convulsions, onset of tonic flexion, onset of Hind limb tonic extension (HLTE) and reduction in mortality were measured. Animals were treated with extracts at a dose of 900mg/kg orally. The results were compared with control group and test for analysis of variance and significance was done (ANOVA, Dunnett's test). **Results:** Aqueous extract of *Aegle marmelos* leaves and Ethanolic extract of *Nigella sativa* seeds possess anticonvulsant property in Pentylenetetrazole (PTZ) induced seizure model, Crude extract of *Benincas ahispida* fruits did not show statistically significant anticonvulsant property. **Conclusions:** *Aegle marmelos* leaf and *Nigella sativa* seed possess anticonvulsant properties in pentylenetetrazole induced seizures, this is in accordance with many other published reports on the same herbs with different animal models and indicates effectiveness in human absence seizure patients.

Key words: Pentylenetetrazole (PTZ) induced seizure test, Anticonvulsant, *Aegle marmelos*, *Nigella sativa*, *Benincasa hispida*, Sodium valproate, Ethosuximide, Swiss albino mice

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INTRODUCTION

Indian Vedic literature, primarily Ayurvedic scripts have mentioned about many herbal preparations and are being used in the treatment of epilepsy, but most of them are not scientifically validated. Many organizations like WHO and ICMR have expressed the urgent need for validation of indigenous medicines as these could be potential alternative to fill in the treatment gap due to lack of sufficient supply and further which may help in the discovery of novel active principles and targets. In this research study we have selected three medicinal plant parts *Nigella sativa* seeds, *Aeglemar melos* leaves and *Benincasa hispida* fruits, for they are cited in ethnomedicine to possess antiepileptic activities but have no scientific validation. Hence we felt it would be worthwhile to scientifically study and validate their antiepileptic properties.

Aegle marmelos (Linn) correa, commonly known as bael (or bel) is a moderate-sized, slender and aromatic tree.

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It is indigenous to India and is abundantly found in the Himalayan tract, Bengal, Central and South India. It is extensively planted near Hindu temples for its wood and leaves which are generally used for worship. Leaves of this plant is used along with many other plant materials for the treatment of continuous fever; convulsions; constipation and watery diarrhoea.[1,2] Nomenclature:[1] Botanical name: *Aegle marmelos* (L.) *Corr.,* Syn.: *Crataeva Marmelos Linn.,* Family: Rutaceae, Vernacular names: English: Wood apple, Bael tree., Sanskrit: Bilwa, Shriphal., Hindi: Shriphal, Bel. Pharmacological activities of *Aegle marmelos* leaves: Antifungal Activity,[2,3] Anti-inflammatory activity,[4-6] Antidiabetic activity,[7-9] Antidiarrhoeal activity,[10,11] Analgesic activity,[12,14] Radioprotective effect,[13] Anxiolytic and antidepressant effects.[14,15] Studies on *Aegle marmelos* leaves aqueous extract report Pb (II) ion adsorption from aqueous solution by leaves,[16] contraceptive efficacy in male albino rats,[17] anti-pyretic and antidiarrhoeal,[18] Anti-Ulcer,[19] Anxiolytic[20] and Abortificient effects in albino rats.[21]

Benincasa hispida[22-24]

The Sanskrit word kusmanda literally means that, fruit, which does not contain heat at all. *Benincasa hispida* (Thunb.) Cogn (Cucurbitacecae) fruit is widely used as a vegetable in India and other tropical countries. The fruit *B. hispida* is an important ingredient of "Kushmandalehyam" (Ayurvedic medicine) which is widely used in epilepsy and other nervous disorders,[25] and as anorectic.[26] Some of the important isolated constituents of *B. hispida* reported were triterpenes, sterols and glycoside and volatile oils.[27] Nomenclature: *Benincasa hispida*, (synonym – *benincasa cerifera*), Family - Cucurbitaceae,Genus - Benincasa. Activities reported from pharmacological studies are Gastroprotective,[28,29] antinociceptive,[29] anticompulsive.[30]

