ISSN: Print -0976-9234

Journal of Pharmaceutical Negative Results

Volume No. 17
Issue No. 1
Jan - Apr 2025

ENRICHED PUBLICATIONS PVT. LTD

JE-18, Gupta Colony, Khirki, Extn, Malviya Nagar, New Delhi-110017
PHONE : - + 91-8877340707
E-Mail : info@enrichedpublications.com

ISSN: Print -0976-9234

Journal of Pharmaceutical Negative Results

Aims and Scope

Journal of Pharmaceutical Negative Results (www.pnrjournal.com) [ISSN: Print -0976-9234, Online - 2229-7723] – (An official publication of Association of Indian pharmacist-AIP, Published by ResearchTrentz). The journal is a peer-reviewed journal developed to publish original, innovative and novel research articles resulting in negative results. This peer-reviewed scientific journal publishes a theoretical and empirical paper that reports the negative findings and research failures in pharmaceutical field. Submissions should have a negative focus, which means the outputs of research yielded in negative results are being given more preference. All theoretical and methodological perspectives are welcomed. We also encourage the submission of short papers/communications presenting counter-examples to usually accepted conjectures or to published papers. This Journal is a biannual publication.

ISSN: Print -0976-9234

ISSN: Print -0976-9234

Journal of Pharmaceutical Negative Results

(Volume No. 17, Issue No. 1, Jan - Apr 2025)

Contents

Beta vulgaris: A dearth of antidepressant activity in mice

Ashok Agrawal, Sunil Bavaskar, Pankaj Salunkhe, Nadeem Manyar, Dnyaneshwar Kale, Pravin Kawtikwar
Shree Sureshdada Jain Institute of Pharmaceutical Education and Research Center, Jamner, Maharashtra, India

A B S T R A C T

The present study was undertaken to investigate antidepressant activity of ethanolic extract of Beta vulgaris [BV], by using forced swim test [FST] and tail suspension test [TST] in mice. Imipramine [10 mg/kg, i.p.] used as the standard drug significantly decreased immobility time in both TST and FST, while BV [50 and 100 mg/kg, i.p.] significantly increased immobility time in TST and FST. This suggests that BV has depressant activity and not antidepressant activity.

Key words: Beta vulgaris, forced swim test, tail suspension test

INTRODUCTION:

Depressive affect or feeling is a normal response to disappointment, loss or other painful events of human life. According to the WHO World Health Survey, depression produces the greatest decrement in health when compared with the chronic diseases angina, arthritis, asthma and diabetes[1] and by the year 2020 will be second only to cardiovascular illness in contributing to the total disease burden imposed on humankind worldwide.[2] Depressive affects are self-limited and do not usually significantly interfere with a person's functional capacity unless they become long-lasting.[3] Moreover, it has been postulated that in some situations the depressive mood might even be useful and may have offered a selective advantage in humans' evolutionary history, by disengaging former goals and reallocating resources.[4] A metabolic disorder of monoamine neurotransmitters in the central nervous system (CNS) is believed to be the main biochemical symptom of depression.

Beta vulgaris, a native of the coasts of Mediterranean, is extensively cultivated in Europe, America and many parts of India.[5] Aqueous and ethanolic extracts of Beta vulgaris have been reported to possess free-radical–scavenging activity, reducing the radical cations and phase II enzymeinducing activities in a murine hepatoma cell in vitro study. Further, the phenolic amides isolated from the seeds of Beta vulgaris have been shown to produce an inhibitory effect on lipopolysaccharide-induced nitric oxide production in experimental isolated tissues in a dose-dependent manner.[6] There are some reports indicating the potential hepatoprotective, antioxidant and anti-inflammatory activities of Beta vulgaris, though without any scientific proof.[7]

MATERIALS AND METHODS

Plant material and extraction

The roots of Beta vulgaris [Family: Chenopodiaceae] were authenticated by Dr. Tanveer A. Khan at the Department of Botany, M. J. College, Jalgaon. Five hundred grams of root were macerated with ethanol for 2 days, and the extract was concentrated under reduced pressure. The extract was dried and stored in amber-colored bottle in a refrigerator.

Animals

Male Albino mice weighing 20-25 g were bred in our animal house [at Shree Sureshdada Jain Pharmaceutical Education and Research Center, Jamner, India] and used for the study. Animals were housed in groups of 5 per cage under standard laboratory conditions of temperature [25 ± 2°C] and relative humidity (45%-55%) and a 12/12hour light/dark cycle. They had access to standard pellet chow [Pranav Agro Industries Ltd., Sangali] and water. All experiments were carried out between 8:00 a.m. and 04:00 p.m. Food, but not water, was withdrawn 4 hours prior to administrations of extracts and drug, till completion of the experiment. The institutional animal ethical committee [IAEC] approved the protocol of this study.

Drugs

The drugs used were Beta vulgaris (BV) 50 and 100 mg/ kg i.p. and imipramine [10 mg/kg i.p.]. All drugs were administered 30 minutes before the start of experiments.

Tail suspension test

In the tail suspension test, mice suspended by the tail show initial struggling, followed by periods of immobility that increase in duration across the 6-minute test. The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al., as a facile means of evaluating potential antidepressants. [8] Mice were hung individually 58 cm above the floor by adhesive tape placed approximately 1 cm away from the tip of the tail. Immobility was recorded during the 6-minute period, in which immobility during the initial 2-minute period was discarded. The animal was considered to be immobile when it did not show any movement of body and hanged passively.

Forced swim test

Behavior despair was proposed as a model to test for antidepressant activity by Porsolt.[9,10] Mice were forced to swim individually in a glass jar [25×12×25 cm3] containing fresh water of 15 cm height and maintained at 25°C. After an initial 2-minute period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the

water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 minutes of the total of 6 minutes' duration of the test. The changes in immobility duration were studied after administering drugs in separate groups of animals.

