RESEARCH ARTICLE

Hematopoietic Potential of Polysaccharides Isolated from *Angelica sinensis* against ACE Inhibitor Induced Anemia in Albino Rats

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A B S T R A C T

Angiotensin converting enzyme (ACE) inhibitors are widely used in treatment of cardiovascular diseases. Their long term use causes development of anemia in patients with these diseases. Present study was designed to evaluate the protective hematopoietic potential of *Angelica sinensis* polysaccharides (ASP) against ACE inhibitor-induced anemia along with their toxic effects on kidney and liver cells. Adult male albino rats were randomly divided into five equal groups (n=6). Group I was control group and group II was administered with ACE inhibitor (20 mg/kg/day) to induce anemia. In group III, ACE inhibitor was administered (20 mg/kg/day) in combination with erythropoietin (EPO, 100 IU/kg/each). Group IV was administered with ASP at the dose rate of 1 g/kg/day. In Group V, ACE inhibitor (20 mg/kg/day) was administered in combination with ASP (1 g/kg/day). After 28 days, blood and tissue samples were collected for hematological and histopathological analysis respectively. The results showed that ACE inhibitors significantly reduced (P<0.05) the hemoglobin value (Hb), packed cell volume (PCV), red blood cell (RBC) count, mean corpuscular volume (MCV) and *mean corpuscular hemoglobin* (MCH) values. In group treated with ASP, there was significant (P<0.05) increase in Hb value and RBC count. The combination of ACE inhibitor and ASP led to the significant (P<0.05) reduction in Hb, PCV, RBC count, MCV and MCH values. While histopathological examination of liver and kidney cells showed a mild degree of toxicity in ASP treated group.

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INTRODUCTION

Angiotensin converting enzyme (ACE) inhibitors are extensively prescribed to decrease the blood pressure (BP), prevent the emergence of kidney diseases in patients with diabetes mellitus (DM) and in congestive heart failure (CHF). ACE plays an essential role in the conversion of angiotensin I (Ang I) into angiotensin II (Ang II) which acts as a vasoconstrictive substance and increases the blood pressure by inhibiting the excretion of sodium and water with increased production of aldosterone. Thus any agent that stops the activity of ACE will inhibit the conversion of Ang I into Ang II and will reduce the blood pressure. They decrease the rate of myocardial infarction, stroke and death in a wide range of high risk patients (Campbell *et al*., 2009; Gad and El-Maddawy, 2014). Other pathway is bone marrow renin angiotensin system that involves hematopoiesis

(Haznedaroglu and Beyazit, 2013). Therapy with ACE inhibitor may be linked with decreasing erythrocyte production in various clinical settings (Inoue *et al.,* 2008) via substance P and Ang II (Lin *et al.,* 2011). ACE inhibitors are linked with high risk of decreased hemoglobin among older hospitalized patients with heart failure (Laudisio *et al.*, 2012; Javed *et al.*, 2015).

Each cell in the body demands proper amount of oxygen to live while iron is an important substance for transportation of oxygen (Zahra *et al.,* 2013). An enhanced understanding of link among the use of ACE inhibitors and progress of anemia can add considerably in the treatment of DM, CHF and hypertensive patients (Ajmal *et al.,* 2013). In anemia, there is decrease in the amount of red blood cells (RBCs), hemoglobin (Hb) concentration or oxygen-binding capacity of Hb (Kouadio *et al.*, 2013) and lead to weakness, fatigue, difficult concentrating or poor work productivity

(Kassebaum *et al.*, 2014). Erythropoiesis and endogenous erythropoietin (EPO) production is assisted by Ang II while ACE inhibitors may aggravate anemia by decreasing Ang II levels. ACE inhibitors also augment plasma N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) by inhibiting the degradation of Ac-SDKP through ACE. Augmented plasma Ac-SDKP inhibits the cycling of hematopoietic stem and progenitor cells (Hayashi *et al.*, 2001).