Nigella sativa

Nigella sativa commonly known as karayal is an annual flowering plant, native to southwest Asia. Seeds and their oil have a long history of folklore usage in various systems of medicines and are used in food as well as medicine. Leaves of *Aegle marmelos Corr* with the fruits of *Nigella sativa* and *Piper nigrum* could be used to treat convulsions.[31,32] Nomenclature: Family: *Ranunculaceae*, Genus: *Nigella*, Species: *sativa*, Vernacular names: *Nigella sativa* commonly known as karayal, English: Small Fennel, Black Cumin Sanskrit: Kalonji, Kalajira, Kalajaji, Mugrela,Upakuncika. Pharmacological actions: Antimicrobialactivity,[33-35] Hepatoprotectiveactivity,[36-38] Hypolipidemicactivity,[37-40] Anticanceractivity,[38-40] Antidiabetic and effects in metabolic syndrome,[41] Gastroprotective activity.[42,43]

MATERIALS AND METHODS

Ethical approval

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC Reg.no 347/CPCSEA), Yenepoya University and care of laboratory animals were taken as per CPCSEA guidelines.

Collection of the plant materials

Green leaves of *Aegle marmelos* were collected locally from Someshwar, Mangalore, Karnataka, *Nigella sativa* seeds and *Benincasa hispida* fruits were procured from Central Market, Mangalore.

Authentication

The test herbs were authenticated by Taxonomist, Prof. Krishnakumar, Department of Applied Botany, Mangalore University, Karnataka, India. One voucher specimen was submitted to Department of Pharmacology, Yenepoya Medical College Mangalore, Karnataka, India.

Extraction

The collected leaves of *A.marmelos* were washed, air-dried, powdered and extracted with Soxhlet apparatus, in portions of 200g, with distilled water at 90°C temperature for 3 days. After exhaustive extraction, the collected aqueous extract was dried on a water bath at 60°C and kept under refrigeration. The final yield of the extraction was 12.5%.

Nigella sativa shade dried seeds were powdered and extracted with Soxhlet apparatus, in portions of 200g, with 99% ethanol at 60°C temperature for 3 days. After exhaustive extraction, the collected ethanolic extract was dried on a water bath at 50°C and kept under refrigeration. The final yield of the extraction was 25%.

Benincasa hispida fresh fruit pulp juice was filtered and dried on a water bath at 60°C.

Preparation of Test drugs and extracts: 0.2 % Dimethyl sulfoxide (DMSO) was used uniformly for all the extracts and drugs as vehicle.For control group plain 0.2% DMSO was selected.

Qualitative phytochemical analysis of the test extracts were done with Preliminary chemical reaction tests[44]and Liquid chromatography–mass spectrometry (LC/MS).[45-47]

Experimental animals

Male Swiss albino mice aged 3-4 months, weighing 25-30g were used in the study.Experimental Animals were housed (Animal house, Yenepoya University, Reg. no 347/CPCSEA) in polypropylene cages and maintained at temperature (25 ±2° C) and light (light period, 06.00–

18.00) in a controlled room with relative humidity of 50–55%. For the automated illumination control, a timer device was designed and installed in the animal house. Food and water were provided *ad libitum*. Experiments were carried out between 15:00 and 19:00 h.

Grouping and dosage

50 male Swiss albino mice were grouped into five groups, on control and three test groups (*Aegle marmelos* 900mg/kg, *Benincasa hispida* 900mg/kg, *Nigella sativa* 900mg/kg. Two standard groups (Sodium valproate 100mg/kg, Ethosuximide 125mg/kg). Route of administration was oral for all the treatmentgroups.

Procedure

1. Mice were brought to the laboratory and allowed them to acclimatize to the laboratory for at least 30 min before test.
2. Test extracts were diluted with 0.2 % Dimethyl sulfoxide.
3. Mice were selected, weighed, and unique identifying mark on each animal was placed. Mice were returned to their holding cages to await dosing. Care was taken not to mix mice that were housed in different home cages to avoid untoward behavioural reactions (e.g., aggression).
4. 0.5ml Vehicle (0.2 % Dimethyl sulfoxide) was administered to each mouse of the control group. Administration was staggered (1 to 3 min intervals) to maintain the same time between compound administration and testing for each animal.
5. Mice were returned to their holding cages as soon as they were treated with the test compound or vehicle.
6. After the post-treatment time (One hour) has elapsed, by grasping the mouse firmly by the nape of the neck, pentylenetetrazole at 60mg/kg/body weight intraperitoneally (i.p) was administered.
7. Following PTZ injection, mouse was immediately placed into a separate observation cage to monitor the course of the seizure.
8. The blockade of clonic convulsions or an increase in convulsion latency (of different phases) was recorded.

