Antidopaminergic effects of leucine and genistein on shizophrenic rat models

Palle Suresh, *M. Pharm,* ***Akondi B. Raju,*** *M.Pharm, PhD.*

ABSTRACT

الأهداف: تقييم أثر الوسين والجنسين على الجهاز الدوباميني في نموذج الفئران المصابة بالفصام.

الطريقة: دراسة أثر الوسين والجنسين على الابومورفين الناتج عن السلوك الغطية والهالوبيريدل المسبب للجمدة، وصدمة القدم المسبب للتعدي والابومبروفين المسبب للنشاط الحركي. أعطي كلا من الوسين (0.7 جرام/كلغ) والجنسين (30 جرام/كلغ) لمدة 30 دقيقة قبل عمل الاختبار في الفئران. أجريت هذه الدراسة الحالية خلال الفترة من أبريل 2011م حتى سبتمبر 2011م في ارانجال، ولاية اندرا براديش، الهند. عبرت النتائج بالمعدل + معدل الخطأ المعياري وأجري التحليل الإحصائي للبيانات باستخدام اختبار الأنوفا ثم اختبار بنفوريني. كما أن القيمة الإحصائية اقل من 0.05 تعتبر قيمة مهمة إحصائياً.

النتائج: قلل الوسين والجنسين عدد الضربات ورفع من خفاء الضربات في صدمة القدم المسببه للتعدي. كما قللت من ابومبروفين (5 ملغ/كلغ)، والابومبروفين و سلوك الغطية (10 ملغ/كلغ). والنشاط الحركي بالمقارنة مع مجموعة التحكم الإيجابية. يحفز العلاج بالوسين والجنسين هالوبيريدل بشكل إحصائي المسبب للجمدة بالمقارنة مع المجموعة التي استخدمت الهالوبيريدل.

خاتمة: أن استخدام الوسين والجنسين يقلل من النشاط الدوباميني بالمقارنة مع الاستخدام المرتبط. تشير هذه النتائج أن الوسين والجنسين له دور طبي في السيطرة على الأمراض النفسية.

Objectives: To evaluate the effect of leucine and genistein on the dopaminergic system in a rat model of schizophrenia.

Methods: Behavioral effects of leucine and genistein on apomorphine induced stereotyped behavior, haloperidol induced catalepsy, foot shock induced aggression, and apomorphine induced locomotor activity were conducted. In each of these tests, the leucine (0.7g/kg p.o.) and genistein (30mg/kg i.p.) were administered 30 minutes before performing the test in rats. Each experiment has 6 groups of rats with 6 rats in each group. The current study was conducted between April 2011 and September 2011 at the Department of Pharmacology, St.Peters Institute of Pharmaceutical Sciences, Warangal, Andhra Pradesh, India. The results were expressed as mean ± S.E.M. and the statistical analysis of data was carried out using one-way analysis of variance (ANOVA), followed by Bonferroni multiple comparison test. Probability level (P) less than 0.05 was considered statistically significant.

Results: Leucine and genistein significantly (p<0.05) reduced the number of fights and increased latency to fights in foot shock-induced aggression; it also decreased apomorphine (5mg/kg, i.p.) induced stereotyped behavior and apomorphine induced (10mg/kg, s.c.) locomotor activity when compared with the positive control group. Pretreatment with leucine and genistein significantly (p<0.01, 55.5±5.898 minutes) potentiated the haloperidol induced catalepsy compared with the haloperidol treated group.

Conclusion: The individual administration of leucine and genistein had less anti dopaminergic activity when compared with their combined administration. These results suggest that leucine and genistein may have a potential clinical application in the management of psychiatric disorders.

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From the Department of Pharmacology, St. Peters Institute of Pharmaceutical Sciences, Vidyanagar, Hanamkonda, Warangal, India.