Herbal medicines have been used since the beginning of human beings on this planet. They comprise active ingredients in complex chemical combinations established as crude fractions which are extracted from aerial or underground parts of the plant (Chawla *et al.*, 2013). The advancement of indigenous medicines and the use of medicinal plants carry significant financial assistances in the treatment of several ailments (Kumar *et al.*, 2013). *Angelica sinensis* is a conventional Chinese herbal medicine with an extensive history of use in Korea, China and Japan (Hook, 2014). It is one of the best medicines from the family *Umbelliferae* (Liu *et al.*, 2012). One of the active constituents is polysaccharide, separated from *A. sinensis* has been found to be involved in activation of hematopoietic cells and muscle tissues and augmenting hematopoiesis by aggregating the release of some hematopoietic growth factors such as EPO (Liu *et al.*, 2012). *Angelica sinensis* polysaccharide (ASP), one of the major active constituents of *A. sinensis* plant is used for treatment of anemia. Earlier studies designated that ASP increases plasma iron levels by inhibiting hepcidin expression (Wang *et al.*, 2011). ASP has been used as a health-supporting agent in cancer patients under chemo-radiation treatment and anemic patients (Lee *et al.*, 2012) and also enhances thrombopoiesis and hematopoiesis (Liu *et al.*, 2010).

Keeping these facts in mind, this study was designed to evaluate the protective hematopoietic potential of ASP against ACE inhibitor-induced anemia along with their toxic effects on kidney and liver cells.

MATERIALS AND METHODS

Experimental animals: Thirty healthy adult male albino rats weighing about 200-300 g were purchased from the local market of Faisalabad, Pakistan. The animals were housed at 25±2°C in a well-ventilated condition under 12 h light and dark cycles in animal room of Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad. The rats were provided with normal feed (rat chow) twice a day for 28 days and drinking water *ad-libitum*. The experiments were carried out in accordance with the guidelines of the Directorate of Graduate Studies and Institutional Animal Ethical Committee.

Drugs: ACE inhibitor: Tablet Zestril® (Lisinopril 5mg) was purchased from Searle Pakistan Limited, Karachi, Pakistan that was used to induce anemia. Synthetic antianemic drug: Injection Eritrogen® (Recombinant human EPO 4000 IU/ml subcutaneously).

Plant material: The dry roots of *A. sinensis* were collected from local herbal market of Faisalabad,

Pakistan, identified and authenticated from the Department of Botany, University of Agriculture, Faisalabad. *A. sinensis* roots were washed thoroughly with distilled water to remove extraneous material. The roots body were dried in shade and pulverized with an electrical grinder and powdered material was passed through mesh sieve and then stored in air tight container.

Preparation of *Angelica sinensis* polysaccharides (ASP): Aqueous extraction of *A. sinensis* root's body were carried out in the Institute of Pharmacy, Physiology and Pharmacology, UAF, Polysaccharide fraction was isolated by the ethanol precipitation method as described by Cho *et al.* (2000) and modified by Ye *et al.* (2003). One hundred grams of roots of *A. sinensis* root's body were boiled for 3-4 hours period with water for a total of 12 hours. After each period of 4 hour boiling, the water extract was collected and residue was boiled again with water for another 4 hour period. All extracts were finally pooled and mixed with a concentrated ethanol solution (final concentration 75% v/v) to precipitate the polysaccharide enriched fraction. Precipitates were dried to a thick semi-solid mass of brown color and stored at ambient temperature until further use.

Experimental protocol: The rats were randomly divided into five groups with six rats in each group. Group I served as control and received normal diet throughout the experiment. Group II (untreated control) received Zestril® (ACE inhibitor,20 mg/kg/day orally) for 28 days, Group III (treated control) received Zestril® 20 mg/kg/day orally for 28 days+ Eritrogen®(EPO) 100 IU/kg at each alternate day for 28 days subcutaneously. Group IV (treated I) and V (treated II) received ASP1 g/kg/day and Zestril® (ACE inhibitor) 20 mg/kg/day+ASP orally, respectively for 28 days.

Blood Sampling and analysis: After the treatment period, blood was collected from jugular vein of rats and poured into ethylene diamine tetra-acetic acid (EDTA) tubes for hematological parameters including RBC, Hb concentration and PCV by standard methods (Rahman *et al.,* 2012). Erythrocytic indices including MCV and MCH were calculated.

