SOME SERUM BIOCHEMICAL CONSTITUENTS OF MARES DURING DIFFERENT PHASES OF REPRODUCTIVE CYCLE

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ABSTRACT

Serum glucose, total proteins, calcium and inorganic phosphorus were determined in estrual, pregnant, infertile and regular breeder mares, each group comprising of 10 animals. The mares were grouped on the basis of rectal examination, history and records. There was a significant (P<0.05) difference in the serum glucose levels between the pregnant and estrual mares, pregnant and regular breeder mares, infertile and regular breeder mares and between infertile and estrual mares. The highest values of serum glucose were recorded in regular breeder mares, whereas the lowest values were recorded in infertile mares. The highest values for total proteins were recorded in regular breeder mares and the lowest in pregnant mares, difference being significant (P<0.05) between the pregnant and estrual and pregnant and regular breeder mares. The highest values of serum calcium were recorded in estrual mares and lowest for regular breeder mares. There was a significant (P<0.05) difference between the estrual and pregnant mares, estrual and infertile mares and between estrual and regular breeder mares. The highest values of serum inorganic phosphorus were recorded for regular breeder mares whereas the lowest values were recorded in pregnant mares. The analysis of data indicated non significant (P>0.05) difference in the serum phosphorus levels among all the groups of mares.

Key words: Serum, biochemical constituents, reproductive cycle, mares.

INTRODUCTION

Pakistan has an equine population of approximately 4.6 million, including 0.3 million horses, 4.1 million asses, and 0.2 million mules (Anonymous, 2003). The horses are currently used as work animals, for riding or show purposes and for producing mules, which are ideally suited to physical work in hard and hilly areas.

The fertility status of mares in Pakistan has been extremely low compared with the world average. The causes of infertility in mare include hormonal imbalance, genetic disabilities, infectious pathology of genital tract and environmental factors including the nutritional management of brood mare (Sane *et al.*, 1994). The beneficial role of minerals, protein intake and blood glucose level in achieving the optimum fertility has been reported in bovine (Husnain, *et al.*, 1981), ovine (Robert, 1982), equine (Frape, 1986) and in canine (Leathem, 1959).

The body condition of mares at the time of breeding can influence the rate of conception (Henneke *et al.*, 1984). Henneke *et al.* (1983) reported that mares in thin body condition at foaling had reduced conception rates, longer postpartum intervals to conception and more cycles per conception. High incidence of early pregnancy loss in pubertal mares is related to immaturity, inadequate nutrition and physical stress (Mitchell and Allen, 1975). Holtan and Hunt (1983) demonstrated that dietary protein concentrations influence circulating progesterone values and, therefore, have implications for mare fertility. The mares that received high quality protein ovulated earlier in the breeding season (Van-Niekerk and Van-Niekerk, 1997a). High quality protein stimulated FSH and LH production during the late transitional period (Van-Niekerk and Van-Niekerk, 1997b). Deficiency of minerals and vitamins causes anoestrous, leading to infertility; under-nourished mares are prone to embryonic mortality (Sane *et al.*, 1994). According to Church and Pond (1974), based on available data on animal nutrition, it can be inferred that macro mineral metabolism and nutrition, with some exceptions, is similar among animal species and therefore observations on one species may be extrapolated to others.

The literature is scanty on the relationship between the levels of biochemical constituents in serum and fertility in equines. As far ascertained, no work has been reported in Pakistan. While attempting to delineate the causes of infertility in mares, it was felt that study of the biochemical constituents of mare serum might be helpful. The present study was therefore, designed to

determine serum levels of total proteins, glucose, calcium and inorganic phosphorus in mares during different reproductive phases i.e. in regular breeder, estrual, infertile and pregnant mares.

MATERIALS AND METHODS

Animals

This study was conducted in the Chenab Breeding Area, Faisalabad. A total of 40 mares kept by the local horse breeders (stud guarantees) were used. Mares from those farms were included in the study where the managemental practices did not vary greatly among themselves.

Examination of animals

A complete history of each mare was obtained from the owners and verified from the records. All the mares were examined per rectum for their current status of reproductive cycle and sexual health. The mares were divided into four groups of 10 mares each.