Analysis of the result

1. Latency for onset of different phases of convulsion (jerky movements, Straub's tail, clonus, tonic flexion and tonic hind limb extension) and mortality per group were compared with control group using Dunnett's test after ANOVA.

RESULTS

Preliminary phytochemical analysis indicates the presence of steroids, flavonoids, tannins, saponins, glycosides, carbohydrates, alkaloids in aqueous extract of *Aegle marmelos* leaves (AmAE). In ethanolic extract of *Nigella sativa* seeds(NsEE) glycosides, carbohydrates, alkaloids were present. In Crude extract *of Benincasa hispida* fruits(BhE) terpenoids, steroids, carbohydrates, alkaloids were present. (Table 1).

Liquid chromatography–mass spectrometry (LC/MS) analysis and matching of the mass charge ratio (m/z, Da) with existing databases identified the presence of Lupeollinoleate, Skimmianine, Eugenol in aqueous extract of *Aegle marmelos* leaves. In ethanolic extract of *Nigella sativa* seeds two compounds were identified, nigellidine&nigell amine-N-oxide.

Pentylenetetrazole (PTZ) induced seizure model; aqueous extract of *Aegle marmelos* leaves and ethanolic extract of *Nigella sativa* seeds possess anticonvulsant property. Crude extract of *Benincasa hispida* fruit did not show statistically significant anticonvulsant property. (Table 2).

As seen in the graph Ethanolic extract of *Nigella sativa* seeds showed effect on all the phases of Pentylenetetrazole(PTZ) induced seizure but Aqueous extract of *Aegle marmelos*

Table 1: Preliminary phytochemical analysis

	TE	ST	FL	TA	PH	SA	G	CA	AL
Am (AQ)	-	+	+	+	-	+	+	+	+
Ns (ETH)	-	-	-	-	-	-	+	+	+
Bh (CRUDE)	+	+	-	-	-	-	-	+	+

Am: *Aegle marmelos*, Ns: *Nigella sativa*, Bh: *Benincasa hispida*. TE: Terpenoids, ST: Steroids, FL: Flavonoids, TA: Tannins, PH: Phenols, SA: Saponin, G: Glycosides, CA: Carbohydrates, AL: Alkaloids, FL: Flavonoids

Table 2: Test for anticonvulsant property in pentylenetetrazole (PTZ) induced seizure model

Treatment	First jerk	Onset- straubs tail	Onset- clonic convulsions	Onset - tonic flexion	Onset - HLTE
NsEE 900 mg/kg	80.±6.4***	84±13.13***	156±37.14***	636±138.26***	637±138.48***
AmAE 900 mg/kg	56±2.24	54±16.88*	61±6.78	378±44.47***	379±43.068***
BhE 900 mg/kg	40±6.15	53±7.79	68±16.19	52±10.8	82±18.89
Control	32±1.28	41±5.13	45±4.89	46±12.9	48±13.48
Sodium valproate	122±12***	183±165***	1472±385***	-	-
Ethosuximide	-	-	-	-	-

NsEE: *Nigella sativa* Ethanolic Extract, AmAE: *Aegle marmelos* Aqeous Extract, BhE: *Benincas hispida* crude extract. *p value<0.05, ***p value<0.001. Tests –ANOVA followed by Dunnet's test

leaves exhibited weak effect on delaying initial phases of PTZ induced convulsions(First jerky movement, Straub's tail, Clonus) compared to the effect on delay in onset of tonic phase. (Figure 1).

DISCUSSION

In the year 1944 Everett and Richards demonstrated that seizures induced by pentylenetetrazole could be blocked by trimethadione and phenobarbital but not by phenytoin. A year later Lennox reported that trimethadione was effective in decreasing or preventing petitmal (i.e., absence seizures) attacks in 50 patients and that it was not effective or worsened generalised tonic clonic seizures. This established Pentylenetetrazole induced seizure test as a model for absence seizures. In a way this was reconfirmed based on the reports that drugs effective against absence seizures blocked the clonic phase of pentylenetetrazole induced seizures. In rodent models involving maximal or supramaximal convulsive stimuli, tonic extension of the forelimbs or hind limbs is the typical convulsive endpoint. During the tonic extensor phase of a seizure, the forelimbs are rigid and extended caudally against the ventral surface of the body, while the hind limbs are rigid and extended horizontally and away from the prone or supine body in a caudal direction. This tonic extensor phase is characteristic of seizures evoked by maximal electroshock or high doses of chemoconvulsants.[48,49]