Histopathological analysis: Formalin fixed kidney and liver biopsies were processed in graded ethanolic concentrations and fixed in paraffin blocks. Their fragments were arranged perpendicular to the plane of the section in the block and 6 micrometer thick transverse fragments were cut and mounted on glass slides and stained with hematoxylin and eosin (H and E stain). Microscopy was completed on Olympus PM-10 ADS automatic light microscope (Olympus optical Co., Tokyo, Japan) with a 20X and 40X objectives.

Statistical analysis: Results were expressed as two way analysis of variance (ANOVA) and statistical differences among different treatment groups were determined by Duncan's Multiple Range test while $P < 0.05$ was considered as significant.
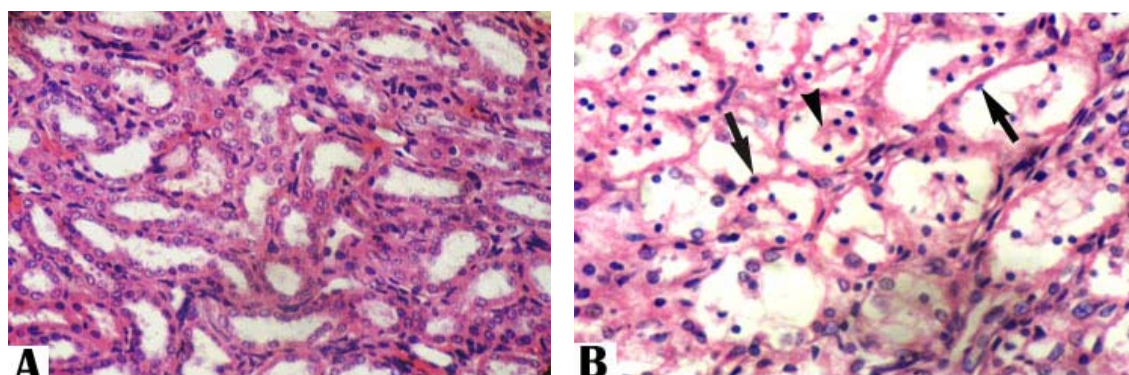
Fig. 1: Photomicrograph of control group (A) showing normal tubular epithelium having nuclei with nucleolus and chromatin material in renal parenchyma. B) Kidney of rat treated with ASP 1g/kg/day oral administration for 28 days showing condensation and pyknotic changes in the nuclei of tubular epithelial cells in renal parenchyma (arrow) and caste present in renal tubules (arrow head). 400X, H&E Staining.
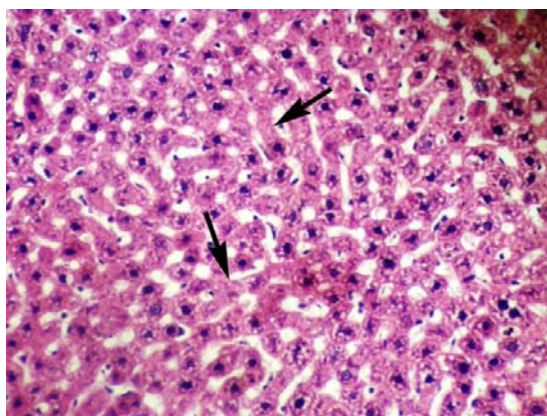

Fig. 2: Liver of rat treated with ASP 1g/kg/day orally for 28 days showing individual cell necrosis (arrow) in hepatic parenchyma. 400X, H&E Staining.

RESULTS

Effects on hematological parameters: Effects on RBCs count (million/mm^3), PCV (%), Hb (g/dl) MCV (fl) and MCH (pg/cell) values of control and treated groups of albino rats after 28 days are presented in Table 1. ACE inhibitor significantly (P<0.05) lowered the Hb (9.1±0.35 g/dl), PCV (31.3±1.04 %), RBCs count (5.6±0.44 million/mm^3), MCV (55.8±0.83 fl) and MCH value (16.2±0.17 pg/cell). Treated 1 group (group IV) which was administered with ASP, significantly (P<0.05) increased the Hb value (17.1±0.65 g/dl), RBCs count (9.9±0.39 million/mm^3) but significantly (P<0.01) reduced the MCV value (41.3±0.93 fl) while the effect on MCH value (17.2±0.25 pg/cell) remained non-significant as compared to control group. Unexpectedly, Hb (8.95±0.18 g/dl), PCV (30.1±0.39 %), RBCs count (5.5±0.09 million/mm^3), MCV (54.7±0.25 fl) and MCH value (16.3±0.11 pg/cell) decreased significantly (P<0.05) in group V treated with ACE inhibitor + ASP as compared to control group after 28 days.

