International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2021.400.407

Research Article
Evaluation of Skeletal Muscle Relaxant Activity of Apigenin in Animal Experimental Models

[1]Syed Mohammed Basheeruddin Asdaq, [1]Basheerahmed Abdulaziz Mannasaheb, [1]Ahmad Alanazi, [1]Bader Almusharraf, [1]Naif Alanazi, [1]Khalid Saad, [1]Sami Alanazi, [1]Khalid Abduallah, [1]Saad Alrashid, [1]Faisal Alanazi, [2,3]Abdulhakeem S. Alamri, [2,3]Walaa F. Alsanie and [2,3]Majid Alhomrani

[1]Department of Pharmacy Practice, College of Pharmacy, AlMaarefa University, Diriyah, Riyadh 13713, Saudi Arabia
[2]Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Taif University, Taif, Saudi Arabia
[3]Centre of Biomedical Sciences Research (CBSR), Deanship of Scientific Research, Taif University, Saudi Arabia

Abstract

Background and Objective: Numerous natural bioactive compounds have demonstrated functional activity, implying that they could play a significant role in the treatment and management of a variety of chronic illnesses. Flavonoids, which include apigenin, make up the biggest group of naturally occurring polyphenols. Skeletal muscle relaxants are medications that relieve undesired spasms without impairing consciousness or reflexes. This study was done to evaluate the skeletal muscle relaxant property of Apigenin (APG) in experimental animal models. **Materials and Methods:** Three doses of apigenin (10, 25 and 25 mg kg^{-1}) were selected and their muscle relaxant activity was compared against standard diazepam 5 mg kg^{-1} using climbing, chimney and modified Kondziela's inverted tests. The results obtained were analyzed using an analysis of variance (ANOVA) and a post-ANOVA Tukey multiple comparisons test. **Results:** Animals treated with 25 and 50 mg kg^{-1} of APG exhibited significant difficulty in climbing up the chain, possibly due to relaxation of the muscles. Similarly, response time in the chimney test was significantly increased in those animals who received a high dose of APG (50 mg kg^{-1}) demonstrating the loss of alertness and retarded muscle tone resulting in difficulty with muscle coordination. Additionally, in inverted tests, animals treated with a high dose of APG had a significantly decreased duration of holding capacity on the mesh. A fall in muscle contraction leads to a decrease in holding time. **Conclusion:** Thus, our findings demonstrate that apigenin, when given orally to mice, has a dose-dependent skeletal muscle relaxant effect.

Key words: Apigenin, skeletal muscle relaxant, climbing test, chimney test, inverted test, diazepam, spasmolytics

Citation: Asdaq, S.M.B., B.A. Mannasaheb, A. Alanazi, B. Almusharraf and N. Alanazi *et al.*, 2021. Evaluation of skeletal muscle relaxant activity of apigenin in animal experimental models. Int. J. Pharmacol., 17: 400-407.

Corresponding Author: Syed Mohammed Basheeruddin Asdaq, Department of Pharmacy Practice, College of Pharmacy, AlMaarefa University, Diriyah, 13713, Riyadh, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Skeletal muscle relaxants are medications that lessen undesired spasms or spasticity without impairing consciousness or voluntary movements. They are useful in a variety of neurological and painful musculoskeletal conditions[1]. The usage of muscle relaxant medications can be traced back to the 16th century. Neuromuscular blocking medicines had become widely used as muscle relaxants in anaesthesia and surgery[2]. Approximately 450 m people suffer from a neurological or behavioural illness, according to a World Health Organization report (WHO, Geneva: 2001). This accounts for 12.3% of the worldwide illness burden[3]. Muscle spasms, discomfort and hyperreflexia are all treated using drugs that influence skeletal muscle function. Neuromuscular blockers and spasmolytics are the two major components of this category of medicine. The farmer is more often used during surgical procedures while the latter is used to ameliorate musculoskeletal pain[4]. Nevertheless, spasmolytics are not indicated as a first-line treatment for acute low back pain because they are no more effective than paracetamol or Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) and their efficacy is less than antidepressants in fibromyalgia[5,6]. The possibility of addiction and interaction with other medications, together with the occurrence of dizziness and drowsiness, adds to the grounds for their limited usage. Therefore, it is imperative to promote credible research on exploring a muscle relaxant that is devoid of abuse and other negative implications of present-day muscle relaxants and equally possesses high efficacy to subvert muscular pain and other neuromuscular disturbances.

