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Synthesis and Evaluation of Antianxiety Activity of Novel Coumarin Derivatives on Swiss Albino Mice

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Abstract : This research highlights phobic behavior of swiss albino mice in an elevated plus maze and open field test as a model to evaluate the anxiolytic effect of synthetic coumarin derivativesi.e4-Methyl-2*H*-chromen-2-one and its substituted derivatives. The pechmann condensation provides route for synthesis of coumarin and its derivatives. For evaluating the antianxiety activity, swiss albino mice were divided into different groups and they were treated with different doses of the test compound and standard drug. 30 minutes after administration of test or standard drug antianxiety activity was checked on apparatus for 5 minutes. The data was subjected to analysis of variance by taking mean and standard error to the mean using Tukey's post-hoc test. In elevated plus maze, all the three tested compounds at high dose revealed increase in time spent in open arm and preference to open arm as compared to control. In open field test, all the three coumarin derivatives showed a significant increase in the number of square crossed and number of rearing as compared to control was observed. All of the changes were statistically highly significant. From the results, it can be concluded that the synthetic coumarin derivatives showed anxiolytic effects when administrated at high dose.
Keywords : Antianxiety, Elevated Plus Maze, Open Field Test, Coumarin, Heterocyclic compounds.

Introduction

There are numerous synthetic drugs which have stimulating or calmative effects on the central nervous system[1] but coumarin are the compounds which are gaining popularity because they have more therapeutic effects and less side effects.

Coumarins are the organic chemical compounds belonging to benzopyrone chemical class which isnow days widely used in pharmaceutical industries[2]. It has two six-membered rings fused together, with one of the rings being a benzene ring and the other containing alkene functionality and an ester functional group. Coumarins play an important role in both natural systems like plants and also in medicinal applications as drug molecules[3].

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In pharmacological and biological tests, coumarins and their derivatives arereported to have anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, anticardiovascular, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, skin burn and neuroprotective properties[4-12]. Natural coumarins obtained from plants *Ducrosia anethifolia*[13], *Biebersteinia multifida*[14], *Melilotusofficinalis*[15]etc. are the example of coumarins which possess antianxiety. These plants show anxiolytic activity by inhibiting monoamine oxidase enzyme and other reason is that there is binding site of coumarin derivatives with the benzodiazepine site of $GABA_A$ receptor. Despite a history of use of natural coumarins as medicine for the treatment of neuropsychiatric disorders, less synthetic coumarins has been investigated so far to substantiate these therapeutic claims and no experimental study is available on antianxiety evaluation. Therefore, it was envisioned to investigate the anxiolytic effect of different synthetic coumarins and their derivatives.

In the present study, coumarin 4-Methyl-2*H*-chromen-2-one and its substituted derivatives has been evaluated for their anxiolytic potential. The Pechmann condensation provides route for synthesis of coumarin and its derivatives. For studying anxiolytic effects, three synthetic compounds have been synthesized (a) 4-Methyl-7-nitro-2*H*-chromene-2-one (b) 4,7-Dimethyl-2*H*-chromene-2-one (c) 4-Methyl-6-nitro-2*H*-chromene-2-one.

Materials and Methods

Drugs and chemicals

Diazepam was obtained from local supplier. Sulphuric acid and ethylacetoacetate was ordered fomLobachemie whereas *m*–cresol and polyethylene glycol were procured from (S. D. Fine-Chem Ltd, Mumbai).*p*-nitrophenol and *m*- cresol were obtained from (Spectrochem Pvt. Ltd). Diazepam was used in concentration of 0.5 mg in 10 ml of distilled water and all the compounds were dissolved in polyethylene glycol and distilled water and were administered to mice according their weights.