Facts that would aid in logical analysis of the results from Pentylenetetrazole induced seizure test are,[50-52]

a) Majority of drugs effective in Pentylenetetrazole induced seizure test are found to be useful in human absence seizures, but predictive power of the model to clinical use is less compared to the Maximal Electroshock induced Seizure test, i.e. further tests specific for absence seizure before clinical trials are required.
b) Neuronal Calcium channel modulators suppress in Pentylenetetrazole induced seizures and a less potent activity by GABAergic drugs; this is similar to human absence seizures.
c) Pentylenetetrazole is reported to be $GABA_A$ receptor antagonist which correlates with behavioural features of Pentylenetetrazole induced seizures. With increasing doses, Pentylenetetrazole is reported to induce phases of seizure in the following order, clonic convulsions, tonic convulsions, and tonic hind limb extension. The clonicphase indicates inhibitory GABAergic currents disrupted by Pentylenetetrazole and tonic phase is seen when sufficient blockade of GABAergic system is attained.
d) There are reports that activation of $GABA_B$ receptor in the globus pallidus may gate pentylenetetrazole-induced tonic seizures. Further $GABA_B$ receptor activation serves to inhibit globus pallidus activity, which "closes the gate," thereby explaining the antiseizure effect on tonic seizure activity.
e) Different phases of Pentylenetetrazole (PTZ) induced seizure correlate with progressive distribution and effect of PTZ to spinal cord and different areas of brain. First jerky movements and Straub's tail -Spinal cord below C6, Clonus – Brainstem (inferior colliculus), Tonic extensor phase – Brainstem (nucleus reticularis pontisoralis).[36]

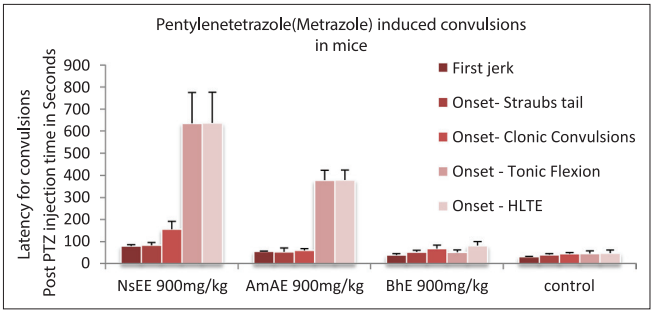

Figure 1: Comparison of anticonvulsant property in Pentylenetetrazole (PTZ) induced seizure model

Pentylenetetrazole interferes with the GABAergic inhibitory control which aids several neuronal circuits propagate action potentials leading to seizure initiation. In case of Pentylenetetrazole, the site of initiation is reported to be the Limbic system. With further blockade of more and more GABA ergic inhibition by Pentylenetetrazole, seizure propagates through brain stem and probably when this reach brainstem through cerebellum the Tonic Phase characterized by spasm of muscles appears. Tonic phase appear at supra maximal doses without a clonic phase or without much demarcation between the phases in the control group where lag between clonic convulsion and tonic convulsion was only 1-4 seconds. From the time of Pentylenetetrazole administration the mean latency for clonic convulsion was 45 seconds and for tonic phase it was 46 seconds in control group. In NsEE treated group onset of clonic phase was after 2½ minutes (mean) and tonic phase was after 10½ minutes (mean). In case of AmAE clonic phase was after one minute (mean) and tonic phase was after 5 minutes (mean). This clearly shows that the extracts treated group differs in seizure behaviour from control groups.

We would propose that *Aegle marmelos* leaves and *Nigella sativa* seeds possess antiepileptic properties and could be useful in human patients. The possible mechanism of action as indicated by the seizure inhibitory pattern and theories discussed in previous paragraphs could be,

Competitive $GABA_A$ receptor agonist with or without $GABA_B$ receptor activation or a less probable weak calcium channel blocker activity.

The findings of the study support the traditional uses of these medicinal plants as antiepileptic medication. Ethanolic extract of *Nigella sativa* seeds possess CNS depressant effects in addition to anticonvulsant properties. Aqueous extract of *Aegle marmelos* leaves is more potent in Pentylenetetrazole induced seizure test and Ethanolic extract of *Nigella sativa* seeds in Maximal Electroshock induced Seizure (MES) test when compared to the previous MES experiments in our lab,[51,52] this indicates the probability of Sodium/Calcium channel mediated mechanism of action for Aqueous extract of *Aegle marmelos* leaves and GABAergic Mechanism for Ethanolic extract of *Nigella sativa* seeds.