Natural products have been widely exploited as viable alternatives in the development of novel medications and therapeutic agents for the treatment of ailments. Apigenin (5,7,4-trihydroxyflavone) is a bioflavonoid that has been studied extensively for a variety of its pharmacological potential. It is present in many plants as an aglycone moiety of several naturally occurring glycosides which were previously used as a yellow crystalline solid to dye wool. It can be found in a variety of fruits and vegetables, but the most common sources are parsley, celery, celeriac and chamomile tea[7]. Apigenin, which makes up 68% of total flavonoids in chamomile flowers, is particularly plentiful[8]. Apigenin is produced from the flavone synthesis pathway and the general phenylpropanoid pathway[9].

Apigenin has been explored for several biological activities in the recent past. It has received particular interest due to its antioxidant, anti-inflammatory, antithrombotic and vasorelaxant potential[10-12]. Further, this bioflavonoid is also reported to have anti-cancer properties by inducing apoptosis through different apoptotic pathways[13-15]. Apigenin was also discovered to have a variety of pharmacological effects on the central nervous system. Apigenin has been shown to possess antidepressant and sedative properties[16,17]. Although earlier studies have exhibited antispasmodic activity of apigenin[18], both as an isolated fraction and plant extract, there is no data about the effects of apigenin on skeletal muscle relaxant activity in *in vivo* experimental models.

As stated above, there is a need for a safer and more effective muscle relaxant that could be commonly prescribed without any possibility of abuse. Since apigenin has been reported for potent antidepressant, anxiolytic[19,20] as well as *in vitro* smooth muscle relaxant properties, it is worth investigating its possible role as a skeletal muscle relaxant in intact animal models. Hence, this study is designed to explore the role of apigenin in animal experimental models for skeletal muscle relaxant properties.

MATERIALS AND METHODS

Study area: The study was carried out in the Pharmacology laboratories of the College of Pharmacy, AlMaarefa University, Riyadh, Saudi Arabia from October, 2019-January, 2020.

Chemicals and drugs: Methylcellulose and sodium chloride for the experimental purpose were purchased from Al-Majharia international trading establishment, Saudi Arabia, whereas, glasswares and other required equipment were supplied by Gulf Scientific Glass Industry limited, Saudi Arabia and Scientific equipment trading Establishment, Saudi Arabia, respectively. APG was purchased from "Beijing Mesochem Technology Co., Ltd. (Beijing, China)" (purity: 98.20 percent by HPLC). All other chemicals required for this study were procured from standard companies and details of the specification were maintained for future use.

Experimental animals: Wistar albino mice (total 90, 30 for each model) weighing between 20-25 g housed at 25±5°C in an air-conditioned animal room under standard conditions were used in this experiment. The mice had free access to standard laboratory feed and water and were acclimatized to laboratory conditions 48 hrs before the experimental protocol to minimize non-specific stress if any. The study

protocol was approved by the Research Committee of the College of Pharmacy, AlMaarefa University, Riyadh, Saudi Arabia.

Experimental protocol: The animals were divided into five groups with animals in each group. Group I and II were control and standard groups that received distilled water and diazepam 5 mg kg^{-1} [21] (p.o), respectively. Group III, IV and V were administered with low (10 mg kg^{-1} p.o), medium (25 mg kg^{-1} p.o) and high (50 mg kg^{-1} p.o) doses of apigenin, respectively.

Experimental models

Acute toxicity study and dose selection: Three doses of apigenin (10, 25 and 25 mg kg^{-1}) were selected based on acute oral toxicity studies published earlier[21]. The evaluation of muscle relaxant potency of standard and test drugs was done by using standardized experimental models[22].

Climbing test: This test was initially proposed by Yemitan and Adeyemi[23]. Mice were taught to climb a 50 cm chain by placing their forepaws on the chain's free end. A clamp-on of a laboratory table is used to suspend the chain (90 cm from the ground). A normal mouse grips the chain with its forepaws and, once free, places its two feet on the chain and climbs until it reaches a marked position 2 cm from the top of the chain. Mice were given a day of conditioning before the experiment. Each mouse was given three chances to climb the 50 cm chain. Mice that reached the target in less than 30 sec were chosen for further testing. The length of time it takes to ascend a chain is used as a measure of muscle relaxation. Muscle relaxant activity was measured at 30, 60, 120 and 240 min after drug administration.

