Discussion


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It is known that in healthy older individuals many physiologic functions are maintained in the basal resting state but decrease in functions occur in the system of most of the organs and in homeostatic mechanisms when the existing system faces a challenge or undergoes a stressful situation. Even with high advancement of age, despite the prevalence of disease an interaction of disease on one hand and physiological control mechanism on the other continue to occur. A disease process depends on the degree of disturbance of functions of the organ system and on perturbation of homeostatic mechanism.

Theories of ageing are of two categories increase in damage to molecules or decrease in regulation of specific genes. DNA undergoes a continuous change in response to exogenous agents or different intrinsic processes. If double strandedness of DNA is maintained there is a possibility of repair of change by specific repair enzymes. Hypotheses have been put forward that biological ageing is due to somatic mutagenesis which can be due to greater susceptibility to mutagenesis or due to deficiency in repair mechanism. It has been suggested that the longevity of the individual has correlation with concentration of DNA repair enzymes. Another theory suggests that errors occur not only in
DNA but also in RNA and protein synthesis, each of these augment each other that culminates in error catastrophe. In view of the fact that protein synthesis is the final common pathway it appears that protein synthesis is the most important source for age dependent errors.

It has been suggested that the major by product of oxidative metabolism like, superoxide radicals which react with DNA, RNA, proteins and lipids, lead to cellular damage and ageing. Had it been so, scavenging enzymes like Vitamin C and Vitamin E could protect the cell from oxidative damage but no concrete evidence has been put forward to show that there occurs errors of scavenging enzymes with ageing nor treatment with Vitamin C or Vitamin E could increase longevity in experimental animals.

That general metabolic activity is largely regulated by the thyroid hormone is well known and it is also true that metabolic clearance rate of thyroid hormone goes down with advancement of age when the hypothalamus pituitary axis is intact it is but natural that thyroxine levels are maintained and the normal metabolic activity is continued but in the present observation, it has been found that the thyroid activity (Serum $T_3$ & $T_4$) is gradually reduced to a low level with a corresponding rise in serum TSH level in both groups of aged individuals, but with Asparagus racemosus or Mucuna pruriens treatment Serum $T_3$ and $T_4$ levels had increased alongwith a fall in TSH level. The present result indicates that
with Asparagus racemosus or Mucuna pruriens treatment general metabolic process is rejuvenated and it is expected that this increased thyroid activity will cause correction of errors that occur in DNA, RNA and protein synthesis mechanism and thereby postponement of senile changes.

In the present experiment status of thyroid activity, levels of serum gonadotrophin (FSH & LH) and testosterone, serum lipid levels, serum creatinine and serum biogenic amines levels have been analysed following treatment with Satavari (Asparagus racemosus) root extracts and Alkushi (Mucuna pruriens) seed extracts in male individuals in two subgroups, one between the age of forty to fifty nine years and the other between the age of sixty to seventy five years. The parameters were analysed with a view to assess the pattern of changes with the advancement of age. The purpose of the present experiment was also to assess whether these extracts could bring about postponement of ageing by alteration of different parameters towards normal levels. The analysis of parameters have been made one month after cessation of treatment with a view to ascertain whether the changes brought about by these extracts were maintained even after cessation of treatment. In the present experiment, each individual had served as his own control and the treatment was given to them for thirty days. The different parameters were assessed on zero day, one month after treatment and also after two months following cessation of treatment by two different compounds.
It is interesting to note that secretion of serum triiodothyronin ($T_3$) and thyroxine ($T_4$) had increased significantly following both *Asparagus racemosus* root extract and *Mucuna pruriens* seed extract treatment for one month. Although they were treated for thirty days, thyroid activity continued to be high even after two months (Tables 1 & 2; 15 and 16). A fall in serum thyroid stimulating hormone (TSH) level following both *Asparagus racemosus* root extract and *Mucuna pruriens* seed extract treatment (Tables 3 & 17 and histograms 3 & 17) corroborates the result on thyroid activity. It is indicated therefore that both the above treatment increased the metabolic activity of cells of individuals in both subgroups. The thyroid hormones (T3 and T4) increase transcription by certain genes in the nucleus and in order to accomplish this, these hormones initially bind directly with receptor proteins in the nucleus of the cell, the receptor being protein molecules located within the chromosomal complex and they probably control the function of genetic promoters or operators. The activation of genetic mechanism leads to formation of hundreds of various types of intracellular proteins many of which are responsible for promoting intracellular metabolic activities in almost all cells of the body and while remaining bound to intracellular receptors can continue to express their controlled functions for days, weeks or months.

Thus it appears that increase in metabolic activity at cellular level by the *Asparagus racemosus* root extract and *Mucuna pruriens* seed
extract, metabolic environment of the body change altogether towards normally and thereby the extracts bring about the rejuvenation of cells.