Group I comprised of the mares that were confirmed to be pregnant for at least 60 days at the time of examination. In group 2 the mares that were found to be in estrus at the time of sample collection were included. The estrual status was confirmed by visual signs, rectal palpation and by stallion testing method. The mares that had not produced any foal within last 30 months were taken as infertile and were included in group 3. These mares did not reveal any palpable abnormality of the genital tract at the time of rectal palpation. The mares that had produced at least two foals in last 30 months and at present were apparently free of any reproductive problem were considered as regular breeders and were included in group 4.

Collection of serum samples

The blood samples from all the mares in different groups were collected for harvesting serum while observing all hygienic measures and 20 ml of blood from each mare was collected by jugular venipuncture. The blood was centrifuged at 2000 rpm (Labofuge I, Labsco, Germany) for 10 minutes; the serum was separated and stored at -20°C until used for analysis.

Determination of blood constituents

A drop of fresh blood at the time of blood collection was used to determine glucose level with the help of glucometer (Tidex, Model 5890, Japan). The glucose values were reflected in mg/dl by the glucometer, hence were later converted into millimoles per litre .The serum calcium level was determined by using the commercial kit (Medi Diagnostics Italy, Calcium (OCPC) Plus, Lot No: 2251) with spectrophotometer (Micro Lab 300, Merck). These values obtained in mg/dl were later converted into millimoles per litre. Serum inorganic phosphorus level was determined by using the commercial kit (Human GmBH. D-65205, Wiesbaden, Germany. Lot No: H031) on spectrophotometer (Micro Lab 200, Merck). The values obtained in mg/dl were later converted into millimoles/litre. The serum total proteins level was determined by using the commercial kit (Total Proteins Liquid Plus, Giesse Diagnostics, Italy, Code 0042) on spectrophotometer (Micro Lab 200). The values obtained in grams/dl were later converted into grams/litre.

Statistical analysis

Mean values (± SD) of serum calcium, total proteins, inorganic phosphorus and glucose of mares in different groups were subjected to one way analysis of variance, using randomized complete block design (Steel and Torrie, 1980). Duncan's multiple range test (Duncan, 1955) was used to compare the means.

RESULTS AND DISCUSSION

Glucose

The mean (± SD) serum glucose levels (m.moles/litre) for pregnant, estrual, infertile and regular breeder mares were 4.37 ± 0.67, 5.33 ± 0.88, 4.49 ± 0.47 and 5.53 ± 0.94, respectively. There was no difference ($P > 0.05$) in the glucose levels between the pregnant and infertile mares and between estrual and regular breeder mares. There was a significant ($P<0.05$) difference between the pregnant and estrual mares, pregnant and regular breeder mares, infertile and regular breeder mares and between infertile and estrual mares (Fig. 1a).

Frape (1986) reported the serum glucose levels of 4.4 m.moles per litre, whereas Johnston (1994) reported a range of 3-6 m.moles/litre for horses. The blood samples used in this study were random samples. The mean values of glucose for all the groups of mares in this study fall within the range of blood glucose levels given in literature as mentioned above.

The blood glucose levels in both pregnant and infertile mares were significantly lower than those in estrual and regular breeders. Fowden *et al.* (2002) reported that there was significant uptake of glucose by the gravid uterus, fetus and uteroplacental tissues at both mid and late gestation. The comparatively lower levels of blood glucose in pregnant mares may be explained in the light of their study. Not much information is available about the role of low blood glucose levels causing infertility in mares. Sticker *et al.* (1995) reported that reduction in energy level reduced

significantly the levels of IGF-I within 24 hours in mares. Butler (2000) has reported that negative energy balance in cows delays the time of first ovulation through inhibition of LH pulse frequency and low levels of blood glucose, insulin and IGF-I collectively restrain estrogen production by dominant follicle and reduce fertility. Beam and Butler (1999) also provided evidence of primarily a hypothalamic locus for the modulation of LH secretion during negative energy balance. Positive relationship between changes in energy balance and function of dominant follicle support the identification of IGF-I.