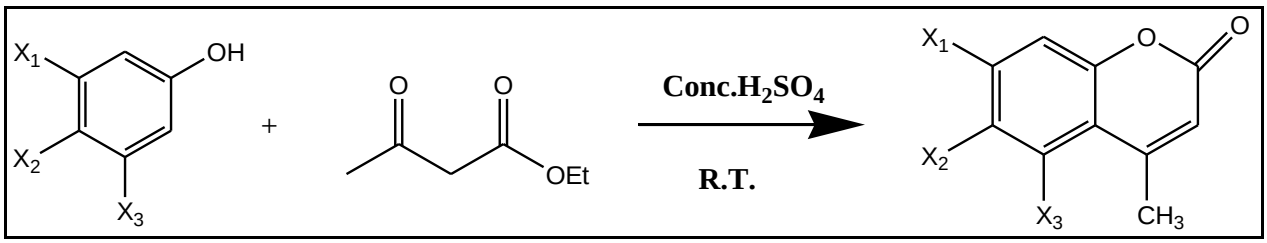
4-Methyl-7-nitro-2*H*-chromene-2-one (38.42mg/kg, 76.84mg/kg), 4,7-Dimethyl-2*H*-chromene-2-one (49.4mg/kg, 98.8mg/kg), 4-Methyl-6-nitro-2*H*-chromene-2-one (38.42mg/kg, 76.84mg/kg) and Diazepam were given by *i.p.* route. Control mice received vehicle (polyethylene glycol and distilled water). The effects of the drugs were estimated 30 min after the administration of the dose. The entire tests were carried out between 8:00 a.m-14:00p.m. In each experiment, apparatus was cleaned using 5% ethanol before introducing the next animal to preclude the possible cueing effects of odors left by previous subjects.

Animals

Swiss albino mice (weighing 15-30g) were taken from Animal House of the G.H.G Khalsa College of Pharmacy and kept in polypropylene cages at 22±1 °C on a 12-h light/dark cycle. Water and food were available *ad libitum*. Institutional Animal Ethics Committee (LAEC) (Registration no: 1801/PO/Re/S/15/CPCSEA) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare and Government of India. Groups of five mice were randomly assigned to different treatment groups and tested in a counter balance order. Eight groups were made with five animals in each group. Control group received vehicle, positive control received Diazepam (2 mg/kg i. p.) while other group receive low and high dose of 4-Methyl-2*H*-chromen-2-one and its substituted derivatives.

Synthesis of the 4-Methyl-2*H*-chromen-2-one and its substituted derivatives.

For synthesis of coumarin derivatives, concentrated sulphuric acid (50ml) was taken in double necked round bottomed flask (250ml) and it was cooled to 10°C. In another beakerphenol (*m*-nitrophenol,*m*-cresol,*p*-nitrophenol)was dissolved in freshly distilled ethylacetoacetate (6.8ml,0.05M). Then, mixture of phenol (1a-c) and ethylacetoacetate (2) was added drop wise to cold concentrated sulphuric acid solution with constant stirring. The temperature was maintained always below 10^o C. After adding all the portions of the mixture, the solution was kept aside for 12 hours, without further cooling. Then, the reaction mixture was poured into crushed ice (150 g). The precipitates obtained were filtered and dried[16-17].



(1a-c) (2) (Scheme-1) (3a-c)

Figure 1: Chemical reaction that leads to formation of coumarins

Table 1: Various substitutions on coumarin nucleus (3a-c)

Compounds	**X_1**	**X_2**	**X_3**
A	NO_2	H	H
B	CH_3	H	H
C	H	NO_2	H

Identification and characterization of compounds synthesized was carried by melting point, solubility, thin layer chromatograpy, ^{1}H-Nuclear Magnetic Resonance Spectroscopy.

Dosing protocol

Animals were divided into eight groups with five animals in each group. Group I was taken as control group and it was treated with polyethylene glycol and distilled water solution. Group II was taken as standard group and dose 2mg/kg was given. Animals of group III receives the low dose of compound 4-Methyl-7-nitro-2*H*-chromene-2-one (3a) and dose was 38.42mg/kg. High dose 76.84mg/kg of same compound was given to group IV. Compound 4,7-Dimethyl-2*H*-chromene-2-one (3b) was injected to group V and group VI with the low (49.4mg/kg) and high dose (98.8mg/kg) respectively. Similarly, groups VII and group VIII were treated with low dose (38.42mg/kg) and high dose (76.84mg/kg) of compound 4-Methyl-6-nitro-2*H*-chromene-2-one (3c). Allthe compounds were dissolved in polyethylene glycol and distilled water solution. Dose of compounds were given according to the weight of the animal.