Histopathological examination of kidney and Liver: In ASP treated group, renal parenchyma indicated mild to moderate degree of nuclear change that included condensation and pyknosis of nuclei (Fig. 1b) which indicates necrotic changes in renal parenchyma in

comparison with the kidney of control group (Fig. 1a). Individual cell necrosis was also present in hepatic parenchyma (Fig. 2).

DISCUSSION

Ang II exhibits a physiological mechanism in stimulation of erythroid progenitor cells proliferation. It increases the secretion of EPO from kidney that will act on bone marrow to stimulate the differentiation of proerythroblast and basophilic erythroblast and ultimately increasing the production of erythrocytes (Vlahakos *et al.*, 2010). ACE inhibitors may aggravate anemia by decreasing Ang II levels (Hayashi *et al.,* 2001; Tomovska *et al.,* 2013). These reported data and conclusions have associations for clinical management of chronically ill patients with inhibitors of renin-angiotensin system (Cole *et al.*, 2000). Anemia contributes to the worsening of cardiovascular diseases in many cases, therefore, the management of anemia is necessary for controlling the patient's overall condition (Ajmal *et al.,* 2013). The link between CHF and anemia may be associated with high mortality (Anand *et al.*, 2004).

In manifestation of anemia, recombinant human EPO and iron supplements use is not so much affective and safe from detrimental effects (Hou *et al.,* 2012). ASPs were considered to be active for increasing hematopoiesis by elevating the release of EPO (Liu *et al*., 2012). In this study, blood samples were taken to check the effect on hematological parameters after 28 days of study. In addition to this, all rats were sacrificed, their abdomens were opened and kidneys and livers were dissected out for histopathological examination to evaluate the safety or toxicity level of *A. sinensis* on kidney and liver.

In ACE inhibitor treated group, the Hb, PCV, RBCs count, MCV and MCH values decreased as compared to the control group that showed anemia in rats of untreated control group. This effect on hematological parameters was due to the depression of renin-angiotensin system by ACE inhibitors, as this system is involved in regulation of hematopoiesis (Hayashi *et al*., 2001). These results showed that ACE inhibitor induced microcytic and hypochromic anemia which is similar to the study of Inoue *et al.* (2008) who found that therapy with an ACE inhibitor may be linked with decreasing erythrocyte production.

Table 1: Hematological parameters in control and treated groups of adult male albino rats (n=6) after 28 days.

Parameters	Groups				
	I	II	III	IV	V
Hb (g/dl)	12.0±0.58	9.1±0.35*	17.9±0.20*	17.1±0.65*	8.95±0.18*
PCV (%)	42.1±1.95	31.3±1.04*	61.0±0.77*	40.9±0.93	30.1±0.39*
RBC count (million/mm³)	6.83±0.34	5.6±0.44*	10.0±0.10*	9.9±0.39*	5.5±0.09*
MCV (fl)	61.5±0.73	55.8±0.83**	61±0.55	41.3±0.93**	54.7±0.25**
MCH (pg /cell)	17.5±0.22	16.2±0.17**	17.7±0.04	17.2±0.25	16.3±0.11**

Values (mean±SEM) in a row bearing single asterisk (P<0.05) or double asterisk (P<0.01) differ significantly as compared to control (group I).

In ASP treated group, the Hb and RBCs count increased while MCV value decreased but unlike the ACE inhibitor treated group (untreated control), change in MCH value was non-significant. Increase in RBCs count and decrease in MCV value resulted in compensatory effect on the change in hematocrit value, thus, it remained non-significant. In this situation, RBCs were referred to as normochromic. As various studies have shown that ASP can be used as a health-supporting agent in anemic patients by increasing plasma iron levels through inhibition of hepcidin expression (Wang *et al*., 2011; Lee *et al*., 2012).