Butler and Smith (1989) has reported that in cows lower availability of glucose may also decrease LH pulsatility or limit ovarian responsiveness to gonadotrophins. This has also been reported in cows that lower blood glucose levels lowered first service conception rate and fertility (Randel, 1990), and higher blood glucose levels decreased service period (Jonsson *et al.*, 1997), and increased first service conception rate (Plym-Forshell *et al.*, 1991). It is possible that the lower blood glucose level in infertile mares in this study was a contributory factor towards their infertile status.

Total proteins

The mean (± SD) total protein values (grams/litre) in pregnant, estrual, infertile and regular breeder mares were 48.5 ± 16.7, 66.5 ± 15.2, 58.7 ± 13.7 and 67.6 ± 6.2, respectively. There was no difference in the levels of total proteins between the estrual, infertile and regular breeder mares and between pregnant and infertile mares. However, there was a significant ($P<0.05$) difference between the pregnant and estrual and pregnant and regular breeder mares (Fig. 1b).

Frape (1986) reported the average serum total protein levels in horses to be 62 grams/litre. The total protein values in pregnant mares were lower than those reported by Frape (1986). It has been reported by Benjamin (1979) that during pregnancy the total protein values in serum are lower. The levels of total serum protein were comparatively lower in infertile mares when compared with those in estrual and regular breeder mares.

The role of quantity and quality of protein intake on reproduction in mares has been documented. Van-Niekerk and Van-Niekerk (1997a) reported that high quality protein stimulated FSH and LH production during the late transitional periods. With high quality protein, cyclic pattern of FSH release started 4 to 6 weeks earlier than the mares on low quality protein. Van-Niekerk and Van-Niekerk (1997b) also reported that the mares that received high quality protein ovulated 2 to 3 weeks earlier compared with those that were on low quality protein intake. The quality of protein intake also affected the estrus in mares during transitional period. However, Van-Niekerk and Van-Niekerk (1997c) also indicated that daily total protein intake had no effect on blood protein concentrations, whereas Benjamin (1979) narrated that protein intake affected the serum total proteins. Van-Niekerk and Van-Niekerk (1998) also mentioned that mares fed on low quality protein diet had higher incidence of early embryonic loss before 90 days, resulting in infertility. The comparatively lower levels of total serum proteins in infertile mares in this study may be explained in the light of above narrated studies.

Calcium

The mean (± SD) calcium values (m.moles/litre) in pregnant, estrual, infertile and regular breeder mares were 2.19 ± 0.44, 2.56 ± 0.40, 1.98 ± 0.25, and 1.86 ± 0.41, respectively. There was no difference between the pregnant, infertile and regular breeder mares. There was a significant ($P<0.05$) difference between the estrual and pregnant mares, estrual and infertile mares and between estrual and regular breeder mares (Fig. 1c).