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Address correspondence and reprint request to: Dr. Akondi B. Raju, Department of Pharmacology, St. Peters Institute of Pharmaceutical Sciences, Vidyanagar, Hanamkonda, Warangal, AP 506001, India. Tel. +918 008757878. Fax. +918 702567304. E-mail: drraju2020@gmail.com

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Schizophrenia is a common and debilitating illness characterized by chronic psychotic symptoms and psychological impairments that strikes at some of the most advanced functions of the human brain. It is a highly prevalent mental illness in our society that inflicts an enormous burden of distress on the affected individuals and families. Genetics, early environment, neurobiology, psychological, and social processes appear to be important contributory factors. Symptoms can be divided into 3 main categories:[1] 1) Psychotic or positive that includes hallucinations, delusions, and thought disorder. 2) Deficit or negative symptoms that consist of severe disturbances in social interaction, motivation, expression of affect, and spontaneous speech.[2] 3) Cognitive impairment affects executive function, attention, memory, and general intellectual functioning.[3] A hyperdopaminergic state is one of the underlying theories for the occurrence of schizophrenia. There is, however, accumulating evidence that schizophrenia is a dopamine dysregulating disorder. Striatal dopamine activity mediated by dopamine (D2) receptors is increased while prefrontal dopamine activity, transmitted by dopamine (D1) receptor activity, is decreased in schizophrenia.[4] The strategy for discovering new antipsychotic drugs remains based on the dopamine hypothesis.[5] In order to improve the pharmacotherapy of schizophrenia,[6] it is necessary to discover and develop several important central nervous acting drugs[7] from traditional remedies, which may provide pathways to discover innovative antipsychotic medications.

Isoflavones are the most well-known type of phytoestrogens. Genistein, the primary soybean isoflavone, has been shown to be beneficial for human health. It has a chemical structure similar to steroidal estrogen (**Figure 1**).

The hydroxyl group and aromatic ring are important for binding effectiveness. However, very few studies have been conducted to investigate the effect of genistein on the CNS. Genistein can mimic the pharmacological actions of the endogenous steroid estrogen, with which it has structural similarities.[8] Genistein can bind to the estrogen receptors and activates the number of estrogen-responsive genes in vitro.[9,10] Estrogens play a significant role in the pathogenesis of psychosis.[11] Treatment with estrogens has been reported to offer benefits in schizophrenia.[12] Unlike synthetic estrogen derivatives that are associated with serious adverse effects, phytoestrogens are considered to be safer.[13] Abnormal levels of amino acids have been reported in patients with schizophrenia and have also been investigated as a biomarker to monitor antipsychotic treatment. The synthesis of biogenic amines (dopamine, norepinephrine, serotonin, and histamine) is related to the availability of their amino acid precursors tyrosine, tryptophan, and histidine.[14] The levels of these substrates in the CNS are influenced by the blood concentration of valine, leucine, isoleucine, and phenylalanine, which have affinity for the same carriers of tyrosine and tryptophan to cross the blood-brain barrier.[15] Administration of competing neutral amino acid (for example, leucine, tryptophan) reduces brain tyrosine and its rate of conversion to dihydroxyphenylalanine (DOPA). Changes in amino acid plasma concentrations might affect the susceptibility to psychotic disorders and influence their treatment outcome. Hence, in the current study, we evaluated the effect of leucine and genistein on the dopaminergic system in a rat model of schizophrenia.

Methods.

Animals. Sixty Albino rats of Wistar strain, weighing 200-250 g were used. The animals were kept under a controlled 12 hour light-dark cycle with food and water available ad libitum throughout the experiment. Animals used in this study were maintained in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSCEA). The current study was conducted between April 2011 to September 2011 at the Department of Pharmacology, St.Peters Institute of Pharmaceutical Sciences, Warangal, Andhra Pradesh, India and approved by Institutional Animal Ethics Committee (IAEC) vide No. 023/SPIPS/WGL/IAEC/2011.

Reagents. The following drugs were used in the present study: Haloperidol (HAL) (RPG Life Sciences, Mumbai, India); Apomorphine (APO) (Sigma-Aldrich, St. Louis, MO,USA), Leucine (L) (Himedia, Mumbai, India); and Genistein (G) (Bioprex Labs, Mumbai, India).