Since it is observed that *Aegle marmelos* leaves and *Nigella sativa* seeds possess anticonvulsant properties there is good scope for,

- Isolation and screening of active constituents for their anticonvulsant properties, other pharmacokinetic and pharmacodynamic properties, drug interaction profiles and toxicity profiling.
- Isobolographic analysis of drug interactions between traditional preparations or the extracts of *Aegle marmelos* and *Nigella sativa* with established drugs with detailed pharmacodynamic and pharmacokinetic parameters would be worthwhile.
- Clinical trials of traditional preparations with these plant parts or with their extracts to test the usefulness in treatment of epilepsy.
- *Benincasa hispida* fruit could be screened with 6HZ model, Kindled seizure models for antiepileptogenic effects, and with different extraction methods.

REFERENCES

1. Nadkarni KM, ed. [Indian materiamedica]; Dr. KM Nadkarni's Indian materiamedica: with Ayurvedic, Unani-Tibbi, Siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & indexes. Vol. 2.Popular Prakashan, 2012.
2. Rana BK, Singh UP and Taneja V. Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos*. Journal of Ethnopharmacology 1997; 57(1): 29-34.
3. Balakumar S, Rajan S, Thirunalasundari T and Jeeva S. Antifungal activity of *Aegle marmelos* (L.) Correa (Rutaceae) leaf extract on dermatophytes. Asian Pacific Journal of Tropical Biomedicine 2011; 1(4):309-312.
4. Angajala G, Ramya R and Subashini R. In-vitro anti-inflammatory and mosquito larvicidal efficacy of nickel nanoparticles phytofabricated from aqueous leaf extracts of *Aegle marmelos* Correa. Actatropica 2014; 135: 19-26.
5. Patil RH, Kumar N, Babu RL and Sharma SC. Anti-inflammatory activity of Vitexnegundo, Boswelliaserrata and *Aegle marmelos* leaf extracts in LPS treated A549 cells. International Journal of Phytopharmacy 2015; 5(2): 12-20.
6. Arul V, Miyazaki S and Dhananjayan R. Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos* Corr. Journal of Ethnopharmacology 2005; 96(1): 159-163.
7. Panda S and Kar A. Evaluation of the antithyroid, antioxidative and antihyperglycemic activity of scopoletin from *Aegle marmelos* leaves in hyperthyroid rats. Phytotherapy Research 2006; 20(12): 1103-1105.
8. Radha R and Amirthaveni M. Hypoglycemic effect of vilvai leaf (*Aegle marmelos*) powder on type II diabetics. Ind J Nutr Dietet 2007; 44(11): 515-521.
9. Arumugam S, Kavimani S, Kadalmani B, Ahmed ABA, Akbarsha MA, and Rao MV. Antidiabetic activity of leaf and callus extracts of *Aegle marmelos* in rabbit. Sci Asia 2008;34: 317-321.
10. Hameed MA, Amir F, Chin KY and Fathima S. Phytochemical screening, Anti-pyretic and antidiarrhoeal activities of the n-hexane and aqueous extracts of the leaves of *Aegle marmelos*. Archives of Pharmacy Practice 2011; 2(3): 90.
11. Rao G and Lakshmi P. Evaluation of Anti diarrhoeal activity of extract from leaves of *Aegle marmelos*. J Appl Pharm Sci 2012; 2(2): 75–78.
12. Shankarananth V, Balakrishnan N, Suresh D, Sureshpandian G, Edwin E and Sheeja E. Analgesic activity of methanol extract of *Aegle marmelos* leaves. Fitoterapia 2007; 78(3): 258-259.
13. Jagetia GC, Venkatesh P and Baliga MS. Evaluation of the radioprotective effect of bael leaf (*Aegle marmelos*) extract in mice. International Journal of Radiation Biology 2004; 80(4): 281-290.
14. Kothari S, Minda M and Tonpay SD. Anxiolytic and antidepressant activities of methanol extract of *Aegle marmelos* leaves in mice. Indian Journal of Physiology and Pharmacology 2010; 54(4): 318-328.
15. Jyothi R, Bhandare B and Satyanarayana V. Evaluation of Antidepressant activity of Methanolic extract of leaves of *Aegle marmelos* (bilwa) in albino mice. In Indian Journal of Pharmacology; 2014; 46(9): S46-S46.
16. Chakravarty S, Mohanty A, Sudha TN, Upadhyay AK, Konar J and Sircar JK, et al. Removal of Pb (II) ions from aqueous solution by adsorption using bael leaves (*Aegle marmelos*). Journal of Hazardous Materials 2010; 173(1): 502-509.
17. Chauhan A and Agarwal M. Assessment of the contraceptive efficacy of the aqueous extract of *Aegle marmelos* Corr. leaves in male albino rats.Human Fertility 2009; 12(2):107-118.
18. Hameed MA, Amir F, Chin KY and Fathima S. Phytochemical screening, Anti-pyretic and antidiarrhoeal activities of the n-hexane and aqueous extracts of the leaves of *Aegle marmelos*. Archives of Pharmacy Practice 2011; 2(3): 90.
19. Madhu C, Hindu K, Sudeepthi CD, Maneela P, Reddy KV and Sree BB. Anti-ulcer activity of aqueous extract of *Aegle marmelos* leaves on rats. Asian Journal of Pharmaceutical Research 2012; 2(4):132-135.
20. Anusha C, Sarumathi A, Shanmugapriya S, Anbu S, Ahmad RS and Saravanan N. The effects of aqueous leaf extract of *Aegle marmelos* on immobilization-induced stress in male albino Wistar rats. International Journal of Nutrition, Pharmacology, Neurological Diseases 2013; 3(1): 11.
21. Gangadhar R and Lalithakumari K. Abortifacient activity of the aqueous extract of the leaves of *Aegle marmelos* (Bel) in albino rats. Indian Drugs 1995; 32(3):129-131.
22. Yoshizumi S, Murakami T, Kadoya M, Matsuda H, Yamahara J and Yoshikawa M. Medicinal foodstuffs. XI. Histamine release inhibitors from wax gourd, the fruits of *Benincasa hispida*