Methods

A) Elevated plus maze

The plus maze apparatus consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof with a plus maze elevated (25 cm) from the floor was used to observe anxiolytic behavior of animals. Standard drug and test drug was administered by intraperitoneal route to the animals. The dose administration schedule was so adjusted that each mice was having its turn on plus maze after 30 min of administration of dose. Each animal was placed in the centre of the elevated plus maze with its head facing the open arms[18].

During this 5 min experiment, behavior of the mice was recorded as a) preference of the animal for its first entry to open/closed arm b) no. of entries into the open/closed arm c) average time spent by the animal in each arm. During the entire experiment, each animal was allowed to socialize. Every precaution was taken to ensure that no external stimuli evoked the animal [19-20].

B) Open field

The open field apparatus was constructed of black plywood and measured 38 x 38 cm with 36 cm walls. Mice were carried to the test room in their home cages and were handled by the base of their tails at all times[21]. Then, dose was given to animal according to their body weigh. 30 minsafter administration of test or standard drug animal was placed in centre of open field and allowed to explore the apparatus for 5 minutes. After the 5 minute test, mice were returned in their home cages and the open field was cleaned with 70 % ethyl alcohol and permitted to dry. During the 5 min experiment behavior of mice was recorded as (a) time spent in centre and periphery (b) number of square cross (c) number of rearings[22-23].

Statistical Analysis

All the results were expressed as mean± standard error mean (SEM). The data of experimental results was statistically analyzed by oneway ANOVA followed by Tukey's multiple comparison post hoc tests[24]. The groups treated with test drug were compared with the respective control (vehicle) group; $p<0.05$ was considered statistically significant.

Results

Present study was conducted to evaluate the potential of 4-Methyl-2*H*-chromen-2-one and its substituted derivatives as anxiolytic through behavioral assay systems i.e. elevated plus maze and open field test. The elevated plus maze, open field was considered to be a valid animal models of anxiety because these models uses natural stimulus that is the fear of a new, brightly light open space and the fear of balancing on a relatively narrow raised platform[25].

Identification of the synthetic coumarin derivatives was done by ^{1}H-Nuclear Magnetic Resonance Spectroscopy. ^{1}H-NMR for test compound 4-Methyl-7-nitro-2*H*-chromene-2-one was (400MHz, Chloroform-d6) (δ_{ppm}): 6.23 (s, C-3, 1H), 2.40 (s, C-4, 3Hs), 8.23-8.10 (m, C-5, 6, 8, 3Hs), for 4,7 -Dimethyl-2*H*-chromene-2-one was (400MHz, Chloroform-d6) (δ_{ppm}): 6.23 (s, C-3, 1H), 2.40 (s, C_4, 3Hs), 8.13-7.70 (m, C-5, 6, 8, 3Hs), 2.28 (s, CH_3 at C7, 3Hs) and for 4-Methyl-6-nitro-2*H*-chromene-2-one was (400MHz, Chloroform-d6) (δ_{ppm}): 6.27 (s, C-3, 1H), 2.43 (s, CH_3 at C_4, 3Hs), 8.63-8.15 (m, C-5, 7, 8, 3Hs).

In elevated plus maze, high doses of synthesized compounds 4-Methyl-7-nitro-2*H*-chromene-2-one (3a) (76.84mg/kg), 4,7 -Dimethyl-2*H*-chromene-2-one (3b) (98.8mg/kg), 4-Methyl-6-nitro-2*H*-chromene-2-one (3c) (76.84mg/kg) induced significant increase in both the number of entries and time spent in open arms in a dose dependent manner compared to vehicle control thereby proving its antianxiety effect. Whereas, low doses of synthesized compounds 4-Methyl-7-nitro-2*H*-chromene-2-one (3a) (38.42mg/kg), 4, 7 -Dimethyl-2*H*-chromene-2-one (3b) (49.4mg/kg), 4-Methyl-6-nitro-2*H*-chromene-2-one (3c) (38.42mg/kg) show moderate antianxiety effect.