Behavioral models. Assessment of apomorphine induced stereotyped behavior in rats. Rats were allowed 30 minutes to become acclimatized to the observation cage, prior to the experiment. The intensity of apomorphine-induced stereotypy was scored blind by an independent observer every 5 minutes for 30 minutes and scored according to the method previously described by Barros and Leite[16] using the following rating scale: 0, absence of stereotyped behavior; 1, intermittent sniffing; 2, constant sniffing; 3, constant sniffing with intermittent licking and/or false-biting; 4,

constant licking or false-licking; 5, constant licking; 6, constant biting and moving round; 7, constant biting and resisted to a small area in the cage. The rats were treated with the leucine (0.7 g/kg p.o.) and genistein (30 mg/kg i.p.) or normal saline and placed individually into the cage. Apomorphine (5 mg/kg i.p.) was given 30 minutes after the leucine and genistein administration and the intensity of stereotyped behavior was recorded.

Haloperidol induced catalepsy. Haloperidol (1 mg/kg) was injected intraperitoneally to rats (n=6) pretreated with leucine (0.7 g/kg p.o.) and genistein (30 mg/kg i.p.). Leucine and genistein were administered 30 minutes prior to the administration of haloperidol. The duration of catalepsy was measured at 30, 60, 120, and 360 minutes using the bar test.[17] Both the forepaws of rats were placed on a horizontal bar raised 9 cm from the table, and the time required to remove the forepaws from the bar was recorded as the duration of catalepsy.

Foot shock induced aggression. Foot shock-induced aggression (FSIA) behavior was induced for 3 minutes by administering a train of impulses through an electronic stimulator to a grid floor. The animals were divided into 6 groups of 6 rats per group. Haloperidol (1 mg/kg, i.p.) as a standard, and leucine (0.7 g/kg p.o.), genistein (30 mg/kg i.p.) were administered 30 minutes prior to the experiment. Aggressive behavior was noted in pairs of rats using 2 parameters, namely, number of fights and latency to fight.[18]

Apomorphine induced locomotor activity. The study was carried out in rats (200-250gm) in 6 groups. The actophotometer equipment was turned on and checked to ensure that all the photocells were working, and each rat was placed individually in the activity cage for 10 minutes. The basal activity score was noted for all the animals. In this experiment the rats were divided into 6 groups (n=6). Haloperidol (1 mg/kg, i.p.) as a standard, and leucine (0.7 g/kg p.o.), and genistein (30 mg/kg i.p.) were administered 30 minutes prior to the experiment, and after 30 minutes each rat was retested activity scores for 10 minutes.[19]

Statistical analysis. Results are expressed as mean ± S.E.M. and the statistical analysis of data was carried out using one-way analysis of variance (ANOVA), followed by Bonferroni multiple comparison test. The statistical analysis was carried out using Graph pad software (GraphPad Software Inc. La Jolla, CA, USA) version 5. A probability level (P) less than 0.05 was considered statistically significant.

Results. ***Apomorphine induced stereotyped behavior.*** Animals were pretreated with leucine (0.7 g/kg), genistein (30 mg/kg), and a combination of leucine and genistein 30 minutes before apomorphine administration. Apomorphine (5 mg/kg i.p.) induced stereotyped behavior was characterized by intermittent or constant sniffing, intermittent or constant licking, and intermittent or constant biting. There was a significant (p<0.05) protection of rats from apomorphine induced stereotyped behavior by administering leucine and genistein. Genistein was more potent when compared with leucine individually, and a combination of both showed significant (p<0.01) protection (**Figure 2**).

Haloperidol induced catalepsy. Animals were pretreated with leucine, genistein, and a combination of leucine (0.7 g/kg, p.o) and genistein (30 mg/kg, i.p) 30

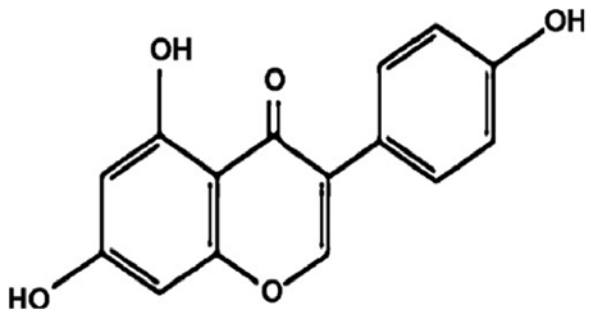

Figure 1 - Chemical structure of genistein. International Union of Pure and Applied Chemistry Name: 5,7-Dihydroxy-3-(4-hydroxyphenyl)chromen-4-one. Genstein found in many plants including soy beans to proved to be beneficial various medical illnesses.[50]