Statistics

All the data are shown as mean ± SEM (standard error of mean). Statistical analysis was performed with one-way ANOVA followed by Dunnett's test. Differences with $P < .05$ were considered statistically significant

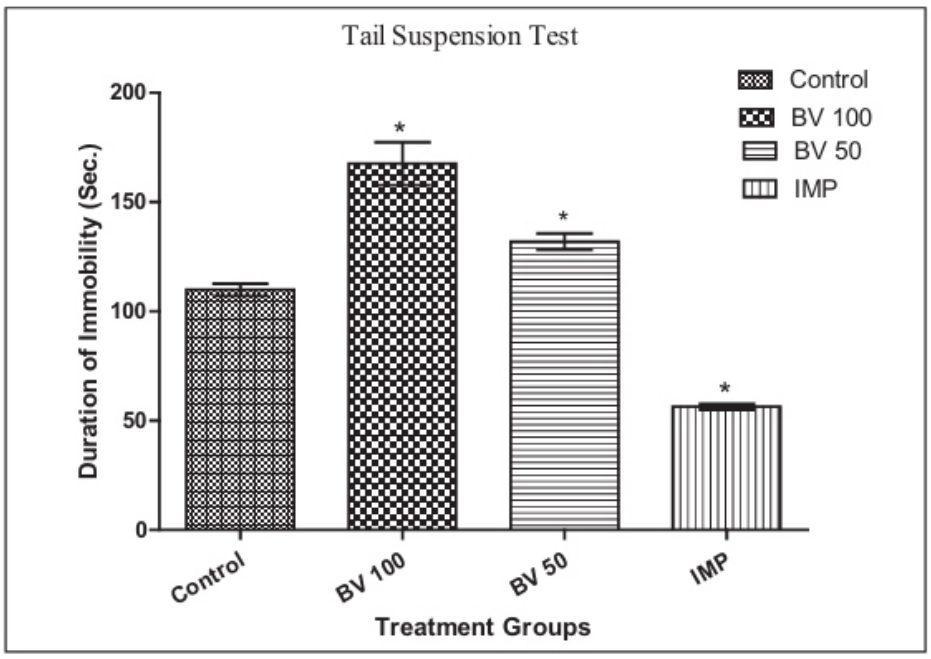


Figure 1: Effect of BV on immobility duration in TST. All the data are shown as mean ± SEM. Statistical analysis was performed with one-way ANOVA followed by Dunnett's test. $P < .05$ was considered statistically significant. *$P < .05$ compared with control.

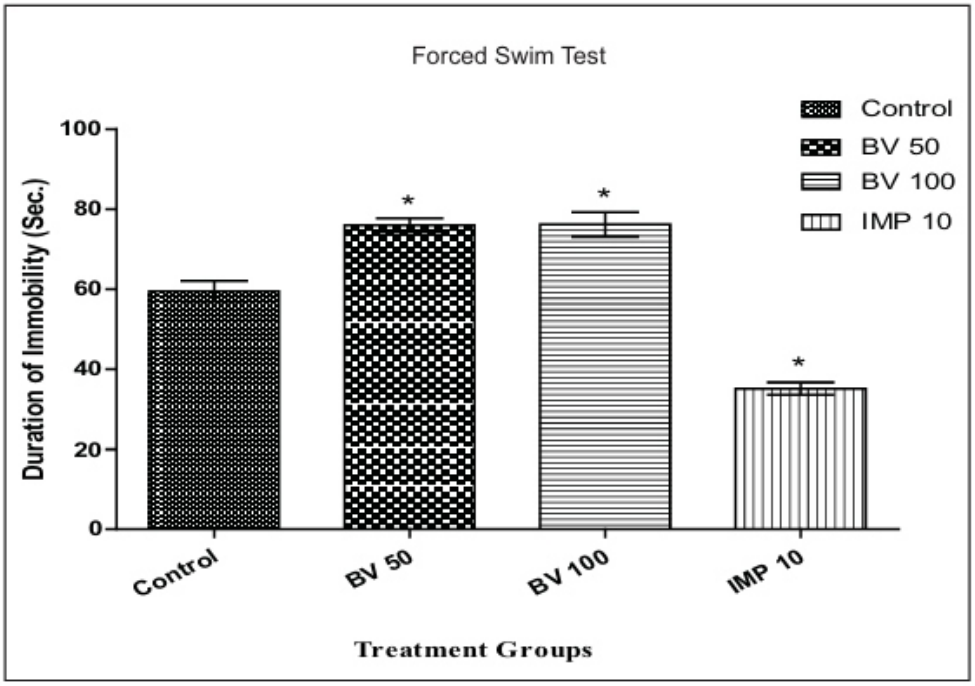


Figure 2: Effect of BV on immobility duration in FST. All the data are shown as mean ± SEM. Statistical analysis was performed with one-way ANOVA followed by Dunnett's test. P < .05 was considered statistically significant. *P < .05 compared with control.

RESULTS

Effect of BV on immobility duration in the tail suspension test In the tail suspension test, BV exhibited an increase in duration of immobility. The results were statistically significant for both dose levels, as shown in Figure 1.

Control

Effect of BV on immobility duration in the forced swim test In the forced swim test, BV exhibited an increase in duration of immobility in the mice. The results were BV 50 statistically significant for both dose levels, as shown in Figure 2.

DISCUSSION

In the present study, BV at both dose levels produced significant depressant effect in mice in both forced swim test (FST) and tail suspension test (TST). Both these models of depression are widely used to screen new antidepressant drugs.[8,9] This immobility, referred to as behavioral despair in animals, is claimed to reproduce a condition similar to human depression.[8,11] It has been argued that the TST is less stressful than FST and has greater pharmacological sensitivity.[12] These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs, including tricyclics, serotonin-

-specific reuptake inhibitors, monoamine oxidase inhibitors and atypical antideppresant drugs.[8,9,13] In conclusion, our results suggest that BV produces depressant effect in mice in both FST and TST, and this effect seems to be mediated most likely through blocking of adrenergic, serotonergic, dopaminergic or GABAnergic system. Thus the results obtained in the present study require further investigation to elucidate the depression induced by Beta vulgaris extracts by using various agonist-antagonist systems. Furthermore, in clinical populations, the possibility of pharmacological interactions between drugs and Beta vulgaris extracts must be considered.

REFERENCES

1. Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: Results from the World Health Surveys. Lancet 2007;370:851-8.

2. Murray CJ, López AD. Evidence-based health policy - lessons from the Global Burden of Disease Study. Science 1996;274:740-3.

3. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th ed. Washington DC: American Psychiatric Association; 2000.

4. Nesse RM. Evolutionary Explanations for Mood and Mood Disorders. In: Stein DJ, Kupfer DJ, Schatzberg AF, editors. Textbook of Mood Disorders. Washington, DC: The American Psychiatric Publishing; 2006. p. 159-75.

5. Chopra RN, Nayar SL, Chopra C. Glossary of Indian medicinal plants. New Delhi: CSIR; 1956. 7.

6. Wettasinghe M, Bolling B, Plhak L, Xiao H, Parkin K. Phase II enzyme-inducing and antioxidant activities of beetroot (Beta vulgaris L.) extracts from phenotypes of different pigmentation. J Agric Food Chem 2002;50:6704-9.