Cogn Yakugakuzasshi: Journal of the Pharmaceutical Society of Japan 1998; 118(5): 188-192.

23. Grover JK, Adiga G, Vats V and Rathi SS. Extracts of *Benincasa hispida* prevent development of experimental ulcers. Journal of Ethnopharmacology 2001; 78(2): 159-164.
24. Huang H, Huang J, Tso T, Tsai Y and Chang C. Antioxidant and angiotension-converting enzyme inhibition capacities of various parts of *Benincasa hispida* (wax gourd). Food Nahrung 2004; 48(3): 230-233.
25. Satpute AD. Ayuveda's contribution to sports medicine. Ancient science of life 1989; 8(3-4): 230.
26. Kumar A and Vimalavathini R. Possible anorectic effect of methanol extract of *Benincasa hispida* (Thunb). Cogn, fruit. Indian Journal of Pharmacology 2004; 36(6): 348.
27. Ghosh K and Baghel M. A Pharmacognostical and Physiochemical study of *Benincasa hispida* with Ayurvedic review.International Journal of Research in Ayurveda & Pharmacy 2011; 2 (6).
28. Rachchh MA and Jain SM. Gastroprotective effect of *Benincasa hispida* fruit extract. Indian Journal of Pharmacology 2008; 40(6): 271-274.
29. Jahan F, Hossain M, Mamun A, Hossain M, Seraj S and Chowdhury A. An evaluation of antinociceptive effect of methanol extracts of *Desmodium gangeticum* (L.) DC. Stems and *Benincasa hispida* (Thunb.) Cogn.leaves on acetic acid induced gastric pain in mice. Advances in Natural and Applied Sciences2010; 4: 365-369.
30. Girdhar S, Wanjari MM, Prajapati SK and Girdhar A. Evaluation of anti-compulsive effect of methanolic extract of *Benincasa hispida* Cogn.fruit in mice. Acta Poloniae Pharmaceutica—Drug Research2010; 67(4): 417-421.
31. Sharma N, Ahirwar D, Jhade D and Gupta S. Medicinal and Pphamacological potential of *Nigella sativa*: A review. Ethnobotanical Leaflets 2009; (7): 11.
32. Corneanu G, Corneanu M, Crăciun C, Ciupină V, Zagnat M and Atyim P. Bioactive Substances from the *Nigella sativa* Seeds. Academy Of Romanian Scientists 2012; 1(1): 13-28.
33. Hanafy MSM and Hatem ME. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). Journal of Ethnopharmacology 1991; 34 (2): 275-278.
34. Morsi NM. Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. Acta Microbiologica Polonica 1999; 49 (1): 63-74.
35. El-Fatatry HM. Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa* L. seeds. Die Pharmazie 1975; 30 (2): 109-111.
36. Kanter M, Coskun O and Budancamanak M. Hepatoprotective effects of *Nigella sativa* L and Urticadioica L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World Journal of Gastroenterology WJG 2005; 42 (11): 6684-6688.
37. Ibrahim RM, Hamdan NS, Mahmud R, Imam MU, Saini SM and Rashid SN. A randomised controlled trial on hypolipidemic effects of Nigella Sativa seeds powder in menopausal women. J Transl Med 2014;(12): 82.
38. Randhawa MA and Alghamdi MS. Anticancer activity of *Nigella sativa* (black seed)—a review. The American Journal of Chinese Medicine 2011;39 (06): 1075-1091.
39. Alenzi FQ, El-Bolkiny YS and Salem ML. Protective effects of *Nigella sativa* oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. Br J Biomed Sci ;2010; 67 (1): 20-28.