When we focus our attention to the effect of *Asparagus racemosus* root extract and *Mucuna pruriens* seed extract on serum gonadotrophins (FSH and LH) and serum testosterone in male population of the two subgroups analysed in the present experiment, it is noted that both Follicle stimulating hormone (FSH) and Luteinising hormone (LH/ICH) had increased significantly. A similar significant rise in serum testosterone concentration was observed following the above treatments. The result indicates that both the treatments could bring about improvement of sexual status even at older age. It is well known that FSH helps to maintain spermatogenic epithelium and Sertoli cells and it is also known that LH/ICH hormone in males have trophic effect on Leydig cells. It is but natural that increase in serum LH/ICH level by its trophic effect on Leydig cells would cause an increase in serum testosterone level which in fact has been observed. With these results on serum gonadotrophins and serum testosterone levels one is tempted to believe that both *Asparagus racemosus* root extract and *Mucuna pruriens* seed extract increase anabolic effects besides gonadal activity. The fact remains that increase in LH/ICH would cause expression of testosterone from the Leydig cells and testosterone in turn would play an essential role for growth and division of germinal cells in forming sperm and increase in FSH would stimulate sertoti cells which would cause conversion of spermatids to sperm.
becomes clear therefore that *Asparagus racemosus* and *Mucuna pruriens* extracts would increase the sexual status of the individual since it is well known that there occurs progressive decline in serum testosterone level with advancement of age in men resulting in andropause. The decline in sexual activity is also associated with reduction of muscle mass and strength. The result of the present study strongly indicates that andropause is likely to be postponed by treatment with these extracts. It also appears that in a man with andropause it is likely that these extracts could reverse the situation and the individuals even at an old age above sixty in likely to undergo rejuvenation process.

There is a saying that a man is as old as his arteries. In this respect the pathology of vascular diseases is an increasingly important aspect of the pathology of old age. Arteriosclerosis is a generic term introduced long back by Lobstein (1833). At various periods, in the study of lipid metabolism, each of the chemically determined category of serum lipids has been shown to be associated quantitatively with arterial disease (Anitschkow 1913). A major technical innovation by Gofman et al (1954) has led to the development of more specific linkage between blood lipid level and atherosclerosis. These lipids are found to be an array of lipoprotein in varying kind of complex lipid fraction having characteristic molecular weight. The risk of vascular disease accident has been shown to be dependent on patients serum lipoprotein level (Jones et al 1951), since elevation of blood lipid level is associated with increased risk of coronary diseases, for all the conditions associated with the thickening
and hardening of arteries occur mostly with the advancement of age. The commonest form of arteriosclerosis is atherosclerosis. The factors most important for causing atherosclerosis is high plasma concentration of cholesterol in the form of low density lipoprotein (LDL) the concentration of which is directly related to consumption of increased saturated fat or increased cholesterol as such in the daily diet. Atherosclerosis has been defined as a variable combination of changes in the intima of arteries consisting of focal accumulation of lipid complex carbohydrates, blood and blood products, fibrous tissue and calcium deposits associated with changes in tunica media. Of the three lipoproteins only one VLDL is initially formed in liver. It contains large quantities of triglyceride formed by the liver in addition to cholesterol and phospholipids. Since lipoprotein lipase hydrolyses a large share of triglyceride into glycerol and fatty acid, releasing these to be stored in the fatty tissues of the body as new triglycerides. The remaining portion of very low density lipoprotein are then called intermediate density lipoprotein (IDL) which are attracted back to the liver cells because of presence of receptors on the liver cell membrane for surface protein in the lipoprotein called apoprotein b. Lipoproteins are then called low density lipoproteins (LDL), centre of which is composed entirely almost of fat soluble esterified cholesterol. Attachment of this protein to the receptor causes entire lipoprotein to be transported inside the cell.

Baseline of serum lipids was determined on a fasting samples of blood levels of LDL cholesterol were measured by the direct LDL method.
(Mcnamara et al 1995). Average lipid value baselines were total cholesterol 228 mg/dl, triglyceride 186 mg/dl, HDL cholesterol 41 mg/dl, non HDL cholesterol 187 mg/dl and direct LDL cholesterol 131 mg/dl. In most other trials serum lipid levels have been determined on fasting samples and LDL cholesterol has been calculated by Friedewald equation (LDL cholesterol = Total cholesterol - HDL cholesterol - VLDL cholesterol), where VLDL indicates very low density dipoprotein (Friedewald et al 1972). This calculation includes intermediate density lipoprotein (IDL) in the LDL fraction, and the average calculated LDL cholesterol would be 150 mg/dl if this equation is applied and with this method which is often practised, LDL cholesterol will be 15% higher than the baseline LDL-cholesterol and naturally this difference could be of some significance for arriving at the exact LDL cholesterol status. Epidemiological surveys indicate that serum cholesterol level is continuously correlated with coronary heart disease risk over a broad range of cholesterol values. This relationship has been observed in many population throughout the world (Law and Wald 1994; Law et al 1994; Law et al 2003). Since serum cholesterol levels correlate highly with total cholesterol in population, the same relation is obvious to exist between LDL cholesterol concentration, and coronary heart disease risk although the association between LDL cholesterol level and coronary heart disease risk is continuous, it is however linear, risk rises more steadily with increase in LDL cholesterol concentration which results in a curvilinear: or
loglinear which means that when the relationship between LDL cholesterol levels and coronary heart disease risk is plotted on a log scale the relationship becomes linear.