7. Duke JA, Bogenschutz-Godwin MJ, Du J, Cellier P. Handbook of medicinal herbs. Boca Raton: FL7 CRC Press; 2002. p. 68.

8. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology (Berl) 1985;85:367-70.

9. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: A primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977;229:327-36.

10. Porsolt RD, Anton G, Deniel M, Jalfre M. Behavioral despair in rats: A new animal model sensitive to antidepressant treatments. Eur J Pharmacol 1978;47:379-91.

11. Willner P. The validity of animal models of depression. Psychopharmacology (Berl) 1984;83:1-16.

12. Thierry B, Steru L, Simon P, Porsolt RD. The tail suspension test: ethical considerations. Psychopharmacology (Berl) 1986;90:284-5.

13. Detke MJ, Rickels M, Lucki I. Active behavior in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl) 1995;

121:66-72.

Insignificant antidiabetic activity of rhizome of Zingiber zerumbet

V. K. Lal, A. Pandey1, P. Tripathi1, R. D. Pandey1
Sagar Institute of Technology and Management, Barabanki, 1Institute of Technology and Management, Gorakhpur, India

ABSTRACT

Objective: To investigate the antidiabetic activity of Z. zerumbet in streptozotocin (STZ)induced diabetic rats. Materials and Methods: The diabetic rats were given aqueous extract of Z. zerumbet (200 mg/kg) and glibenclamide (10 mg/kg) for 21 days, and their hypoglycemic activity and effect on body weight were assessed. Result: The treatment with aqueous extract of Z. zerumbet and glibenclamide both showed hypoglycemic activity but efficacy of Z. zerumbet is not as significant as glibenclamide. Glibenclamide maintain the body weight of rats throughout the study period, whereas the body weight of Z. zerumbet-treated rats significantly falls. Conclusion: This study suggests that aqueous extract of Z. zerumbet shows no significant activity in STZ-induced diabetic rats.

Key words: Antidiabetic activity, glibenclamide, Z. zerumbet

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders with different underlying etiologies, each characterized by hyperglycemia due to under utilization of glucose.[1] The pharmacological agents currently used for treatment of type-2 diabetes include sulphonylureas, biguanide, thiazolidinediones, and acarbose. These agents however have restricted usage due to several undesirable side effects and fail to significantly alter the course of diabetic complications.[2] The limitation of currently available oral antidiabetic agents either in terms of efficacy or safety coupled with emergenic of the disease into a global epidemic have encouraged a concerted effort to discover drugs that can manage type-2 diabetes more efficiently. [3] Also with increasing incidence of diabetes in rural population throughout the world and due to adverse effects of synthetic medicine, there is a clear need for development of indigenous, inexpensive new source for antidiabetic crude or purified drugs.[4] Because ancient time plants have been exemplary sources of medicine, Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45, 000 plants species and among them, several thousands have been claimed to possess medicinal properties. Research conducted in last few decades on plants mentioned in ancient literature or used traditionally for diabetes has shown antidiabetic property. The recommendation of the WHO committee on diabetes mellitus encouraging research on hypoglycemic agents of plant origin used in traditional medicine has greatly motivated

research in this area.[5]

Currently, several hundred plants have been reported to have beneficial effects in the treatment of diabetes.[6-8] In the present study, we selected Zingiber zerumbet to examine its hypoglycemic activity because it is mentioned as antidiabetic herbs in Indian traditional system of medicine[9] and other plants like Zingiber officinale of the family Zingiberaceae have proved antidiabetic activity.[10]

Z. zerumbet (L) smith, a member of the family Zingiberaceae is well known as Van Adrak. The plant is widely cultivated in village garden in the tropics for its medicinal properties and as a marketable spice.[11] It grows in the edge of the forest, village thickest in the partial shade. It is distributed in India, Bangladesh, Malaysia, Nepal, and Sri Lanka.[12] It has been reported that plants from this family have anti-inflammatory, anti-ulceration, antioxidant, and antimicrobial properties. The volatile oil of the rhizome has been shown to contain zerumbone and camphene.

The rhizome of Z. zerumbet has been subject of many studies, especially in India. Only a very few scientific studies have been conducted so for on medicinal aspect of this plant. These include inhibition of prostaglandin synthesis, antipyretic and analgesic activity.[13] The purpose of this research was to experimentally assess the antidiabetic effect of aqueous extract of rhizome of

Z. zerumbet in streptozotocin (STZ)-induced diabetic rats and compare it with glibenclamide as a reference standard.

MATERIALS AND METHODS

Collection of plant

Rhizome of Z. zerumbet was collected from their natural habitats in and around Munderwa (Basti). The plants were authenticated by comparison with the herbarium and voucher specimen (Voucher specimen No.-97769) was lodged in the departmental herbarium of National Botanical Research Institute, Lucknow.

Preparation plant extract

For extraction, 1 kg fresh rhizome of Z. zerumbet was used. Rhizome was washed with water, cut into small pieces, dried for 1 to 2 days. Aqueous extract from rhizome was prepared with distilled water in Soxhlet apparatus.

Experimental animals and streptozotocin induction of diabetes

Healthy mixed Wistar rats weighing 200 to 300 g of 3 to 4 months of age were obtained from animal house of I.T.M., GIDA, Gorakhpur. Diabetes was induced by injecting STZ to overnight-fasted rats in a dose of 55 mg/kg i. p. in 0.1M citrate buffer, pH 4.5. All animal procedures have been approved and prior permission from the Institutional Animal Ethical Committee was obtained as per the prescribed

guidelines. Animals were kept in cages and fed with recommended diet, that is, 100 mg/kg pellets and water ad libitum. They were kept in this condition for three weeks before use for acclimatization to new environment.

Extract and drug administration

Extract was suspended in distilled water administered orally through oral feeding tube at a dose of 200 mg/kg body weight. The dose of the extract is determined from preliminary study in our laboratory. Glibenclamide was administered at a dose of 10 mg/kg body weight.

Experimental design

In the present experiment, 24 rats (18 diabetic, 6 normal rats) were used. These rats were divided into four groups. Six rats were used in each group. All treatments were conducted for 21 days.

Group-I - Normal control rats were administered distill water Group-II - Diabetic control rats were administered distill water

Group-III - Diabetic rats were administered Glibenclaimide

Group- IV - Diabetic rats were administered extract of Z. zerumbet.

Determination of blood glucose

Glucometer (Accu-Chek, Roche Diagnostic, Germany) was used for determination of blood glucose level of rats. Blood samples were obtained from the cut tail-tick of conscious rats. Basal and 48 hours postinduction blood glucose level of the animal were recorded. Thereafter, the extract and drug were administered daily for 21 days. Blood glucose levels were measured on 0, 7th, 14th, and 21st day. Body weights were monitored.