Table 2: Number of entries and time spent in open arm on elevated plus maze

Group	Treatment	Dose	No.of entries in open arm	Time spend in open arm(sec)
Group I	Vehicle control	_____	3±0.63	30±1.14
Group II	Standard-Diazepam	2mg/kg	11±0.63***	85±1.00***
Group III	3a (L)	38.42mg/kg	6±0.63*	36±0.94*
Group IV	3a (H)	76.84mg/kg	9±0.40***	50±0.84***
Group V	3b (L)	49.4mg/kg	7±0.44**	37±1.09**
Group VI	3b (H)	98.8mg/kg	10±0.54***	65±0..94***
Group VII	3c (L)	38.42mg/kg	7±0.44*	35±1.41*
Group VIII	3c (H)	76.84mg/kg	8±1.04***	49±1.22***

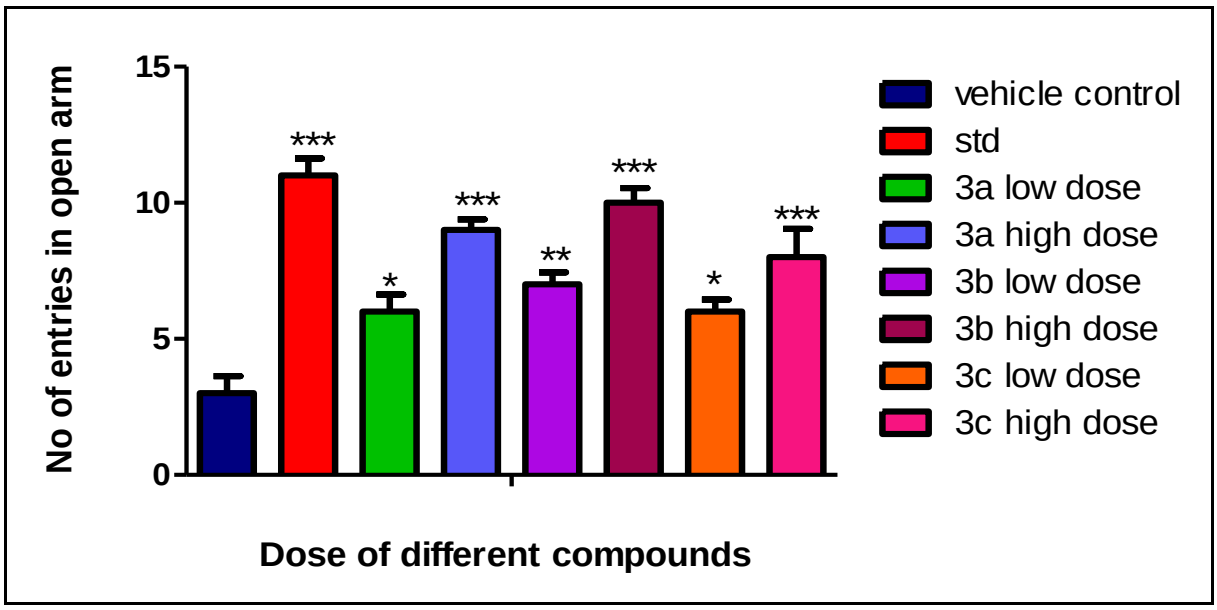


Fig. 2: Number of entries in open arm are expressed as mean ± SEM (n=5); *** P<0.001, ** P<0.01, *p<0.05 as compared to control.