40. Kumara SS and Huat BT. Extraction, isolation and characterisation of antitumor principle, alpha-hederin, from the seeds of *Nigella sativa*. Plantamedica 2001; 67 (1): 29-32.
41. El-Dakhakhny M, Mady N, Lembert N and Ammon HP. The hypoglycemic effect of *Nigella sativa* oil is mediated by extrapancreatic actions. Plantamedica 2002; 68(5): 465-466.
42. El-Abhar HS, Abdallah DM and Saleh S. Gastroprotective activity of *Nigella sativa* oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats. Journal of Ethnopharmacology 2003; 84 (2): 251-258.
43. Kanter M, Demir H, Karakaya C and Ozbek H. Gastroprotective activity of *Nigella sativa* L oil and its constituent, thymoquinone against acute alcohol-induced gastric mucosal injury in rats. World Journal of Gastroenterology: WJG 2005; 11(42): 6662-6666.
44. Tiwari P, Kumar B, Kaur M and Kaur GandKaur H. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia2011; 1 (1).98-106.
45. Umadevi K, Vanitha V and Vijayalakshmi K. Physicochemical Evaluation, Phytochemical Screening And Chromatographic Fingerprint Profile of *Aegle marmelos* (L.) LEAF EXTRACTS. World Journal of Pharmaceutical Research 2012; 1 (3): 813-837.
46. Avula B, Wang Y, Ali Z and Khan I. Quantitative determination of chemical constituents from seeds of *Nigella sativa* L. using HPLC-UV and identification by LC-ESI-TOF. Journal of AOAC International 2010; 93 (6): 1778-1787.
47. Kumar C, Mythily R and Chand Raju S. Extraction and mass characterization of sugars from ash gourd peels (*Benincasa hispida*). Rasayan Journal of Chemistry 2012; 5 (3): 280-285.
48. Reigel CE, Dailey JW and Jobe PC. The genetically epilepsy-prone rat: an overview of seizure-prone characteristics and responsiveness to anticonvulsant drugs. Life Sci 1986; 39:763-774.
49. Nehlig A and Boehrer A. Effects of remacemide in two models of genetically determined generalized epilepsy, the GAERS and the audiogenic Wistar AS. Epilepsy Res 2003; 52:253-261.
50. Loscher W and Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res1988;2: 145-181.
51. Rejeesh, EP, Lovelyn J and Rao SN. Anticonvulsant activity of *Aegle marmelos* leaf aqueous extract using Maximal Electroshock induced Seizure model in Mice. International Journal of Universal Pharmacy and Bio Sciences 2013; 2(2):39-45.
52. Rejeesh, EP, Lovelyn J and Rao SN. Anticonvulsant activity of Nigella Sativa ethanolic extract in Maximal Electroshock Seizure model.International Journal of Universal Pharmacy and Bio Sciences 2013; 2(5):36-44.

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RE - Concept and design of the study, reviewed the literature, conducted experiment, data collection and analysis, manuscript preparation and critical revision of the manuscript; **LJ -** reviewed the literature, manuscript preparation and critical revision of the manuscript; **KK -** Critical revision of the manuscript.

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