Statistical analysis

All the data were expressed as SEM ±. Statistical analysis was carried out using one-way ANOVA followed by Bonferroni multiple comparison test. The criterion for statistical significance was P less than 0.001.

RESULTS

In STZ-induced diabetic rats, there was a significant ($P<0.001$) increase in blood glucose level and significant ($P<0.001$) decrease in body weight. The effect of Z. zerumbet on blood glucose level and body weight was determined by comparison of normal, diabetic control, Z. zerumbet-treated diabetic rats and glibenclamide (Reference Standard)treated diabetic rats. Blood glucose was measured before and during the treatment.

It is clear from the data in Figure 1 that the blood glucose level of diabetic control rats continued to increase during the 21 days of the experiment. In contrast, the Z. zerumbet-treated diabetic rats not exhibited significantly reduced glucose levels during the experiment period when compared with the control diabetic rats. After 21 days of treatment with Z. zerumbet extract, the blood glucose level were not significantly reduced (P>0.001) in comparison with diabetic control rats.

Table 1: Effect of administration of feeding the aqueous extract of Zingiber zerumbet rhizome and glibenclamide on body weight

Group	Body weight (g)	
	Before treatment	After treatment
Untreated control	194 ± 1.88	220.5 ± 1.839
Diabetic control	202.66 ± 2.33	168.5 ± 2.513###
Diabetic + Glibenclaminde (10 mg/kg)	206.66 ± 1.745	222.33 ± 1.96***
Diabetic + extract (200 mg/kg)	197 ± 2.176	190.4 ± 1.476

All values are expressed as mean ± S.E.M (n = 6), ###*P*<0.001 as compared with untreated control. One-way ANOVA followed by Bonferroni multiple comparison test, ***P<0.001 as compared with diabetic control

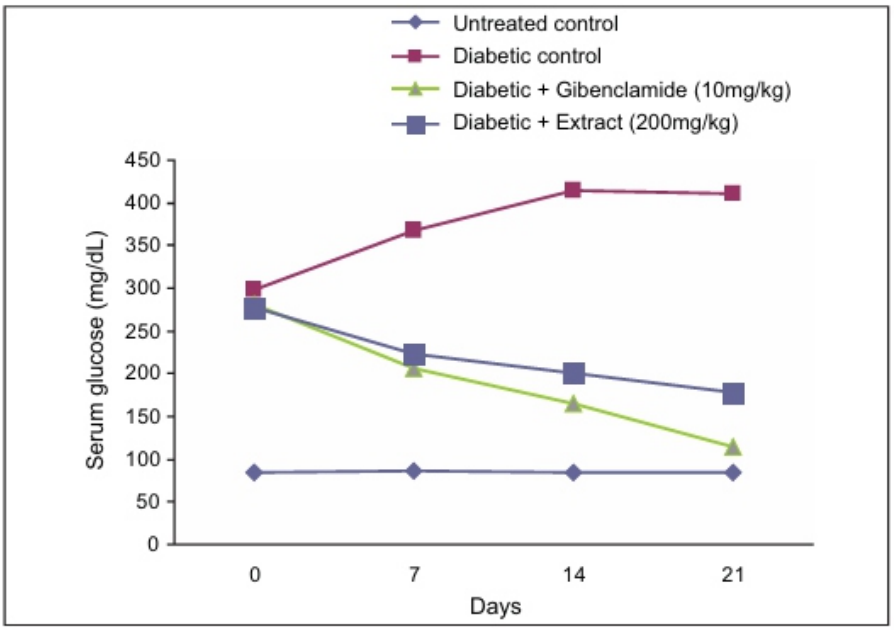


Figure 1: Effect of administration of feeding the aqueous extract of Zingiber zerumbet rhizome and glibenclamide on serum glucose estimation in diabetic rats

However, glibenclamide-treated rats showed significant reduction (P<0.001) in blood glucose level throughout the study period in comparison with diabetic control rats. So, in STZ-treated diabetic model, Z. zerumbet slightly reduced the blood glucose but the hypoglycemic effect is not as significant (P>0.001) as reference standard glibenclamide. Result also showed that body weight of hyperglycemic rats treated with Z. zerumbet and also of untreated hyperglycemic rats was significantly reduced but when treated with glibenclamide, no significant reduction in body weight was observed [Table 1].

DISCUSSION

STZ is a valuable agent for the production of diabetes because it allows the consistent production of diabetic states with mild, moderate, or severe hyperglycemia, where animals with mild or moderate diabetes have provided an opportunity to study the influence of oral hypoglycemic agents, and STZ-induced diabetic rats have been widely used as a model for diabetes mellitus in experimental animal.[14] Result in this study showed that antidiabetic activity was found in Z. zerumbet but it is not as significant as glibenclamide. It has been known that Z. zerumbet is mentioned in folk medicine to treat diabetes in India.[10]

The result from this study does not support the usage of this plant as a beneficial practice in folk medicine in the treatment of diabetes. Result also showed significant reduction in body weight of hyperglycemic rats treated with Z. zerumbet. This suggests that extract of Z. zerumbet cannot prevent weight loss in hyperglycemic rats as observed in hyperglycemic rats treated with glibenclamide.

REFERENCES

1. Greenberg RA, Sacks DB. Screening for diabetes: Is it warranted? Clin Chim Acta 2002:315;61-9.

2. Rang HP, Dale MM. The Endocrine System Pharmacology. 2nd ed. United Kingdom: Longman Group Ltd; 1991. p. 504-8.

3. Ranjan C, Ramanujam R. Diabetes and insulin resistance associated disorders: Disease and the therapy. Curr Sci 2002;83:1533-8.

4. Alam K, Mahpara S. Role of diet, nutrients, spices and natural products in diabetes mellitus Pak J Nutr 2003;2:1-12.

5. World Health Organization. The WHO Experts Committee on Diabetes Mellitus. Technical Report Series, World Health Organization, Geneva; 1980.

6. Hill JO, Peters JC. Biomarkers and functional foods for obesity and diabetes. Br J Nutr 2002:88:S213-8.

7. Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol 2003;84:105-8.

8. Srinvasan K. Plant foods in the management of diabetes mellitus: Spices as beneficial antidiabetic food adjuncts. Int J Food Sci Nutr 2005;56:399-414.

9. Akhani SP, Vishwakarma SL, Goyal RK. Anti-diabetic activity of Zingiber officinale in streptozotocin-induced type I diabetic rats. J Pharm Pharmacol 2004;56:101-5.