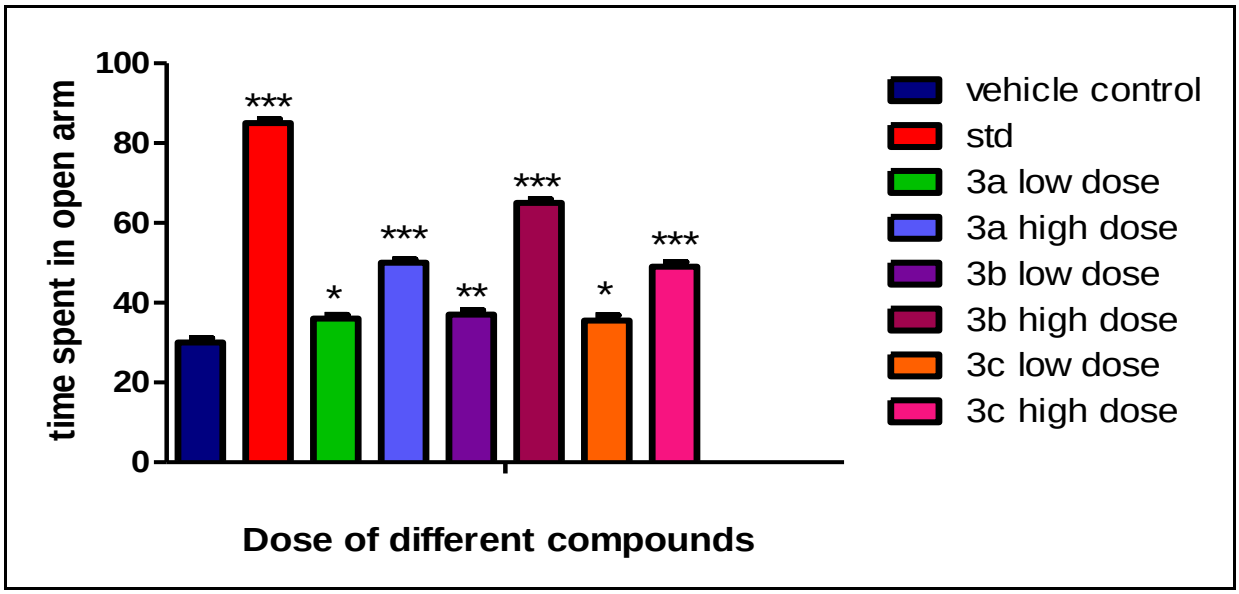


Fig. 3: Time spent in open arm is expressed as mean ± SEM (n=5); *** P<0.001, ** P<0.01, *p<0.05 as compared to control.

In open field test, high doses of synthesized compounds 4-Methyl-7-nitro-2*H*-chromene-2-one (3a) (76.84mg/kg), 4,7 -Dimethyl-2*H*-chromene-2-one (3b) (98.8mg/kg), 4-Methyl-6-nitro-2*H*-chromene-2-one (3c) (76.84mg/kg) induced significant increase in both the number of square crossed and numbers of rearing in a dose dependent manner compared to vehicle control thereby proving its antianxiety effect.Whereas, low doses of synthesized compounds 4-Methyl-7-nitro-2*H*-chromene-2-one (3a) (38.42mg/kg), 4, 7 -Dimethyl-2*H*-chromene-2-one (3b) (49.4mg/kg), 4-Methyl-6-nitro-2*H*-chromene-2-one (3c) (38.42mg/kg) show moderate antianxiety effect.

Table 3: Number of square crossed and rearing **in open field**

Group	Treatment	Dose	No. of square cross	No. of rearing
Group I	Vehicle control	_____	50±0.54	10±0.94
Group II	Standard -Diazepam	2mg/kg	83±0.83***	25±1.81***
Group III	3a (L)	38.42mg/kg	53±0.44*	16±1.30*
Group IV	3a (H)	76.84mg/kg	65±0.89***	21±1.019***
Group V	3b (L)	49.4mg/kg	54±0.63**	17±1.22**
Group VI	3b (H)	98.8mg/kg	76±0.70***	22±1.09***
Group VII	3c (L)	38.42mg/kg	53±0.44*	12±1.22*
Group VIII	3c (H)	76.84mg/kg	60±0.31***	19±1.14***