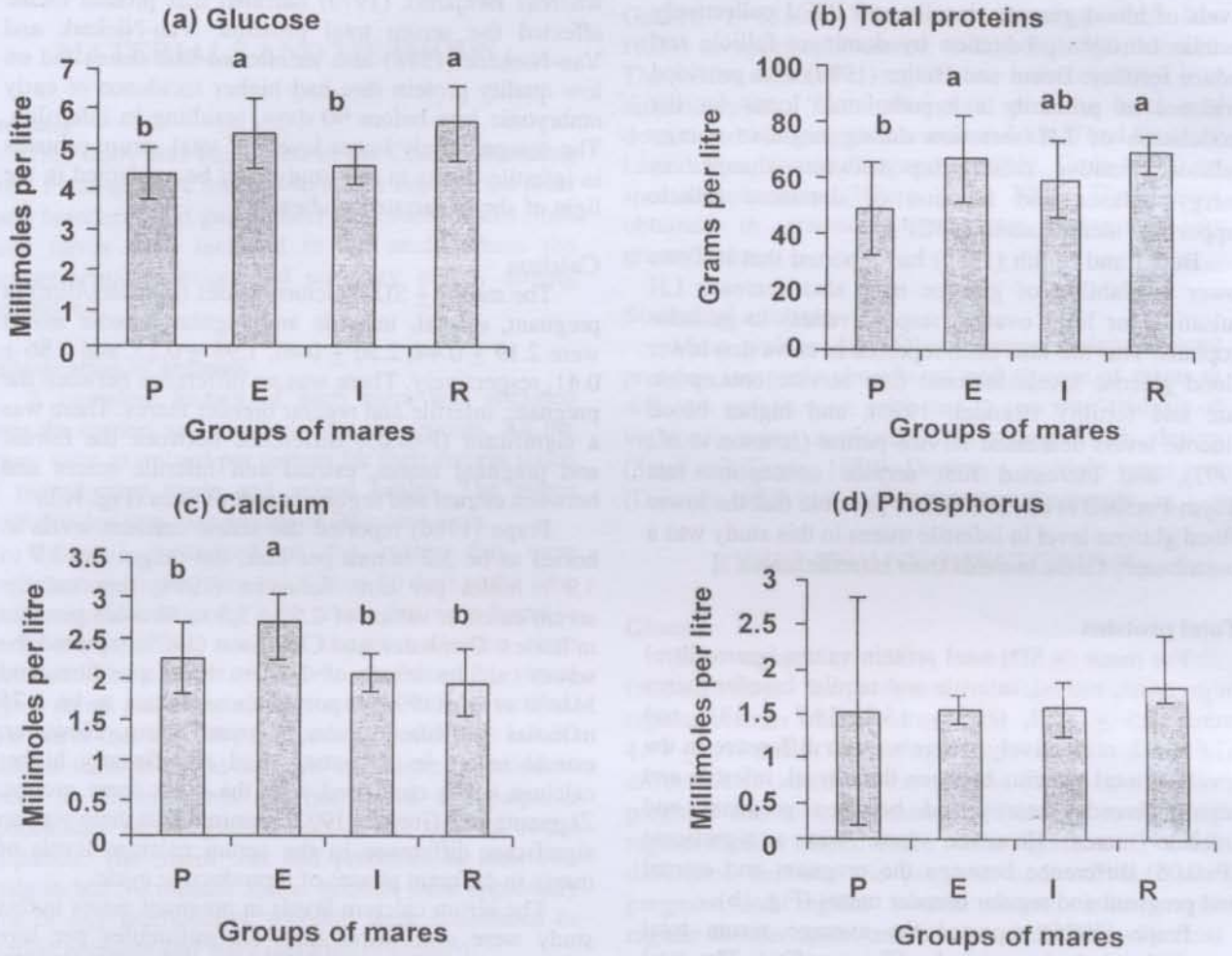
Frape (1986) reported the serum calcium levels in horses to be 3.2 m.mol per liter, the range was 2.9 to 3.9 m.moles per litre. Johnston (1994) reported the serum calcium values of 2.5 to 3.5 millimoles per litre in horses. Cymbaluk and Christison (1989) reported the serum calcium levels of 2.77 m.moles per litre, and Martin *et al.* (1996) reported these values to be 2.75 m.moles per litre in non pregnant mares. However, estrual mares in this study had significantly higher calcium levels compared with the other three groups. Zepperitz and Gurtler (1992) reported that there was no significant difference in the serum calcium levels of mares in different phases of reproductive cycle.

The serum calcium levels in pregnant mares in this study were also lower than 3.1 millimoles per litre reported for pregnant mares by Martin *et al.* (1996). Harvey *et al.* (1993) reported that serum calcium levels did decrease slightly during pregnancy in mares. Similar findings in goats have been reported by Mbassa and Poulsen (1991) that serum calcium level decreased in late gestation in goats.

Inorganic phosphorus

The mean (± SD) values (m.moles/litre) of inorganic phosphorus in pregnant, estrual, infertile and regular breeder mares were 1.51 ± 1.30, 1.53 ± 0.16, 1.54 ± 0.29 and 1.98 ± 0.38, respectively. However, the analysis of data indicated non significant difference in the serum phosphorus levels among all the groups of mares (Fig. 1d).