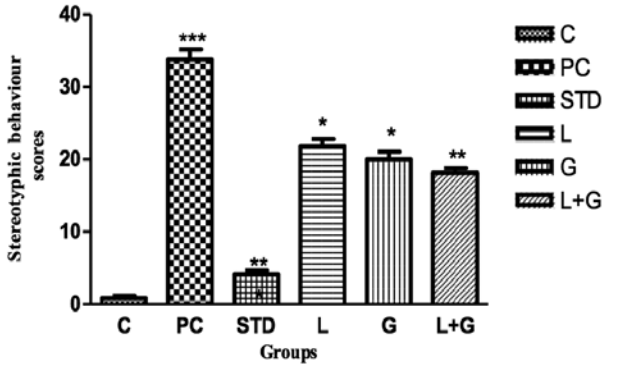

Figure 2 - Effect of leucine and genistein on apomorphine (5 mg/kg i. p.) induced stereotyped behavior in rats. Effect of leucine and genistein on apomorphine(5 mg/kg i. p.) induced stereotyped behavior in rats. Each point is mean ± S.E.M. The above experiment has 6 groups of rats with 6 rats in each group. One way analysis of variance followed by Boenferoni's multiple comparison test revealed significant difference between control and various groups. *p<0.05, **p<0.01, ***p<0.001 versus vehicle. C - control group, PC - positive control group, STD - standard drug received group L - leucine, G - Genistein

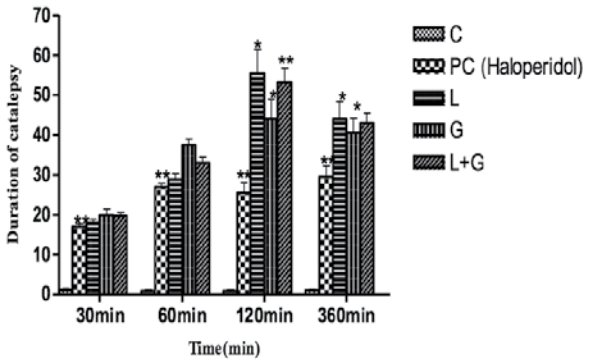

Figure 3 - Effect of leucine and genistein on haloperidol induced catalepsy in rats. Each point is mean ± S.E.M. The above experiment has 6 groups of rats with 6 rats in each group. One way analysis of variance followed by Boenferoni's multiple comparison test revealed significant difference between control and various treatment groups. *p<0.05, **p<0.01 versus vehicle. C - control group, PC - positive control group, STD - standard drug received group L - leucine, G - Genistein

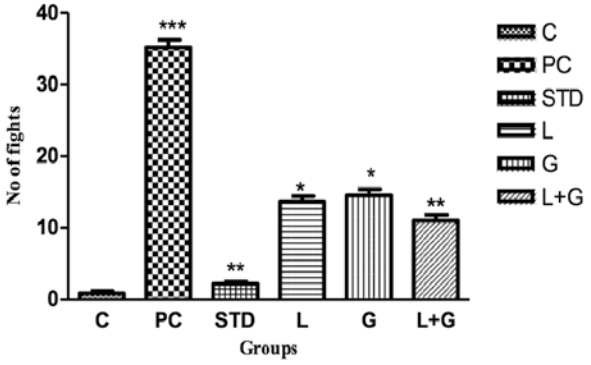

Figure 5 - Effect of leucine and genistein on foot shock induced aggression in rats. Each point is mean ± S.E.M. The above experiment has 6 groups of rats with 6 rats in each group. One way analysis of variance followed by Boenferoni's multiple comparison test revealed significant difference between control and various treatment groups. *p<0.05, **p<0.01, ***p<0.001 versus vehicle. C - control group, PC - positive control group, STD - standard drug received group L - leucine, G - Genistein