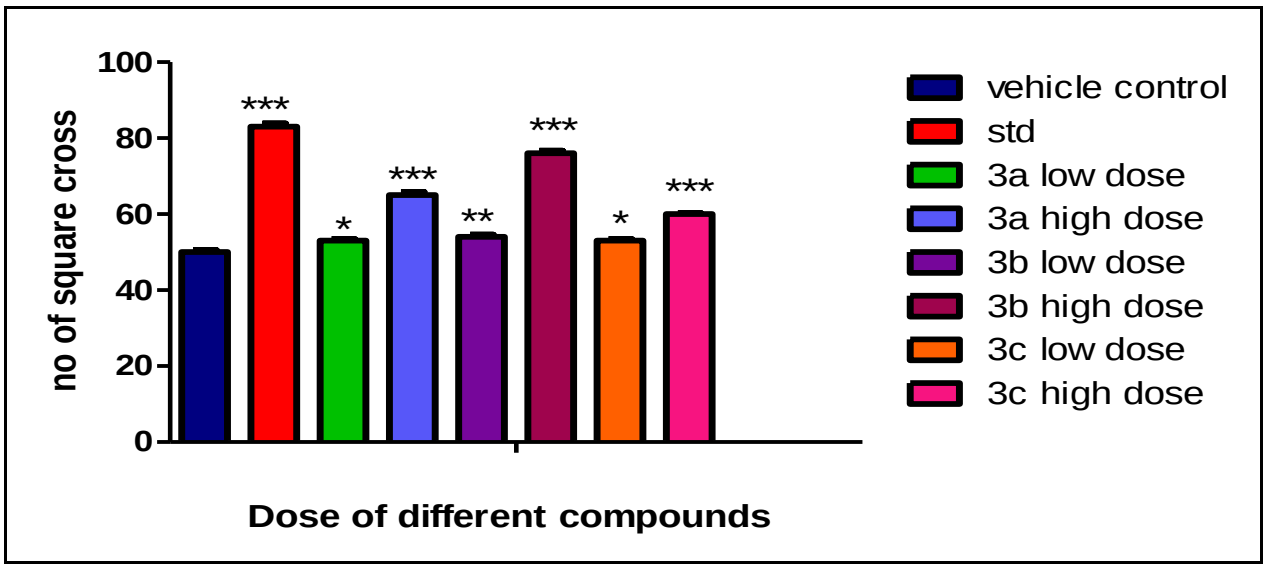


Fig. 4:Number of square crossed are expressed as mean ± SEM (n=5); *** P<0.001, ** P<0.01, *p<0.05 as compared to control.

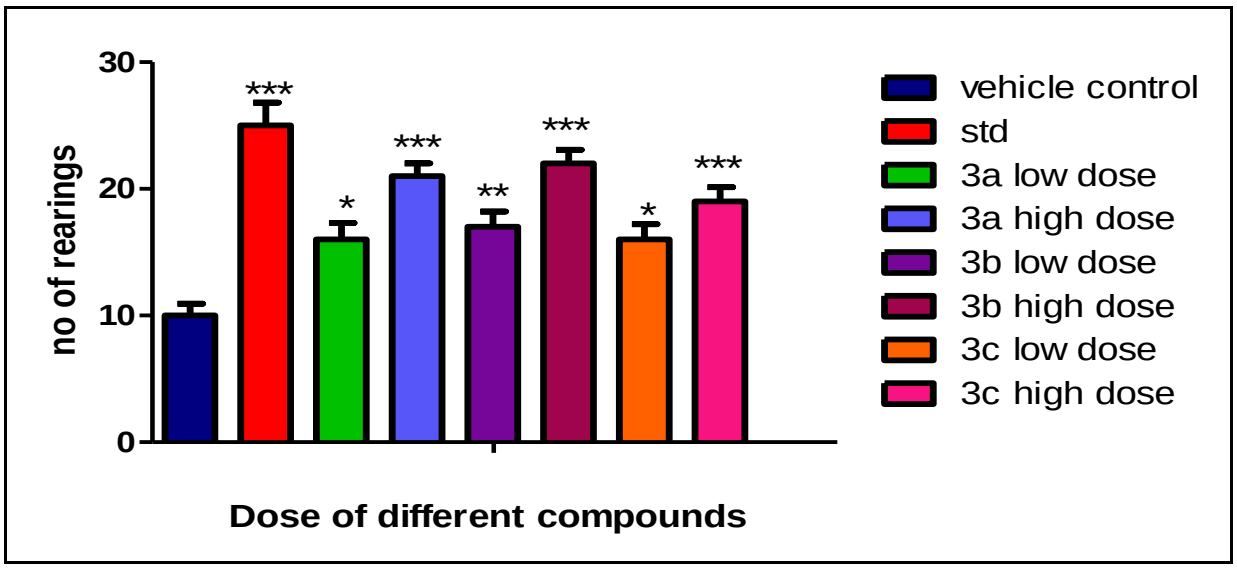


Fig. 5: Number of rearings are expressed as mean ± SEM (n=5); *** P<0.001, ** P<0.01, *p<0.05 as compared to control.