Chimney test: This is one of the commonly used tests to evaluate tranquillizing and muscle relaxant activity[24]. The cylinders were 30 cm long Pyrex glass cylinders. Mice weighing 16-18 g had an internal diameter of 22 mm, while mice weighing 18-20 g had an internal diameter of 25 mm and mice weighing 20-22 g had an internal diameter of 28 mm. A mark is 20 cm from the base of each tube. The tube was initially kept in a horizontal position. A mouse with its head forward was introduced near the end of the tube, near the mark. The tube is shifted to a vertical position when the mouse reaches the other end of the tube, which it is pushed towards with a rod if necessary. The time it took the mouse to climb backwards out of the cylinder at the top was recorded. Mice were given a day of conditioning before the experiment. Each mouse was given three chances to climb backwards, each with a one-minute break between them. Mice that climbed backwards in 30 sec were used in the experiment. The mice's inability to climb backwards out of the tube within 30 sec was used as the endpoint for determining muscle relaxant action. Mice that had previously been examined were uniformly divided into groups (Groups I-V) and given the appropriate treatment. Muscle relaxant activity was measured after 30 min, 1, 2 and 4 hrs after drug administration.

Modified Kondziela's inverted screen test: The inverted screen is a 43 cm^2 wire mesh made up of 12-remillimetre squares of 1 millimetre-diameter wire[25-27]. A 4 cm deep wooden beading surrounds it (which pre-vents the occasional mouse which attempts to climb onto the other side). The Mouse was placed in the middle of the wire mesh screen, which was reversed (180°) and a stopped clock was started, with the mouse's head falling first. Mice were used in the experiment who was hung upside down for 60 sec. Muscle relaxation was defined as the inability to hang upside-down for 60 sec. Animals that fell from the mesh within 10 sec received a score of '1,' while those who fell between 11 and 25 sec and 26-59 sec received ratings of '2' and '3,' accordingly. Those that fell after 60 sec received a score of 4. Muscle relaxant activity was measured in each group of animals after 30 min, 1, 2 and 4 hrs after drug administration.

Statistical analysis: The climbing test used a two-way multivariate analysis of variance (two-way MANOVA) to see whether there was an impact of two independent variables, drug treatment and recording time and the two dependent variables, distance travelled and time is taken to traverse that distance, on each other. The multivariate statistic, Wilks' Lambda (Λ) was used to interpret the effect of independent variables on dependent variables. Univariate Analysis of Variance was done in the chimney and Modified Kondziela's inverted screen test to check the effect of treatment and time of recording response on a single dependent variable (response). The Post-ANOVA Tukey multiple comparisons were done to check the level of significance. The analysis was done using SPSS-IBM 25 and $p<0.05$ was taken as statistically significant.

RESULTS

Effect of apigenin using climbing test: Previously, trained mice were supposed to climb up the chain for a 50 cm distance in a maximum of 30 sec. Animals who climb up the

Table 1: Descriptive statistics of climbing test

Time (min) post-administration	Treatment	Distance travelled (cm)	Time is taken to travel (sec)
30	Control	43.0±12.13	19.40±4.22
	Diazepam	25.5±6.25***	30.00±0.00
	APG 10 mg kg^{-1}	50.0±0.00	10.00±2.3
	APG 25 mg kg^{-1}	50.0±0.00	16.60±4.22
	APG 50 mg kg^{-1}	43.0±9.59	22.80±14.5
60	Control	50.0±0.00	19.6.0±4.44
	Diazepam	08.6±2.84***	30.00±0.00
	APG 10 mg kg^{-1}	50.0±0.00	15.83±4.75
	APG 25 mg kg^{-1}	46.8±12.56	25.60±5.76
	APG 50 mg kg^{-1}	33.0±5.57*	22.80±4.33
120	Control	48.0±4.47	17.8.0±12.13
	Diazepam	14.5±5.21***	30.00±0.00
	APG 10 mg kg^{-1}	50.0±0.00	16.12±2.47
	APG 25 mg kg^{-1}	36.0±0.20*	16.60±5.94
	APG 50 mg kg^{-1}	28.25±3.59**	19.65±4.23
240	Control	47.0±6.7	18.00±5.4
	Diazepam	15.8±3.49***	30.00±0.00
	APG 10 mg kg^{-1}	42.4±9.87	22.47±4.32*
	APG 25 mg kg^{-1}	28.6±8.08**	22.40±3.22*
	APG 50 mg kg^{-1}	26.6±7.45**	22.80±2.01*