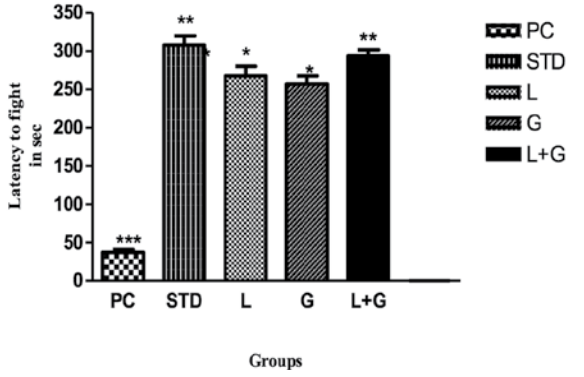

Figure 4 - Effect of leucine and genistein on latency of flights in rats. Each point is mean ± S.E.M. The above experiment has 6 groups of rats with 6 rats in each group. One way analysis of variance followed by Boenferoni's multiple comparison test revealed significant difference between control and various treatment groups. *p<0.05, **p<0.01, ***p<0.001 versus vehicle. C - control group, PC - positive control group, STD - standard drug received group L - leucine, G - Genistein

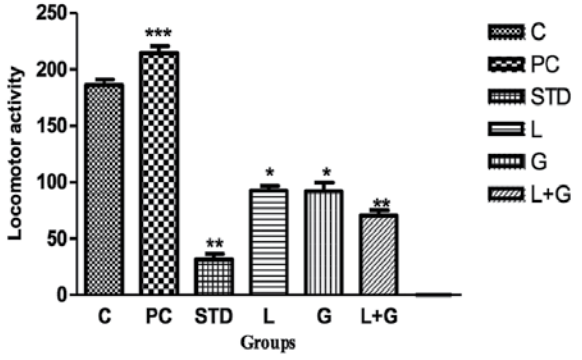

Figure 6 - Apomorphine induced locomotor activity. Each point is mean ± S.E.M. The above experiment has 6 groups of rats with 6 rats in each group. One way analysis of variance followed by Boenferoni's multiple comparison test revealed significant difference between control and various treatment groups. *p<0.05, **p<0.01, ***p<0.001 versus vehicle. C - control group, PC - positive control group, STD - standard drug received group L - leucine, G - Genistein

minutes prior to haloperidol administration. In positive control animals, haloperidol (1 mg/kg i.p.) produced maximum catalepsy at 360 minutes (29.5±2.7425). There was significant (p<0.05) potentiation of rats from haloperidol induced catalepsy by administering leucine and genistein. Genistein was more potent when compared with leucine individually, and a combination of both showed significant (p<0.01) potentiation. The rats treated with leucine (55.5±5.898 seconds), genistein (44±4.933 seconds), and leucine + genistein (53.167±3.582 seconds) exhibited maximum catalepsy at 120 minutes (**Figure 3**).

Foot shock induced aggression. Intraperitoneal administration of haloperidol (1mg/kg, i.p.) showed a significant (p<0.001) decrease in the number of fights and increased the latency to fight compared with positive controls in foot shock induced aggression. The number of fights were less in the leucine and genistein treated groups (p<0.05). As shown earlier, the protection was high with a combination (p<0.01), when compared to individual treatment with leucine and genistein (**Figures 4 & 5**).

Apomorphine induced locomotor activity. Apomorphine (10mg/kg s.c.) significantly (p<0.001)

potentiated the locomotor activity when compared with the control group. Animals were pretreated with leucine, genistein, and a combination of leucine and genistein 30 minutes prior to apomorphine administration. Haloperidol significantly (p<0.01) decreased locomotor activity in apomorphine induced locomotion when compared with positive controls. Leucine, genistein, and a combination of leucine and genistein showed a significant (p<0.01) reduction in locomotor activity compared with positive controls. When leucine and genistein were given in combination, there was a significant (p<0.01, 70.50±4.53 counts or digital score) decrease in locomotor activity compared with the individual leucine and genistein treated groups (**Figure 6**).