Discussion

Previous studies suggest that coumarin and their derivatives are potent inhibitors of the enzyme monoamine oxidase. There are investigations indicating that the modulation of monoamine oxidase (MAO) inhibitors modulate monoamine levels in the brain (dopamine, serotonin, and norepinephrine) and provoke behavioral modifications in mice thus, exerting an anxiolytic effect. Another study reported that there is binding of coumarin derivatives with the BZD site of the $GABA_A$receptors. They are ligands for benzodiazepines binding sites on $GABA_A$ receptors which are thought to potentiate $GABA_A$ receptors and increase opening of chloride ion channel thus responsible for hyperpolarization. All these findings advocate that the mechanism underlying the anxiolytic activity of the coumarin and its derivatives may occur through the inhibition of MAO-A, increase in the GABAnergic action and increase in serotonin level respectively and phenolic compounds act as a ligand for receptors exerting anxiolytic and slight sedative effects[26-27].

Thus, it can be concluded that 4-Methyl-2*H*-chromen-2-one and its substituted derivatives has potential antianxiety effect which may be due to inhibition of monoamine oxidase enzyme or may be due to binding with $GABA_A$ receptor[28]. However, further studies are needed to explore the exact mechanism of action of these synthesized compounds.

Conclusion

From our research studies, it can be concluded that high doses of 4-Methyl-2*H*-chromen-2-one and its substituted derivatives possess potent anxiolytic activity. However, moderate effect was observed for low dose of the synthetic compounds. Further studies can be conducted to explore an exact mechanism of action of these synthesized compounds.

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Conflict of Interest:

Authors have no conflict of interest.

References

1. Schulz V, Hänsel R, Blumenthal M, Tyler VE. Rational phytotherapy: A reference guide for physicians and pharmacists. Springer Science & Business Media, 2004 Jul 15.
2. Farinola N and Piller, N.Pharmacogenomics: Its role in re-establishing coumarin as treatment for lymphedema. Lymphatic Research and Biology, 2005, 3 (2), 81–86.
3. Aoyama Y, Katayama T, Yamamoto M, Tanaka H, Kon K. A new antitumor antibiotic product, demethylchartreusin. Isolation and biological activities. *The Journal of Antibiotics*, 1992, 45(6), 875–878.
4. Canning C, Sun S, Ji X, Gupta S, and Zhou K. Antibacterial and cytotoxic activity of isoprenylated coumarianmammea A/AA isolated from Mammea Africana. Journal of ethnopharmacol, 2013, 147, 259-262.
5. Srikrishna D, Godugu C, Dubey P. A Review on Pharmacological properties of Coumarins. Mini reviews in medicinal chemistry, 2018 Feb 1, 18(2), 113-41.
6. Venugopala KN, Rashmi V, Odhav B.Review on natural coumarin lead compounds for their pharmacological activity. BioMed research international, 2013, 2013, 14.
7. Kostova I, Manolov I, Nicolova I, Konstantinov S, Karaivanova M.New lanthanide complexes of 4-methyl-7-hydroxycoumarin and their pharmacological activity. European journal of medicinal chemistry, 2001 Apr 1,36(4), 339-47.
8. Nofal ZM, El-Zahar MI, Abd El-Karim SS. Novel coumarin derivatives with expected biological activity. Molecules, 2000 Feb 16,5(2), 99-113.