Values are given as Mean±SEM, n = 6, APG: Apigenin, *p<0.05, **p<0.01 and ***p<0.001 when compared to the control group (Chi-square test)

chain quickly is an indication of the absence of muscle relaxant property of the treatment. At the end of 30 min post-administration of apigenin (APG), the distance travelled in a given time was not significantly altered in animals treated with any of three doses of apigenin (APG) when compared to the normal control group. Also, the time taken by these three groups is not significantly different from the normal control group, indicating the absence of muscle relaxant property of APG at 30 min after administration (Table 1).

At the end of 60 min of administration, a high dose (50 mg kg^{-1}) of APG showed a significant reduction in the distance travelled in a given time compared to control group animals, whereas, other doses of APG failed to show any significant variation. When animals were tested for their climbing ability on the chain at the end of 2 and 4 hrs, both medium and high doses (25 and 50 mg kg^{-1}) exhibited significantly reduced travelled distance in 30 secs when compared to the corresponding control group.

The group of animals that received diazepam showed a significant reduction in the distance travelled in 30 secs at all recording times (30, 60, 120 and 24 min) compared to the control group, signifying their potential to cause relaxation of the muscles. However, there was a slight improvement in travelling distance at the end of 2 hrs and that remained almost consistent till the end of 4 hrs. This group of animals had low muscle coordination ability and hence were termed as strong muscle relaxants (Table 1).

The two-way multivariate analysis of variance (two-way MANOVA) demonstrated a significant impact of treatment on the dependent variable. All major four multivariate tests were checked for the impact of independent variables (drug administered) on dependent variables (distance travelled and time taken to travel). The four tests that were used were Pillai's trace test, Hotelling's trace, Wilk's Lambda and Roy's Largest root test.

Based on the results given by all four above tests, the p-value was 0.001, which means that there is a statistically significant effect of treatment of animals on both the dependent variable. In other words, some of the treatments in the study groups were able to increase the travel time on the chain. Also, the effect of treatment on travel time is not the same at different times of recording the response. The significant impact of treatment was noted at different recording times by Pillai's trace test ($F = 7.473$, $p = 0.001$), Hotelling's trace ($F = 11.070$, $p = 0.000$) and Roy's Largest root ($F = 22.247$, $p = 0.000$) tests. The most recommended test was Wilks' Lambda (Λ). It also showed a statistically significant interaction effect between the type of treatment given and recording time on the combined dependent variables, $F(8, 150) = 9.231$, $p = .000$, Wilks' $\Lambda = .449$.

Effect of apigenin using chimney test: As shown in Table 2, the response time taken by a group of animals treated with diazepam was significantly higher than the control group at all recording times. No significant change was observed in groups given APG 10, 25 and 50 mg kg^{-1} at 30 min and 60 min recordings. Animals treated with a high dose (50 mg kg^{-1}) of APG showed a trend of muscle relaxant activity at 120 min but

Table 2: Descriptive statistics of chimney test

Time (min) post-administration	Treatment	Response time (sec)	Significance (p-value)
30	Control	3.65±1.03	
	Diazepam	12.11±4.13***	0.001
	APG 10 mg kg^{-1}	3.8±1.01	0.565
	APG 25 mg kg^{-1}	4.8±1.00	0.494
	APG 50 mg kg^{-1}	4.9±1.02	0.387
60	Control	4.8±1.25	
	Diazepam	15.0±0.00***	0.001
	APG 10 mg kg^{-1}	5.3±0.98	0.489
	APG 25 mg kg^{-1}	6.2±1.06	0.298
	APG 50 mg kg^{-1}	8.5±1.10	0.134
120	Control	4.6±0.88	
	Diazepam	15.0±0.00***	0.001
	APG 10 mg kg^{-1}	5.4±0.54	0.423
	APG 25 mg kg^{-1}	6.4±1.02	0.245
	APG 50 mg kg^{-1}	9.2±1.59	0.071
240	Control	5.4±1.02	
	Diazepam	15.0±0.00***	0.001
	APG 10 mg kg^{-1}	5.9±0.87	0.387
	APG 25 mg kg^{-1}	6.8±0.98	0.123
	APG 50 mg kg^{-1}	10.7±1.45*	0.048