Discussion. The estrogen hypothesis suggests that estrogen provides protection from the development of schizophrenia and decreases the severity of negative symptoms.[20,21] Preclinical data supports the involvement of estrogen in the regulation of several neurotransmitter systems (dopamine, serotonin, noradrenalin, and glutamate).[22,23] Beside the direct influence on neurotransmission, estrogen may play a role in schizophrenia by susceptibility gene regulation.[24] Estrogen affects a variety of processes during brain development including neuronal differentiation, survival, and excitability.[25,26]

Apomorphine is specific for the dopaminergic system acting as a receptor agonist. The nucleus accumbens and the caudate-putamen nucleus[27-29] are the important anatomic regions, which are involved in the stereotypic behavior. Dopaminergic over stimulation leads to stereotyped behavior and it can be induced by the dopamine receptor agonist apomorphine,[30,31] or by the dopamine releasing agent, amphetamine.[32] It is a measure of dopamine (D2) receptor reactivity. Apomorphine decreases motor activity at lower doses and causes hyperactivity and stereotypy in high doses.[33-35] Leucine and genistein and its combination decreased the stereotype behavior in rats, suggesting that these compounds are having antidopaminergic activity. Leucine and genistein in combination significantly decreased the apomorphine induced stereotypic behavior compared with individual administration. Most drugs, such as phenothiazines, thioxanthines, and butyrophenones, which are used in the treatment of psychosis are known to have a preference for D2 receptors,[36] and abolished apomorphine induced stereotyped behavior,[16] based on this, further investigation is required to investigate the antipsychotic potential of leucine and genistein.

Catalepsy is a trans like state of self-hypnotic sleep during which there is long term maintenance of an animal in an abnormal posture. The striatum and nucleus accumbens are the major brain structures involved in neuroleptic induced catalepsy, which appears due to blockade of dopamine neurotransmission.[37] In the present study, leucine and genistein and its combination significantly increased the haloperidol increased catalepsy. This potentiation of cataleptic effect suggests the antidopaminergic effect of leucine and genistein. The dopamine agonist apomorphine and amphetamine can exacerbate psychosis in patients suffering from schizophrenia.[38,39] These clinical findings have led to the development of the dopamine hypothesis of schizophrenia.[40] Therefore, the behavioral sensitization of rats to dopamine agonists could be a good experimental murine model for finding newer antipsychotic drugs. Dopamine agonists such as apomorphine induce a strong increase in locomotor activity when injected into rodents. There is evidence that this increased locomotor activity is due to increased dopaminergic activity in the mesolimbic system, particularly, forebrain structures, nucleus accumbens, and olfactory tubercle are known to be involved in mediating locomotor activity. According to existing evidence, the dopamine receptors located in the nigrostraital system are responsible for the stereotyped behavior; whereas induction of locomotor activity is related to the mesolimbic dopamine system.[41,42] Administration of the dopamine antagonist effectively antagonized apomorphine-induced increase in the locomotor activity in experimental animals. It has been shown that the drugs display selectivity for the dopamine receptors.[43] Administration of leucine and genistein in combination compared to their individual administration showed a significant decrease in apomorphine induced locomotor activity suggesting that leucine and genistein having dopamine antagonistic activity. Dopamine levels, measurement of dopamine synthesis, and turnover in the whole brain have increased in aggressive strains of rats, and in rats that have just engaged in aggressive behavior.[44] In the foot-induced aggressive behavior model, the level of dopamine increases in the striatum.[45] A postmortem study[46] showed that the levels of GABA and glutamic acid decarboxylase were low in brain areas such as the striatum and olfactory lobes, and there was increased dopamine levels in rats that exhibited aggressive behavior.

In the present study, the results showed that there was a significant decrease in foot shock induced aggression, which may be mediated by the dopamine antagonistic activity of leucine and genistein. Based

on the results of these behavioral studies, we confirm that the administration of leucine and genistein reduces schizophrenia, and this can be attributed to their antidopaminergic activity. We provided evidence that phytoestrogen genistein, is similar to the action of estrogen and can have positive influences on dopaminergic function via estrogen mediated receptors.

Further studies are required, taking into considering the limitations of this study, which include identification of the exact mechanisms. These further studies will prove the role of leucine and genistein in potential clinical applications in the management of psychiatric disorders.

In conclusion, the administration of leucine and genistein showed a decrease in apomorphine induced stereotyped behavior, foot shock induced aggression, apomorphine induced locomotor activity, and increased haloperidol induced catalepsy. The individual administration of leucine and genistein resulted in lower antidopaminergic activity when compared with their combined administration.

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Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as the use of *P* values, which fails to convey important information about effect size. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the computer software used.