Frape (1986) reported the serum inorganic phosphorus values in horses to be 0.5-1.6, with an average of 1.1 millimoles per litre. The values recorded in this study for pregnant, estrual and infertile mares



Means with different superscripts differ significantly (P<0.05)

Fig 1: Mean values (±SD) of serum inorganic phosphorus, calcium, glucose and total proteins levels in pregnant (P), estrual (E), infertile (I) and regular breeder (R) mares

fall within this range and are slightly higher for regular breeder mares. However, serum phosphorus values for mares in all groups are lower than 2.03 millimoles/litre reported by Cymbaluk and Christison (1989). This study did not demonstrate any difference in the serum phosphorus levels in different groups of mares. The literature is scanty about the serum levels of phosphorus in different phases of reproductive cycle in mares. However, it has been reported in other species that serum phosphorus values differ in different phases of reproductive cycle. Husnain *et al.* (1981) reported that serum phosphorus levels were lower in anestrous than the cyclic buffaloes.

Because of their interdependent role, the function of calcium and phosphorus and hence their values in the serum should be considered together. Serum phosphorus values vary without untoward physiological effects. A diet related clinical disorder, nutritional secondary hyperparathyroidism, is a condition in which serum phosphorus is slightly raised and serum calcium values are slightly depressed. In this study, the calcium to phosphorus ratios for pregnant, esrtual, infertile and

regular breeder mares were 1:0.7, 1:0.6, 1:0.87 and 1:1.1, respectively. Based on the mean values and ranges for serum electrolytes in horses as published by Frape (1986), the calcium to phosphorus ratio ranges from 1:0.2 to 1:0.4 with an average of 1:0.34. The ratio between Ca and P in this study is greater than the average reported by Frape (1986).

It can be inferred from the present study that maintenance of physiological levels of glucose, total proteins, calcium and inorganic phosphorus is a pre-requisite for optimum fertility and reproductive success. Mare is capable of considerable adjustments to variety of situations, but the extremes of excesses or deficiencies of above mentioned biochemical constituents may lead to lowered reproductive efficiency. The studies expanded to larger groups of mares, preferably with controlled managemental conditions, are indicated.

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REFERENCES

Anonymous, 2003. Economic Survey of Pakistan, Government of Pakistan, Finance Division, Islamabad.

Beam, S.W. and W. R. Butler, 1999. Effect of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertil. Suppl; 54: 411-424.

Benjamin, M.M., 1979. Outline of Veterinary Clinical Pathology. 3rd Ed., Lowa State University Press, Ames, Lowa, USA, pp: 286-290.

Butler, W. R. and R. D. Smith, 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci., 72(3): 767-783.

Butler, W. R., 2000. Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci., 60-61: 449-457.

Church, D. C. and W.G. Pond, 1974. Basic Animal Nutrition and Feeding, 3rd Ed. Jhon Wiley & Sons, London, UK. Pp: 181-209.

Cymbaluk, N. F. and G. I Christison, 1989. Effects of dietary energy and phosphorus contents on blood chemistry and development of growing horses. J. Anim. Sci., 67 (4): 951-958.

Duncan, D. B., 1955. Multiple range and multiple F test. Biometrics. 11: 1-42.

Fowden, A. L., A. J. Forhead, K. L. White and P. M. Taylor, 2002. Equine uteroplacental metabolism at mid and late gestation. Exp. Physiol., 85: 539 -545.

Frape, D., 1986. Equine Nutrition and Feeding, Longman Group, UK, pp: 134-155.

Harvey, A. L., B. H. Thomas, C. C. Culbertson and E.V. Collins, 1993. The effect of limiting feeding of oats and timothy hay during work on the nitrogen balance of draft geldings. Proc. Amer. Soc. Anim. Prod. Chicago, USA, 3: 94

Henneke, D. R., G. D. Potter and J. L. Kreider, 1984. Body condition during pregnancy, lactation and reproductive efficiency in mares. Theriogenology, 21: 897 -900.

Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurement and body fat percentage in mares. Equine Vet. J., 15: 371-372.

Holtan, D. W. and L. D. Hunt, 1983. Effect of dietary protein on reproduction in mares. Proc. 8th Equine Nutr. Physiol. Soc. Symp., University of Kentucky, Lexington, USA.

Husnain, Z. U., C. S. Ali, K. M. Ahmad and H. A. Samad, 1981. Studies on relationship between blood mineral level and fertility of buffaloes. Pakistan Vet. J., 1(4): 141-144.