Values are given as Mean±SEM, n = 6, APG: Apigenin, *p<0.05, **p<0.01 and ***p<0.001 when compared to the control group (Chi-square test)

Table 3: Descriptive statistics of modified inverted test

Time (min) post-administration	Treatment	Response time (sec)	Significance (p-value)
30	Control	49.00±1.03	
	Diazepam	23.09±4.13***	0.001
	APG 10 mg kg^{-1}	49.66±1.01	0.979
	APG 25 mg kg^{-1}	44.40±1.00	0.648
	APG 50 mg kg^{-1}	36.98±1.02	0.078
60	Control	43.00±1.25	
	Diazepam	9.87±0.00***	0.000
	APG 10 mg kg^{-1}	45.3±0.98	0.889
	APG 25 mg kg^{-1}	42.2±1.06	0.598
	APG 50 mg kg^{-1}	26±1.10	0.056
120	Control	39±0.88	
	Diazepam	4.88±0.00***	0.001
	APG 10 mg kg^{-1}	53.4±0.54	0.987
	APG 25 mg kg^{-1}	33±1.02	0.068
	APG 50 mg kg^{-1}	18±1.59*	0.023
240	Control	51.87±1.02	
	Diazepam	3.78±0.00***	0.001
	APG 10 mg kg^{-1}	52.9±0.87	0.965
	APG 25 mg kg^{-1}	26.8±0.98*	0.054
	APG 50 mg kg^{-1}	11.65±1.45**	0.010

Values are given as Mean±SEM, n = 6, APG: Apigenin, *p<0.05, **p<0.01 and ***p<0.001 when compared to the control group (Chi-square test)

it was not significant. However, the response time was significantly increased at 240 min recording of those animals who received a high dose of APG (50 mg kg^{-1}).

Effect of apigenin using inverted test: Animals who received diazepam, hold on to mesh for significantly shorter periods when compared to control. The Group of animals who were given a low dose of APG (10 mg kg^{-1}) held the mesh for a long time, almost similar to control groups. Animals who received a moderate dose (25 mg kg^{-1}) demonstrated a trend of activity from 120-240 min when compared to the control. Animals treated with a high dose of APG had a significantly decreased duration of holding capacity on the mesh at 120 and 240 min (Table 3).

DISCUSSION

The research was carried out to explore the role of one of the naturally occurring substances, apigenin (APG), as a muscle relaxant using standardized animal experimental models.

The present study demonstrated a dose-dependent pharmacological effect on decreasing the tone of muscular contraction and reducing muscle strength. The experimental

models used in our study are established and validated by literature as models to check muscle tone, alertness and muscle strength.

The Climbing test is meant for assessing motor balance and muscle coordination[28]. Animals with severely relaxed muscles showed difficulty climbing up the chain, while some animals were able to climb up the chain but took a longer time than the control animals. The Chimney test is an indicator of the alertness of animals. Similar to an earlier study[29], animals with retarted muscle tone exhibited difficulty in muscle coordination. Our results are in agreement with an earlier study using an inverted test where animals were not able to hold on to the mesh in the inverted position upon treatment with agents having muscle relaxant properties and a decrease in the muscle contraction declined the holding time[30].