10. Pullaiah T, Naidu Chandrashekhar K. Antidiabetic plants in India and herbal based antidiabetic research. New Delhi: Regency Publication; 2003. p. 318.

11. Saadiah MS, Halijah I. Proceedings of the National Convention on Herbal medicine. Vol. 21. Kuala Lumpur: Forest Research Institute Malaysia; 1995. p. 205-7.

12. Anonymous. The wealth of India. Raw materials. Vol. 11. India: CSIR; 1976. p. 89-90.

13. Omar R. Ali Rahman Z, Latif MT, Lihan T, Adam JH. Proceedings of the Regional Symposium on Environment and Natural Resources 10-11th April. In: Omar R. Ali Rahman Z, Latif MT, Lihan T, Adam JH, editors. Vol 1. Hotel Renaissance Kuala Lumpur, Malaysia: 2002. p. 692-7.

14. Murphy ED, Anderson JW. Tissue glycolytic and gluconeogenic enzyme activities in mildly and moderately diabetic rats: Influence of tolbutamide administration. Endocrinology 1974:94:27-34.

How to select appropriate statistical test?

Jaykaran

Selection of appropriate statistical test is very important for analysis of research data. Use of wrong or inappropriate statistical test is a common phenomenon observed in articles published in biomedical journals.[1-4] Wrong statistical tests can be seen in many conditions like use of paired test for unpaired data or use of parametric statistical tests for the data which does not follow the normal distribution or incompatibility of statistical tests with the type of data, etc.[5] Because of the availability of different types of statistical software, performing the statistical tests become easy, but selection of appropriate statistical test is still a problem.

Selection of appropriate statistical tests depends on the following three things:

- What kind of data we are dealing with?
- Whether our data follow the normal distribution or not?
- What is the aim of the study?

WHAT KIND OF DATA WE ARE DEALING WITH?

Usually our research data fall in one out of the following four types of data: nominal data, ordinal data, interval data, and ratio data.

Nominal data

In these kinds of data, observations are given a particular name. Like a person is observed to be 'male' or 'female' or Name of drug as generic or brand, etc. Nominal data cannot be measured or ordered but can be counted. These types of data are considered as categorical data but the order of the categories is meaningless. Data that consist of two classes like male/female or dead/alive are called binomial data, and those that consist of more than two classes like tablet/capsule/syrup are known as multinomial data. Data of these types are usually presented in the form of contingency tables like 2×2 tables.

Ordinal data

Ordinal data is also a type of categorical data but in this, categories are ordered logically. These data can be ranked in order of magnitude. One can say definitely that one measurement is equal to, less than, or greater than another. Most of the scores and scales used in research fall under

the ordinal data. For example, rating score/scale for the color, taste, smell, ease of application of products, etc.

Interval data

Interval data has a meaningful order and also has the quality that equal intervals between measurements represent equal changes in the quantity of whatever is being measured. But these types of data have no natural zero. Example is Celsius scale of temperature. In the Celsius scale, there is no natural zero, so we cannot say that 70°C is double than 35°C. In interval scale, zero point can be choosen arbitraly. IQ Test is also interval data as it has no natural zero.

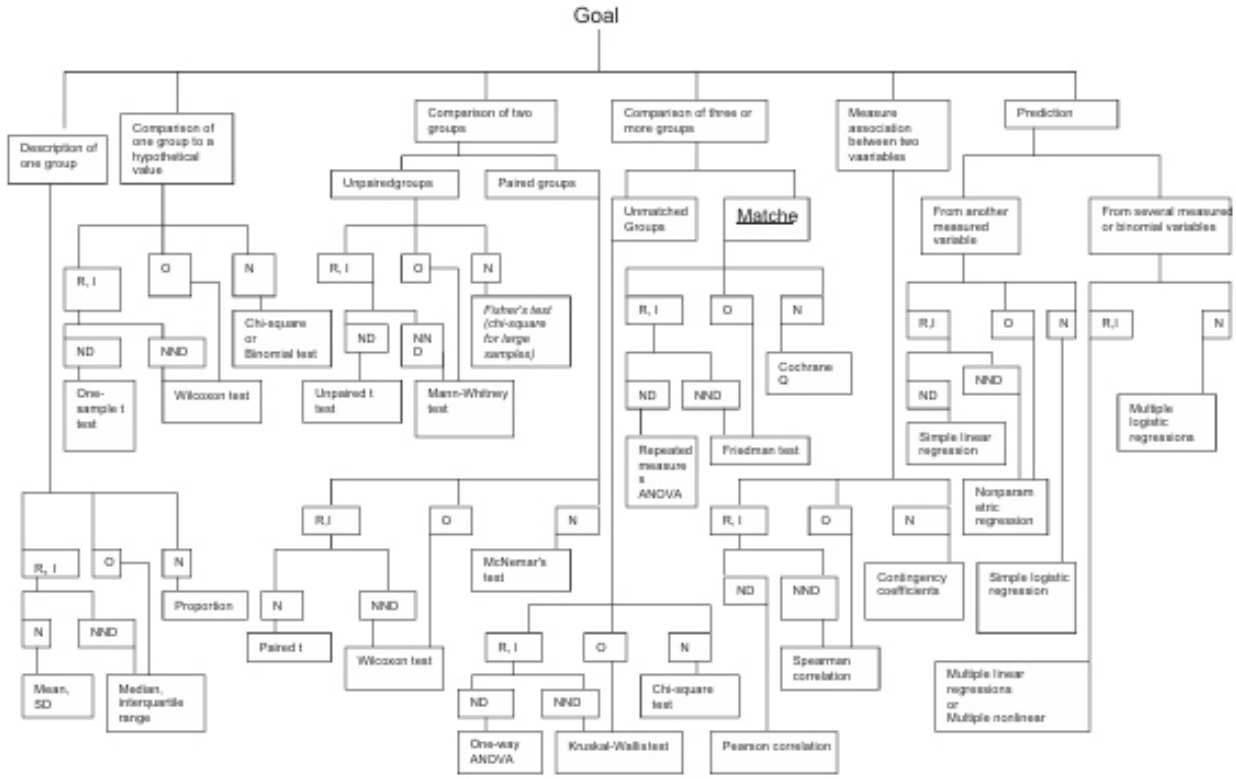
Ratio data

Ratio data has all the qualities of interval data (natural order, equal intervals) plus a natural zero point. This type of data is observed to be used most frequently. [4] Example of ration data is height, weight, length, etc. In this type of data, it can be said meaningfully that 10 m of length is double than 5 m. This ratio hold true regardless of which scale the object is being measured in (e.g., meters or yards). Reason for this is the presence of natural zero.