Johnston, A. M., 1994. Equine Medical Disorders. 2nd Ed. Blackwell Scientific Publications, Oxford, London, UK, pp: 186-188.

Jonsson, N. N., M. R. Mcgowan, T. M. Davison, A. M. Hussain, M. Kafi and A. Matschoss, 1997. Relationships among calving season, heat load, energy balance and postpartum ovulation of dairy cows in a subtropical environment. Anim. Reprod. Sci., 47 (4): 315- 326.

Leathem, J. H., 1959. Reproductive Physiology and Protein Nutrition in Dog. Rutgers Univ. Press, New Brunswick, New Jersey, USA.

Martin, K. L., R. M. Hoffman, D. S. Cornfield, W. B. Ley and L. D. Warnick, 1996. Calcium decreases and parathyroid hormone increases in serum of periparturient mares. J. Anim. Sci., 74(4): 834-839.

Mbassa, G. K. and J. S. Poulsen, 1991. Influence of pregnancy, lactation and environment on some clinical and chemical reference values in Danish Landrace dairy goats (*Capra hircus*) of different parity. I. Electrolytes and enzymes. Comp. Biochem. Physiol. B.; 100(2): 413-422.

Mitchell, D. and W. R. Allen, 1975. Observations on reproductive performance in the yearling mare. J. Reprod. Fertil. Suppl., (23): 531-536.

Plym-Forshell, K., L. Andersson and B. Pehrson, 1991. The relationship between fertility of dairy cows and clinical and biochemical measurements, with special reference to plasma glucose and milk acetone. Zentralbl Veterinarmed A., 38(8): 608-616.

Randel, R. D., 1990. Nutrition and postpartum rebreeding in cattle. J. Anim. Sci., 68 (3): 853-862.

Robert, S. J., 1982. Veterinary Obstetrics and Genital Diseases (Theriogenology). 2nd Ed. CBS Publishers and Distributors, India: 573 -582.

Sane, C. R., A. S. Kaikini, S. B. Kodagali, V. B. Hukeri, B. R. Deshpande, D. P. Velhankar, S. N. Luktuke and V. L Deopurkar, 1994. Infertility in mares, In: Reproduction in Farm Animals, 2nd Ed. Varghese Publishing House, Bombay, India, pp: 301-307.

Steel, R.G.D. and J. H. Torrie. 1980. Principles and Procedures of Statistics. 2nd Ed. McGraw Hill Book Co., New York, USA.

Sticker, L. S., D. J. Thompson, J. M. Fernandez, L. D. Bunting and C. L. De Pew, 1995. Dietary protein and or energy restriction in mares: Plasma growth hormone IGF-1, prolectin, cortisone and thyroid hormone response to feeding, glucose and epinephrine. J. Anim. Sci., 73 (5): 1424-1432.

Van-Niekerk, F. E. and C. H. Van-Niekerk, 1997a. The effect of dietary protein on reproduction in the mare. II Growth of foal body mass of mares and serum protein concentration of mares during the anovulatory, transitional and pregnant periods. J. South Afr. Vet. Assoc., 68 (3): 81-85.

Van-Niekerk, F. E. and C. H. Van-Niekerk, 1997b. The effect of dietary protein on reproduction in the mare. III. Ovarian and uterine changes during the anovulatory periods in the non pregnant mare. J. South Afr. Vet. Assoc., 68 (3): 86-92.

Van-Niekerk, F. E. and C. H. Van-Niekerk, 1997c. The effect of dietary protein on reproduction in the mare IV. Serum progestagen, FSH, LH and melatonin concentrations during the ovulatory periods in the non pregnant mares. J. South Afr. Vet. Assoc. 68 (4): 114-120.

Van-Niekerk, F. E. and C. H. Van-Niekerk, 1998. The effect of dietary protein on reproduction in the mares. VII. Embryonic development, early embryonic death, fetal losses and their relationships with serum progestagen. J. South Afr. Vet. Assoc., 69 (4): 150-155.

Zepperitz, H. and H. Gurtler, 1992. Ionized calcium and total calcium in the blood of cattle, sheep, swine, and horses of different ages, reproductive stages and uses. Abstract. Pub Med.