The centrally acting muscle relaxants, namely Diazepam, were used in our study as a standard agent to compare the effects of APG. Apigenin binds to the central BDZ-R with a K_i in the low micromolar range, exhibiting a competitive type of inhibition in brain synaptosomal membranes. Incongruent with earlier study[31] where APG was shown to possess anxiolytic activity through its competitive activity in the brain, in our study, a high dose of APG showed muscle in-coordination. We speculate about a possible centrally acting muscle relaxant property of APG. The failure of low doses of APG to produce similar activity in this study suggests the availability of a low concentration of APG to penetrate BBB. Our findings are similar to earlier studies[31] where a low dose of APG failed to produce the anxiolytic or sedative effect that is possibly responsible for their muscle relaxant properties. Also, a study carried out earlier showed a concentration-dependent effect of APG on gastric relaxation[32]. However, a 5-fold increase in dosage produced a significant decrease in the climbing ability of animals with a significant increase in time to travel the distance. At high doses, apigenin also had a sedative effect. APG has previously been shown to influence glutamate and ionotropic -aminobutyric acid (GABA) neurotransmission. APG lowered GABA-evoked currents and N-methyl-D-aspartate (NMDA) receptor-mediated responses in a reversible manner. The sedative effects could thus be explained by a decrease in network excitability[33]. The reduction of GABA-activated Cl^- currents by APG in a dose-dependent fashion was also reported in another study where the effect of APG was blocked by co-application of Ro 15-1788, a specific benzodiazepine receptor antagonist[34]. The muscle relaxant efficacy of APG was found to be high at 4 hrs from the time of administration in climbing and chimney tests. In the inverted test, relatively early onset of action was seen for a high dose of APG (from 2 hrs itself) and even a moderate dose of APG also demonstrated a positive trend towards muscle relaxant potential. Classic BDZs, on the other hand, elicit a significant drop in these measures indicating high-intensity muscle relaxation. The effect of BDZs is also spontaneous and noticeable from the first time the response is recorded, but the high dose of APG has a delayed commencement of the action. The delay in the onset of action of APG could be due to decreased dissolution or slow absorption or delayed distribution of APG for producing central effects. Micro- and nano delivery for therapeutic formulations containing APG should be investigated and implemented to better target tissues and organs and improve therapeutic efficacy[35]. The possibility of using a modified delivery system is another option to improve therapeutic outcomes. A study was done earlier demonstrated the possible role of a solid dispersion model in enhancing the dissolution rate and absorption rate of orally administered APG[36]. Additionally, APG obtained from green and innovative technologies such as enzymatic treatment and microwave or ultrasound-assisted extraction may also improve its therapeutic benefits[37].

CONCLUSION

In conclusion, our present results suggest that apigenin, administered orally in mice, has dose-dependent skeletal muscle relaxant properties. Further, as the muscle relaxant property was delayed, it would be good to refine the formulation to facilitate better absorption and greater distribution to enhance the pharmacokinetic profile of the formulation and yield a good pharmacological effect. Additionally, to ascertain the actual mechanism of action, apigenin has to be tested in direct centrally acting models and compared with *in vitro* studies.

SIGNIFICANCE STATEMENT

This study discovered that apigenin possessed a significant dose-dependent muscle relaxant effect that could be beneficial for the treatment of undesired spasms or spasticity without impairing consciousness. The outcome of this study can provide one of the fundamental elements that can be further investigated to get a safe, economical and effective muscle relaxant that is devoid of any abuse and other negative implications of present-day muscle relaxants and equally possesses high efficacy to subvert muscular pain and other neuromuscular disturbances.

ACKNOWLEDGMENT

The authors are thankful to AlMaarefa University, Riyadh, Saudi Arabia, for providing support to do this research. Walaa F. Alsanie would like to acknowledge Taif university for support No. TURSP (2020/53).