WHETHER OUR DATA FOLLOW THE NORMAL DISTRIBUTION OR NOT?

This is the second prerequisite for selection of appropriate statistical test. If you know the type of data (nominal, ordinal, interval, and ratio) and distribution of data (normal distribution or not normal distribution), selection of statistical test will be very easy. There is no need to check distribution in the case of ordinal and nominal data. Distribution should only be checked in the case of ratio and interval data. If your data are following the normal distribution, parametric statistical test should be used and nonparametric tests should only be used when normal distribution is not followed.

There are various methods for checking the normal distribution, some of them are plotting histogram, plotting box and whisker plot, plotting Q-Q plot, measuring skewness and kurtosis, using formal statistical test for normality (Kolmogorov-Smirnov test, Shapiro-Wilk test, etc). Formal statistical tests like Kolmogorov-Smirnov and Shapiro-Wilk are used frequently to check the distribution



R, I = Ratio and Interval data O= Ordinal data N = Nominal data

N= Normal distribution NND = Non normal distribution

Figure 1: Flow chart for selection of appropriate statistical test

of data. All these tests are based on null hypothesis that data are taken from the population which follows the normal distribution. P value is determined to see the alpha error. If P value is less than 0.05, data is not following the normal distribution and nonparametric test should be used in that kind of data. If the sample size is less, chances of non-normal distribution are increased.

WHAT IS THE AIM OF THE STUDY?

This is the third prerequisite of selection of appropriate statistical test. What we want to compare? Whether we want to compare the drug with placebo? Or we want to compare effect of intervention by comparing preintervention endpoints with postintervention endpoints. If a researcher is clear about all the three questions mentioned in previous text, appropriate statistical test can be selected from the flowchart [Figure 1]; this flow chart is modified from the table given on a site of software graphpad (www.graphpad.com).

To understand it better, an example has been discussed below. Incidence of tumors observed in control and treated mice in a preclinical study is as follows:

• Controls - 1 of 14 animals

•Treated - 6 of 14 animals

Use appropriate statistical test to determine if the incidence is significantly different in two groups.

What is the aim of the study?

Our aim is to compare incidence of tumor between two unpaired groups (control vs treated).

What kind of data we are dealing with?

In the present study, the data type is nominal data.

Whether our data follow the normal distribution or not?

There is no need to check distribution in nominal data.

They follow chi-square distribution.

So by looking at the table, we can say that most appropriate test in this condition will be 'Fishers Test' (Chi-Square if large sample size).

REFERENCES

1. Lang T. Twenty statistical errors even you can find in biomedical research articles. Croat Med J 2004;45:361-70.

2. Jaykaran, Yadav P, Chavda N, Kantharia ND. Some issues related to the reporting of statistics in clinical trials published in Indian medical journals: A survey. Int J Pharmacol 2010;6:354-9.

3. Karan J, Kantharia ND, Yadav P, Bhardwaj P. Reporting statistics in clinical trials published in Indian journals: A survey. Pak J Med Sci 2010;26:212-6.

4. Karan J, Goyal JP, Bhardwaj P, Yadav P. Statistical reporting in Indian Pediatrics. Indian Pediatr 2009;46:811-2.

5. Strasak AM, Zaman Q, Karl PP, Gobel G, Ulmer H. Statistical errors in medical research-a review of common pitfalls. Swiss MED Wkly 2007;137:44-9.

Some commonly observed statistical errors in clinical trials published in Indian Medical Journals

Jaykaran

Sir,

Standard of reporting of statistics in clinical trials published in various medical journals is not satisfactory. A growing body of literature points to persistent statistical errors, f laws and deficiencies in clinical trials published in the western and Indian medical journals.[1,2] In this letter, I want to highlight some commonly observed statistical errors which I found in clinical trials published in Indian medical journals, so that readers of medical journals can critically appraise the clinical trials.

One of the major problems is inadequate reporting about the sample size. In clinical trials, sample should be big enough to have a high chance of detecting, as statistically significant, a worthwhile effect if it exists, and thus to be reasonably sure that no benefit exists if it is not found in trial. For sample size calculation in hypothesis testing, the researcher must know the effect size, standard deviation, significance level and power of study. Exact sample size should be calculated during the design phase of clinical trials, preferably with the help of a statistician, and the method of calculation of sample size should be reported in the manuscript.[1]

Another problem is inappropriate/ wrong statistical tests. I observed that even simple statistical tests like “chi-square test” or “the Student t test” are misused. Before applying any statistical test, the assumptions for that statistical test should be fulfilled and reported in the manuscript. If some obscure / less known statistical test is used, then justification for using that test and/ or proper reference should be given. One common problem I observed in the “statistics” section of clinical trials published in Indian medical journals is regarding the “where appropriate” statement. In many clinical trials, it is mentioned that “appropriate statistical tests were used to analyze the data” or “t tests were used for quantitative data, and chisquare test was used for qualitative data.” These kinds of statements should be avoided as they provide insufficient information.[1,3]

Failure to report adjustment for multiple endpoints is a frequently observed problem in clinical trials published in Indian medical journals. Multiplicity of inferences can be because of multi-sample comparisons, interim and subgroup analyses and multiple endpoints. This multiplicity is associated with false positivity, i.e., likelihood of getting a significant result just by chance. If with one statistical test, the chance of a significant result is 5%, then after 20 tests, it will increase to 40%. Investigator should use various methods described to adjust multiple endpoints and hence type I error. Not only

International Conference on Harmonization (ICH) E9 guideline but also Consolidated Standards of Reporting Trials (CONSORT) statement demands use of procedures like Bonferroni correction and composite end point method etc for adjustment of multiple endpoints.[4]

Results are usually reported as "P values" only. It is reported that P values are often misinterpreted; and even if they are interpreted correctly, there are some limitations. Results should be explained as absolute difference between the two groups for the endpoint as well as 95% confidence interval around the difference. This confidence interval should be reported with, or instead of, P value. Confidence intervals tell the reader exactly the range of values with which the data are statistically compatible. In spite of instruction to authors of many Indian medical journals to mention about the reporting of exact P value, many clinical trials published in Indian medical journals report arbitrary P values, like "P < .05" or "P > .05" or "P = NS." Exact P values should be reported rather than arbitrary values.[5]

In clinical trials published in Indian medical journals, "intention to treat" (ITT) principle is usually not followed.