In treated II group, the Hb and PCV levels, RBC count, MCV and MCH levels decreased significantly (P<0.05) as compared to control group that indicated the ineffectiveness of combination of ACE inhibitor + ASP. There was not only reduction in RBC count but also microcytic and hypochromic anemia. It showed that when ASP was given alone, it exerted its anti-anemic effect but when given in combination with an ACE inhibitor, it produced a detrimental effect with ACE inhibitor and lead towards severe anemia. As the drug was given in crude form, this severe anemia might be the result of interaction between ACE inhibitor and ASP or with some other component present in *A. sinensis* that may have effect on hematological parameters in combination with ACE inhibitor. This interaction might be at the time of administration of ACE inhibitor and ASP. However, ASP (10 mg/kg/day) in myelo-suppression mouse model showed increased recovery of platelets and other blood cells which in turn enhanced thrombopoiesis and hematopoiesis (Liu *et al*., 2010).

In case of histopathological examinations of liver and kidney, ASP showed necrotic changes at the cellular level that resulted in mild degree of toxicity on both kidney and liver and it was administered only for 28 days. According to this concept, ASP has a potential to induce hepatic and renal toxicity in 28 days and will induce severe toxicity when administered for a longer period of time. These effects might be due to some changes in environment and genetic makeup. It is a common belief that herbal medicines are free of any side effects because of their natural origins. But dose, frequency and manner of administration are not fully controlled and reviewed as these are usually self-prescribed. Herbs containing chemicals may be natural to the plants but for the human body, they are not natural (Arsad *et al.,* 2014).

Conclusion: ACE inhibitor induced anemia while in case of ASP, Hb level and RBCs count increased. In rats treated with ACE inhibitor + ASP, there was not only reduction in Hb and PCV value and RBCs count but there was also microcytic and hypochromic anemia. It means APS may have effect against anemia but when it is administered simultaneously with ACE inhibitor to cure anemia, it showed unfavorable effect with more complicated anemia, thus it should not be used with ACE inhibitors. Histopathological examination of liver and kidney revealed that ASP showed toxicity within 28 days. These results ended up with a conclusion that ASP will be severely toxic when given for a longer period of time. Therefore, it is not safe for the patients with cardiovascular diseases for longer period of time.

Author's contribution: BA, IJ, TK and JAK conceived and designed the experiment. SKZ performed the whole experiment. AR analyzed the data and drafted the manuscript. All the authors read and approved the final version of the manuscript.

REFERENCES

Ajmal A, Gessert CE, Johnson BP, Renier CM and Palcher JA, 2013. Effect of angiotensin converting enzyme inhibitors and angiotensin receptor blockers on hemoglobin levels. BMC Res Notes, 6: 443.

Anand I, McMurray JJ, Whitmore J, Warren M, Pham A *et al*., 2004. Anemia and its relationship to clinical outcome in heart failure. Circulation, 110: 149-154.

Arsad SS, Esa NM and Hamzah H, 2014. Histopathologic changes in liver and kidney tissues from male sprague dawley rats treated with Rhaphidophora decursiva (Roxb.) Schott extract. J Cytol Histo. doi:10.4172/2157-7099.S4-001.

Campbell DJ, 2009. Renin inhibitors – mechanisms of action.Aust Prescr, 32: 132-135.

Chawla R, Thakur P, Chowdhry A, Jaiswal S, Sharma A *et al*., 2013. Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes. A dreadful lifestyle disorder of 21st century. J Diabetes Metab Disord, 12: 35.

Cho CH, Mei QB, Shang P, Lee SS, So HL *et al*., 2000. Study of the gastrointestinal protective effects of polysaccharides from *Angelica sinensis* in rats. Planta Med, 66: 348-51.

Cole J, Ertoy D, Lin H, Sutliff RL, Ezan E *et al*., 2000. Lack of angiotensin II–facilitated erythropoiesis causes anemia in angiotensin-converting enzyme–deficient mice. J Clin Invest, 106: 1391-1398.

Gad SB and El-Maddawy ZK, 2014. Silymarin improves pancreatic endocrine function in rats. Pak Vet J, 34: 214-218.

Hayashi K, Hasegawa K and Kobayashi S, 2001. Effects of angiotensin-converting enzyme inhibitors on the treatment of anemia with erythropoietin. Kidney Int, 60: 1910-1916.