REFERENCES

1. Naidu, S.V. and V. Rani, 2019. A study to evaluate the skeletal muscle relaxant property of pregabalin and gabapentin in albino rats. Int. J. Basic Clin. Pharmacol., 8: 1381-1386.
2. Veena, N.S., K. Sivaji, G.V. Benerji, M.F. Babu and D.R. Kumari, 2015. Skeletal muscle relaxant property of diazepam by using rotarod on albino mice. Indian J. Basic Appl. Med. Res., 4: 714-721.
3. George, M., L. Joseph and A. Sharma, 2012. Antidepressant and skeletal muscle relaxant effects of the aqueous extract of the *Prosopis cineraria*. Braz. J. Pharm. Sci., 48: 577-581.
4. Charles, R.C. and E.S. Robert, 2003. Modern Pharmacology with Clinical Applications. 6th Edn., Lippincott Williams and Wilkins, Philadelphia, ISBN-13: 978-0781737623, Pages: 832.
5. Chou, R., A. Qaseem, V. Snow, D. Casey, J.T. Cross, P. Shekelle and D.K. Owens, 2007. Diagnosis and treatment of low back pain: A joint clinical practice guideline from the American college of physicians and the American pain society. Ann. Internal Med., 147: 478-491.
6. van Tulder, M.W., T. Touray, A.D. Furlan, S. Solway and L.M. Bouter, 2003. Muscle relaxants for nonspecific low back pain: A systematic review within the framework of the cochrane collaboration. Spine, 28: 1978-1992.
7. Münch, G., M. Venigalla and E. Gyengesi, 2015. Curcumin and apigenin - novel and promising therapeutics against chronic neuroinflammation in Alzheimer s disease. Neural Regener. Res., 10: 1181-1185.
8. Salehi, B., A. Venditti, M. Sharifi-Rad, D. Kręgiel and J. Sharifi-Rad *et al*., 2019. The therapeutic potential of apigenin. Int. J. Mol. Sci., Vol. 20. 10.3390/ijms20061305.
9. Benavente-Garcia, O. and J. Castillo, 2008. Update on uses and properties of citrus flavonoids: New findings in anticancer, cardiovascular and anti-inflammatory activity. J. Agric. Food Chem., 56: 6185-6205.
10. Hansen, E., M. Krautwald, A.E. Maczurek, G. Stuchbury and P. Fromm *et al*., 2010. A versatile high throughput screening system for the simultaneous identification of anti-inflammatory and neuroprotective compounds. J. Alzheimer's Dis., 19: 451-464.
11. Myhrstad, M.C.W., H. Carlsen, O. Nordstrom, R. Blomhoff and J.O. Moskaug, 2002. Flavonoids increase the intracellular glutathione level by transactivation of the γ-glutamylcysteine synthetase catalytical subunit promoter. Free Rad. Biol. Med., 32: 386-393.
12. Zheng, P.W., L.C. Chiang and C.C. Lin, 2005. Apigenin induced apoptosis through p53-dependent pathway in human cervical carcinoma cells. Life Sci., 76: 1367-1379.
13. Shukla, S. and S. Gupta, 2006. Molecular targets for apigenin-induced cell cycle arrest and apoptosis in prostate cancer cell xenograft. Mol. Cancer Ther., 5: 843-852.
14. Liu, X., L. Li, L. Lv, D. Chen, L. Shen and Z. Xie, 2015. Apigenin inhibits the proliferation and invasion of osteosarcoma cells by suppressing the Wnt/β-catenin signaling pathway. Oncol. Rep., 34: 1035-1041.
15. Nakazawa, T., T. Yasuda, J. Ueda and K. Ohsawa, 2003. Antidepressant-like effects of apigenin and 2,4,5-trimethoxycinnamic acid from perilla frutescens in the forced swimming test. Biol. Pharm. Bull., 26: 474-480.
16. Kopustinskiene, D.M., V. Jakstas, A. Savickas and J. Bernatoniene, 2020. Flavonoids as anticancer agents. Nutrients, Vol. 12. 10.3390/nu12020457.
17. Sadraei, H., M. Ghanadian, G. Asghari and N. Sekhavati, 2018. Antispasmodic activity of apigenin and luteolin, two components of dracocephalum kotschyi extract, on rat ileum contractions. J. Herbmed Pharmacol., 7: 100-105.
18. Ali, F., Rahul, F. Naz, S. Jyoti and Y.H. Siddique, 2017. Health functionality of apigenin: A review. Int. J. Food Properties, 20: 1197-1238.
19. Zhao, L., J.L. Wang, R. Liu, X.X. Li, J.F. Li and L. Zhang, 2013. Neuroprotective, anti-amyloidogenic and neurotrophic effects of apigenin in an Alzheimer's disease mouse model. Molecules, 18: 9949-9965.
20. Adebesin, I.F., A.J. Akindele and O.O. Adeyemi, 2015. Evaluation of neuropharmacological effects of aqueous leaf extract of Albizia glaberrima (Leguminosae) in mice. J. Ethnopharmacol., 160: 101-108.
21. El Shoubaky, G.A., M.M. Abdel-Daim, M.H. Mansour and E.A. Salem, 2016. Isolation and identification of a flavone apigenin from marine red alga acanthophora spicifera with antinociceptive and anti-inflammatory activities. J. Exp. Neurosci., 10: 21-29.
22. Yemitan, O.K. and O.O. Adeyemi, 2003. Anxiolytic and muscle-relaxant activities of *Dalbergia saxatilis*. West Afr. J. Pharmacol. Drug Res., 19: 42-46.
23. Ahmad, S.S., S. Priyambada and P. Vijayalakshmi, 2019. Comparative study of muscle relaxant activity of midazolam with diazepam in male albino mice. Int. J. Pharm. Sci. Rev. Res., 58: 39-44.
24. Adeyemi, O.O., O.K. Yemitan and A.E. Taiwo, 2006. Neurosedative and muscle-relaxant activities of ethyl acetate extract of *Baphia nitida* AFZEL. J. Ethnopharmacol., 106: 312-316.
25. Vogel, H.G., 2002. Drug Discovery and Evaluation: Pharmacological Assays. 2nd Edn., Springer-Verlag Berlin Heidelberg, New York, Pages: 1408.
26. Deacon, R.M.J., 2013. Measuring the strength of mice. J. Visualized Exp., Vol. 76. 10.3791/2610.