Meaning of "intention to treat" principle is that all patients randomized into clinical trial are to be accounted for in the primary analysis, and all primary events observed during the follow-up period are to be accounted for as well. If either of these aspects is not adhered to, the analysis of results may easily be biased in unpredictable directions and thus the interpretation of the results compromised.[1,5]

I observed that, in many of the clinical trials baseline comparisons between the two groups for various endpoints are reported with P values for each. In randomized controlled trials, recruitment of subjects is done with proper randomization techniques, hence the differences observed between the groups are considered to be chance f indings. So there is no need for reporting of baseline comparisons. Any difference between the groups should be adjusted by various statistical techniques during the analysis of results, but P values need not be reported.[5]

I believe that, a statistician should be included at an early stage in the clinical trials to prevent these errors. Investigators performing trials should have some background knowledge of biostatistics, and there should be rigorous statistical review of manuscripts before sending them for peer review. Journals should have few statisticians in the editorial team.

REFERENCES

1. Karan J, Kantharia ND, Yadav P, Bhardwaj P. Reporting statistics in clinical trials published in Indian journals: A survey. Pak J Med Sci Q 2010;26:212-6. Available from: http://www.pjms.com.pk/issues/janmar2010/pdf/article44.pdf [last accessed on 2010 Apr 4].

2. Hopewell S, Dutton S, Mee Yu, Chan A. Altman DG. The quality of reports of randomized trials in 2000 and 2006: Comparative study of articles indexed in Pub Med. BMJ 2010;340:c723. Available from http://www.bmj.com/cgi/content/full/340/mar23_1/c723 [last accessed 2010 Apr 2].

3. Strasak AM, Zaman Q, Pfeiffer KP, Göbel G, Ulmer H. Statistical errors in medical research: A review of common pitfalls. Swiss Med Wkly 2007;137:44-9. Available from: http://www.smw.ch/docs/pdf200x/2007/03/smw-11587.pdf [last accessed on 2010 Apr 5].

4. Neuhauser M. How to deal with multiple endpoints in clinical trials. Fundam Clin Pharmacol 2006;20:515-23. Available from: http:// www3.interscience.wiley.com/journal/118553180/full [last accessed on 2010 Apr 4].

5. Tom L. Twenty statistical errors even YOU can find in biomedical research articles. Croat Med J 2004;45:361-70. Available from: http:// www.cmj.hr/2004/45/4/15311405.pdf [last accessed on 2010 Apr 2].

Do medicinal plants possess significant activities?

Sanjib Bhattacharya, Mueen Ahmed Kk1, Vipra Kundoor2

Dear Sir,

The use of medicinal plants and their preparations for the treatment of diseases is as old as mankind, and even today majority of the world population depends on herbal health care practices. Exploring medicinal plants in the context of modern science is the need for optimum and proper utilization of medicinal plants. Research on medicinal plants for biological activities is an area of interest worldwide. Lots of scientific research has been carried out on a large number of medicinal plants, as evident from increase in the number of research articles on biological activity of plants. In spite of increased research on medicinal plants, there is no considerable development in the number of marketable drugs as plant derived chemical entities or herbal formulations with modern standards of safety and efficacy. In this situation, the medicinal properties of medicinal plants become questionable, raising the title question.

Even now, patients favor herbal medications for their perceived efficacy and good safety profile. Although opportunities for developing plant-based drugs with international acceptance are vast, at present it appears that there is lack of integrated research focusing on the development of commercially viable and useful phytomedicines. Majority of the research publications on medicinal plants show significant pharmacological activities of crude or partially purified/fractionated plant extracts in experimental animal models, mentioning the almost common refrain in conclusion: further studies are required to isolate the bioactive compounds and/or further studies are required to clarify the mechanism of examined activity. Unfortunately, publications on such further studies are very much rare on those plants, despite claiming so-called significant activities. In this way, majority of the medicinal plant research starts and ends at this preliminary level of observations. Most of these studies are repetitive, using similar models. Further, in some cases in terms of further study, a medicinal plant is credited with innumerable pharmacological activities without focusing practical therapeutically beneficiary effects. Sometimes, repetition of these studies elsewhere does not show the claimed activity. Hence, the reproducibility of the effects is questionable, especially in case of humans.

If a medicinal plant really possessed certain significant activity, then why there is not any concerted effort toward further definitive chemical pharmacological and clinical studies leading to possible drug development?

Many reasons can be found for this and the challenges in phytopharmacology have been elaborately discussed by the researchers elsewhere. Various problems inherently associated with herbal medicine

include: identification of plants, cultivation, collection and processing, extraction, standardization, toxicological data and poor methodological quality of animal studies. Among them, the last point appears to be important while significant activity of medicinal plants is concerned. Reports show that animal studies are poor predictors of effects in humans.[1] There are several methodological problems associated with phytopharmacological studies in animal models.[2] Some of these include the following.

- Differences in animal species and strains
- None or less availability of suitable higher mammals like cat, dog, and monkey
- Different means for inducing disease or injury with varying similarity to the human conditions
- Variation in dose selection and dosing regimen that is of uncertain relevance to the human conditions
- Lack of proper screening of experimental animals to avoid false positive results
- Choice of comparison treatment (none/vehicle/ positive control)
- Small experimental group with inadequate sample size
- Simple statistical treatment of experimental data

Most of the research publications on medicinal plants are animal studies and majority of these studies suffer at least one of the problems mentioned above.

There are certain complications associated with crude or partially purified plant extracts while detecting their pharmacological properties. These include:

- differences in solvents used for extraction,
- differences in extraction methods,
- presence of tannins and related polyphenols which frequently give false positive pharmacological response both in vitro and in vivo,
- presence of residual solvents in extracts and
- disparity in standardization methods.

Use of isolated compounds can circumvent the above said difficulties. However, purification of extracts or isolation of compounds often leads to decrease or complete loss of pharmacological activities. Expensive and time consuming isolation of constituents frequently leads to re-isolation and re-identification of known or less important compounds like phenolics, sugars, etc.

It is also discouraging to observe that among numerous research articles on medicinal plants, very few are clinical studies. Clinical trials with herbal medicines are very less globally. Evidence to justify the potential use of herbal medicine in mainstream medicine is therefore lacking. The issues, difficulties and possible alternative strategies of clinical trials of herbal medicine have been discussed by several researchers in pursuit of a better outcome.[3]

The cause of raising questions toward activities of medicinal plants may be that most of the work in this field has remained within clinics of traditional practitioners or confined to academic research laboratories and not taken by industries which are strong and dynamic in research and development. There are several international patents granted for different pharmacological activities of medicinal plants and yet these have not been commercially exploited. The earlier successes have been achieved distinctly when the industry effort was intensive. Majority of the drugs (whether natural or synthetic) would not have been developed or their development would have been delayed significantly in the absence of scientific or technical contribution from pharmaceutical companies.