Haznedaroglu IC and Beyazit Y, 2013. Local bone marrow renin–angiotensin system in primitive, definitive and neoplastic haematopoiesis. Clin Sci, 124: 307-323.

Hook IL, 2014. Danggui to *Angelica sinensis* root: are potential benefits to European women lost in translation? A review. J Ethnopharmacol, 152: 1-13.

Hou H, Bao Y, Li Q and Shi W, 2012. Preparation of blood-deficient model and research of *Angelica* polysaccharide on enriching blood in chickens. Evid Based Complementary Altern Med, 5 pages doi:10.1155/2012/965947.

Inoue A, Babazono T and Iwamoto Y, 2008. Effects of the renin-angiotensin system blockade on hemoglobin levels in type 2 diabetic patients with chronic kidney disease. Am J Hypertens, 21: 317-322.

Javed S, Khan JA, Khaliq T, Javed I and Abbas RZ, 2015. Experimental evaluation of nephroprotective potential of *Calotropis procera* (Ait) flowers against gentamicin-induced toxicity in albino rabbits. Pak Vet J, 35: 222-226.

Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N *et al.*, 2014. A systematic analysis of global anemia burden from 1990 to 2010. Am J Hematol, 123: 615-624.

Kouadio MK, Righetti AA, Abé NN, Wegmüller R, Weiss MG *et al.*, 2013. Local concepts of anemia-related illnesses and public health implications in the taabo health demographic surveillance system Côte d'Ivoire. BMC Hematol, 13: 5.

Kumar G, Jalaluddin M, Rout P, Mohanty R and Dileep CL, 2013. Emerging trends of herbal care in dentistry. J ClinDiagn Res, 7: 1827-1829.

Lee JG, Hsieh WT, Chen SU and Chiang BH, 2012. Hematopoietic and myeloprotective activities of an acidic *Angelica sinensis* polysaccharide on human CD34+ stem cells. J Ethnopharmacol, 15: 739-745.

Laudisio A, Marzetti E, Pagano F and Bernabei R, 2012. Introduction of angiotensin-converting enzyme inhibitors induces a fall in hemoglobin levels in elderly patients hospitalized with heart failure: a cohort study. Aging Clin Exp Res, 24: 145-151.

Lin C, Datta V, Duodu DO, Chen X, Fuchs S *et al.*, 2011. Angiotensin-converting enzyme is required for normal myelopoiesis. FASEB J, 25: 1145-1155.

Liu C, Li J, Meng FY, Liang SX, Deng R *et al.*, 2010. Polysaccharides from the root of *Angelica sinensis* promotes hematopoiesis and thrombopoiesis through the PI3K/AKT pathway. BMC Complement Altern Med, 10:79.

Liu J, Zhang Y, You R, Zeng F, Guo D *et al.*, 2012. Polysaccharide isolated from *Angelicasinensis* inhibits hepcidin expression in rats with iron deficiency anemia. J Med Food, 15: 923-929.

Rahman ZU, Khan JA, Khaliq T and Ali A, 2012. Manual of Physiology-I. 5th Ed, University of Agriculture, Faisalabad, Pakistan, pp: 12-55.

Tomovska J, Presilski S, Gjorgievski N, Tomovska N, Qureshi MS and Bozinovska NP, 2013. Development of a spectrophotometric method for monitoring angiotensin-converting enzyme in dairy products. Pak Vet J, 33: 14-18.

Vlahakos DV, Marathias KP and Madias NE, 2010.The role of the renin-angiotensin system in the regulation of erythropoiesis. Am J Kidney Dis, 56: 558-565.

Wang KP, Zeng F, Liu JY, Guo D and Zhang Y, 2011. Inhibitory effect of polysaccharides isolated from *Angelica sinensis* on hepcidin expression. J Ethnopharmacol, 134: 944-948.

Ye YN, So HL, Liu ES, Shin VY and Cho CH, 2003. Effect of polysaccharides from *Angelicasinensis* on gastric ulcer healing. Life Sci, 72: 925-932.

Zahra K, Nawaz M, Khan A and Bacha U, 2013. Hemoglobin, red blood cell count, hematocrit and derived parameters for diagnosing anemia in elderly males. Proc Pak Acad Sci, 50: 217-226.