27. Bawazeer, S., A. Rauf and S. Bawazeer, 2020. Gastrointestinal motility, muscle relaxation, antipyretic and acute toxicity screening of amyrin type triterpenoid (daturaolone) isolated from *Datura metel* Linnaeus (Angel's trumpet) fruits. Front. Pharmacol., Vol. 11. 10.3389/fphar.2020.544794.
28. Luong, T.N., H.J. Carlisle, A. Southwell and P.H. Patterson, 2011. Assessment of motor balance and coordination in mice using the balance beam. J. Visualized Experiments, Vol. 10. 10.3791/2376.
29. Muhammad, N., M. Saeed, H. Khan and I. Haq, 2013. Evaluation of n-hexane extract of viola betonicifolia for its neuropharmacological properties. J. Nat. Med., Vol. 67. 10.1007/s11418-012-0636-0.
30. García-Campos, P., X. Báez-Matus, C. Jara-Gutiérrez, M. Paz-Araos and C. Astorga *et al*., 2020. N-acetylcysteine reduces skeletal muscles oxidative stress and improves grip strength in dysferlin-deficient Bla/J mice. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21124293.
31. Kumar, S., R. Madaan and A. Sharma, 2008. Estimation of apigenin, an anxiolytic constituent, inturnera aphrodisiaca. Indian J. Pharm. Sci., 70: 847-851.
32. Rotondo, A., R. Serio and F. Mulè, 2009. Gastric relaxation induced by apigenin and quercetin: Analysis of the mechanism of action. Life Sci., 85: 85-90.
33. Losi, G., G. Puia, G. Garzon, M.C. de Vuono and M. Baraldi, 2004. Apigenin modulates GABAergic and glutamatergic transmission in cultured cortical neurons. Eur. J. Pharmacol., 502: 41-46.
34. Avallone, R., P. Zanoli, G. Puia, M. Kleinschnitz, P. Schreier and M. Baraldi, 2000. Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. Biochem. Pharmacol., 59: 1387-1394.
35. Huang, Y., X. Zhao, Y. Zu, L. Wang, Y. Deng, M. Wu and H. Wang, 2019. Enhanced solubility and bioavailability of apigenin via preparation of solid dispersions of mesoporous silica nanoparticles. J. Pharm. Res., 18: 168-182.
36. Alshehri, S.M., F. Shakeel, M.A. Ibrahim, E.M. Elzayat and M. Altamimi *et al*., 2019. Dissolution and bioavailability improvement of bioactive apigenin using solid dispersions prepared by different techniques. Saudi Pharm. J., 27: 264-273.
37. Durazzo, A., M. Lucarini, E. Camilli, S. Marconi and P. Gabrielli *et al*., 2018. Dietary lignans: Definition, description and research trends in databases development. Molecules, Vol. 23. 10.3390/molecules23123251.