9. Bonsignore L, Loy G, Secci D, Calignano A. Synthesis and pharmacological activity of 2-oxo-(2H) 1-benzopyran-3-carboxamide derivatives. European Journal of Medicinal Chemistry, 1993 Jan 1, 28(6), 517-20.
10. Wu L, Wang X, Xu W, Farzaneh F, Xu R. The structure and pharmacological functions of coumarins and their derivatives. Current medicinal chemistry, 2009 Nov 1,16(32), 4236-60.
11. Hamaduobeed AL-Hamadaui A.The use of propolis and black cumin in treatment of some wounds and burn infections. Al-Qadisiah Medical Journal, 2010, 6(9), 210-22.
12. Sashidhara KV, Palnati GR, Sonkar R, Avula SR, Avula C, Awasthi C and Bhatia G.Coumarian Chalcone fibrates: A new structural class of lipid lowering agents. European journal of medicine chemistry, 2013, 64, 422-431
13. Hajhashemi V, Rabbani M, Ghanadi A, Davari E. Evaluation of antianxiety and sedative effects of essential oil of Ducrosia anethifolia in mice. Clinics, 2010, 65(10), 1037-42.
14. Monsef-Esfahani HR, Amini M, Goodarzi N, Saiedmohammadi F, Hajiaghaee R, Faramarzi MA, Tofighi Z, Ghahremani MH.Coumarin compounds of Biebersteinia multifida roots show potential anxiolytic effects in mice. DARU Journal of Pharmaceutical Sciences, 2013,21(1), 51.
15. Kaur S, Sharma A, Bedi PM.Bioactivity Guided Isolation, Characterization and Quantification of an Anxiolytic Constituent-Kaempferol, from Melilotus officinalis Aerial Parts. Journal of Biologically Active Products from Nature, 2017 Sep 3,7(5), 379-90.
16. Li TS, Zhang ZH, Yang F and Fu CG.Montmorillonite clay catalysis. Part 7. 1 an environmentally friendly procedure for the synthesis of coumarins via pechmann condensation of phenols with ethyl acetoacetate. Journal of Chemical Research Synopses, 1998, 1, 38-9.
17. Wight AP, Davis ME.Design and preparation of organic− inorganic hybrid catalysts. Chemical reviews, 2002 Oct 9, 102(10), 3589-614.
18. Kulkarni SK and Reddy DS.Animal behavioral models for testing anti-anxiety agents. Methods Find Exp Clinical Pharmacol, 1996, 18, 219–30.
19. Pellow S, Chopin P, File SE, and Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of neuroscience methods, 1985 Aug 1, 14(3), 149-67.
20. Walf AA and Frye CA.The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nature protocols, 2007 Feb, 2(2), 322.
21. Stanford SC.The Open Field Test: reinventing the wheel. Journal of neuroscience methods, 2007,*21*(2), 134-135.
22. Shimada T, Matsumoto K, Osanai M, Matasuda H, Terasawa K & Watanabe H. The modified light/dark transition test in mice: evaluation of classic and putative anxiolytic and anxiolytic drugs. General Pharmacology the Vascular Systemx, 1995, 26(1), 205-210.
23. Gould TD, Dao DT and Kovacsics CE. The open field test. In Mood and anxiety related phenotypes in mice. Humana Press, Totowa, NJ, 2009, 42, 1-20.
24. Cvijovic Z, Radenkovic G, Maksimovic V, Dimcic B. Application of ANOVA method to precipitation behaviour studies. Materials Science and Engineering: A, 2005 Apr 25, 397(1-2), 195-203.
25. Kaur D, Kaur D, Bains N, Chopra A and Arora P. Anti-anxiety evaluation of extracts of stigma maydis (corn silk). International journal of Pharmacy and pharmaceutical sciences, 2015, 7, 307-312.
26. Bruhlmann C, Ooms F, Carrupt PA, Testa B, Catto M, Leonetti F, Altomare C and Carotti A.Coumarins derivatives as dual inhibitors of acetylcholinesterase and monoamine oxidase. Journal of Medicinal Chemistry, 2001 Sep 13, 44(19), 3195-8.
27. Jeong SH, Han XH, Hong SS, Hwang JS, Hwang JH, Lee D, Lee MK, Ro JS and Hwang BY.Monoamine oxidase inhibitory coumarins from the aerial parts of Dictamnusalbus. Archives of pharmacal research, 2006 Dec 1, 29(12), 1119.
28. Singhuber J, Baburin I, Ecker GF, Kopp B and Hering S. Insights into structure-activity relationship of GABAA receptor modulating coumarins and furanocoumarins. Eur J Pharmacol, 2011, 668(1), 57-64.

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