In spite of several doubts and questions regarding the activities of medicinal plants in contemporary therapeutics, the prospect of medicinal plants for health care system should not be considered so bleak in view of the development of some herbal drugs of promising therapeutic utility. Although at present development of pure phytochemicals as drugs is very few, there are several standardized or purified extracts from known medicinal plants (silymarin comes to mind first) showing good safety and efficacy in humans. Standardized plant extracts in formulations are increasingly being accepted in European and American countries.

For several compelling reasons, researchers involved in modern drug discovery processes have started revisiting medicinal plants to reduce the typical innovation deficit faced today. There is no denying that medicinal plants are endowed with significant medicinal properties since time immemorial and have furnished several important drugs. Nowadays there are some publications and patents describing interesting and novel leads form medicinal plants acting at the molecular level mechanisms. Many promising leads like curcumins and withanolides are available; only industry-based integrative research and development is required to yield many more successes like reserpine and artemisinin. Best of public and private sectors comprising academia and industry experts should come together to explore the seemingly low-valued but highly gifted wealth of medicinal plants for the benefit of mankind.

REFERENCES

1. Knight A. Systematic reviews of animal experiments demonstrate poor human clinical and toxicological utility. ALTEX2007;14:125-30.

2. Pandora P. Where is the evidence that animal research benefits humans? BMJ 2004;328:514-17.

3. Guo R, Canter PH, Ernst E. A systematic review of randomized clinicalrials of individualized herbal medicine in any indication. Postgrad Med J 2007;83:633-37.

Instructions for Authors

Essentials for Publishing in this Journal

1 Submitted articles should not have been previously published or be currently under consideration for publication elsewhere.

2 Conference papers may only be submitted if the paper has been completely re-written (taken to mean more than 50%) and the author has cleared any necessary permission with the copyright owner if it has been previously copyrighted.

3 All our articles are refereed through a double-blind process.

4 All authors must declare they have read and agreed to the content of the submitted article and must sign a declaration correspond to the originality of the article.

Submission Process

All articles for this journal must be submitted using our online submissions system. http://enrichedpub.com/ . Please use the Submit Your Article link in the Author Service area.

Manuscript Guidelines

The instructions to authors about the article preparation for publication in the Manuscripts are submitted online, through the e-Ur (Electronic editing) system, developed by **Enriched Publications Pvt. Ltd**. The article should contain the abstract with keywords, introduction, body, conclusion, references and the summary in English language (without heading and subheading enumeration). The article length should not exceed 16 pages of A4 paper format.

Title

The title should be informative. It is in both Journal's and author's best interest to use terms suitable. For indexing and word search. If there are no such terms in the title, the author is strongly advised to add a subtitle. The title should be given in English as well. The titles precede the abstract and the summary in an appropriate language.

Letterhead Title

The letterhead title is given at a top of each page for easier identification of article copies in an Electronic form in particular. It contains the author's surname and first name initial .article title, journal title and collation (year, volume, and issue, first and last page). The journal and article titles can be given in a shortened form.

Author's Name

Full name(s) of author(s) should be used. It is advisable to give the middle initial. Names are given in their original form.

Contact Details

The postal address or the e-mail address of the author (usually of the first one if there are more Authors) is given in the footnote at the bottom of the first page.

Type of Articles

Classification of articles is a duty of the editorial staff and is of special importance. Referees and the members of the editorial staff, or section editors, can propose a category, but the editor-in-chief has the sole responsibility for their classification. Journal articles are classified as follows:

Scientific articles:

1. Original scientific paper (giving the previously unpublished results of the author's own research based on management methods).

2. Survey paper (giving an original, detailed and critical view of a research problem or an area to which the author has made a contribution visible through his self-citation);

3. Short or preliminary communication (original management paper of full format but of a smaller extent or of a preliminary character);

4. Scientific critique or forum (discussion on a particular scientific topic, based exclusively on management argumentation) and commentaries. Exceptionally, in particular areas, a scientific paper in the Journal can be in a form of a monograph or a critical edition of scientific data (historical, archival, lexicographic, bibliographic, data survey, etc.) which were unknown or hardly accessible for scientific research.

Professional articles:

1. Professional paper (contribution offering experience useful for improvement of professional practice but not necessarily based on scientific methods);
2. Informative contribution (editorial, commentary, etc.);
3. Review (of a book, software, case study, scientific event, etc.)

Language

The article should be in English. The grammar and style of the article should be of good quality. The systematized text should be without abbreviations (except standard ones). All measurements must be in SI units. The sequence of formulae is denoted in Arabic numerals in parentheses on the right-hand side.

Abstract and Summary

An abstract is a concise informative presentation of the article content for fast and accurate Evaluation of its relevance. It is both in the Editorial Office's and the author's best interest for an abstract to contain terms often used for indexing and article search. The abstract describes the purpose of the study and the methods, outlines the findings and state the conclusions. A 100- to 250- Word abstract should be placed between the title and the keywords with the body text to follow. Besides an abstract are advised to have a summary in English, at the end of the article, after the Reference list. The summary should be structured and long up to 1/10 of the article length (it is more extensive than the abstract).

Keywords

Keywords are terms or phrases showing adequately the article content for indexing and search purposes. They should be allocated heaving in mind widely accepted international sources (index, dictionary or thesaurus), such as the Web of Science keyword list for science in general. The higher their usage frequency is the better. Up to 10 keywords immediately follow the abstract and the summary, in respective languages.

Acknowledgements

The name and the number of the project or programmed within which the article was realized is given in a separate note at the bottom of the first page together with the name of the institution which financially supported the project or programmed.

Tables and Illustrations

All the captions should be in the original language as well as in English, together with the texts in illustrations if possible. Tables are typed in the same style as the text and are denoted by numerals at the top. Photographs and drawings, placed appropriately in the text, should be clear, precise and suitable for reproduction. Drawings should be created in Word or Corel.

Citation in the Text

Citation in the text must be uniform. When citing references in the text, use the reference number set in square brackets from the Reference list at the end of the article.

Footnotes

Footnotes are given at the bottom of the page with the text they refer to. They can contain less relevant details, additional explanations or used sources (e.g. scientific material, manuals). They cannot replace the cited literature.
The article should be accompanied with a cover letter with the information about the author(s): surname, middle initial, first name, and citizen personal number, rank, title, e-mail address, and affiliation address, home address including municipality, phone number in the office and at home (or a mobile phone number). The cover letter should state the type of the article and tell which illustrations are original and which are not.

Note