Fatty streak or spot is superficial yellow or yellowish gray intimal lesion. The process may start as early as the age of ten. The risk factor in the form of increased LDL cholesterol in plasma can lead to formation of fibrous plaque which is nothing but circumscribed elevated intimal thickening, giving it a firm appearance. An atheromatic plaque in which fatty softening is predominant is called atheroma.

Recently it has been suggested that platelets may play a role in atherogenesis not by contributing materials directly to form plaque but by way of pharmacological effects of agents released by platelet on arterial wall cells. It has been shown that changes in the blood level of low density lipoprotein with age parallel difference in the rate of progression of atherosclerosis with age.

In the present observation it has been found that both *Asparagus racemosus* and *Mucuna pruriens* treatment could reduce the LDL cholesterol level to a significant extent which means that coronary heart disease risk is also lowered under these situations.

Much less is known about the function of high density lipoprotein (HDL) than about low density lipoprotein (LDL). These are formed mainly
in liver but to a lesser extent in intestinal epithelium during absorption of fat from the gut. These lipoproteins are mostly composed of two types of apoproteins, apoprotein A\textsubscript{1} which lie on the outersurface of lipoprotein. These apoproteins do have affinity for receptors other than apoprotein "b" of the apoprotein of low density lipoprotein (LDL). It is likely that HDL can actually absorb cholesterol crystals that are beginning to deposit in the arterial wall and can transfer them to IDL and LDL to be carried back to the liver. Thus it is evident that high density lipoprotein plays a great role in protecting against the development of atherosclerosis.

The potential benefit of rise in HDL cholesterol has evoked considerable interest. It has been documented that one class of drug that modestly raises HDL cholesterol is the Fibrates. Post-Hock Analysis (Rubins 2000, NCEP report and final report of expert panel (2002) of several clinical trials with fibrates indicates that they reduce risk for coronary heart disease events in patients. Another drug that raises HDL cholesterol is nicotinic acid and the combination of statins with nicotinic acid produces a marked reduction LDL cholesterol and striking rise in HDL cholesterol (Bays et al 2003), so as to reduce the risk for coronary heart disease.

In view of the protecting effect of HDL cholesterol against the development of atherosclerosis, it appears all the more interesting to note that HDL cholesterol level had increased in both age groups of male
individuals by treatment of *Asparagus racemosus* root extract and *Mucuna pruriens* seed extracts. The potency of these extracts is proved in view of the maintenance of increased level of High density lipoproteins (HDL) cholesterol in both groups of individuals. In the present analysis we find that both *Asparagus racemosus* and *Mucuna pruriens* extracts could cause a significant increase in HDL cholesterol level and thereby would reduce the risk for coronary heart disease which means that these drugs would be useful for the continuance of well being in aged individuals.

Not only hypercholesterolemia, hypertriglyceridemia also appears to be an important risk factor for atherosclerosis. Hypertriglyceridemia has been shown to be associated with premature atherosclerosis. More than fifty percent of hyperlipidaemic atherosclerotic survivors appear to have simple monogenic familial disorders inherited as autosomal dominant trait - familial combined hyperlipidemia, familial hypertriglyceridemia, familial hypercholesterolemia in descending order of frequency. These simply inherited hyperlipidemia were more frequent in myocardial infarction survivors below age sixty than in those who were older.

In contrast non monogenic forms of hyperlipidemia occur with equal frequency above and below age of sixty (Bierman 1990). This indicates that genes associated with simply inherited hyperlipidemia, accelerated changes seen with age leading to atherosclerosis at an earlier age than usual. All studies indicate that hyperlipidemia is more meaningful risk
factor for coronary heart disease and it operates independently of and in addition to hypertension, diabetes, obesity and other factors. For treatment decision when triglyceride is more than 400 mg/dl patients are considered at higher risk factor for coronary heart disease and should be aggressively treated for lowering the lipidaemic level. In view of significant reduction in serum triglyceride by Asparagus racemosus and Mucuna pruriens treatment, in the present observation it appears that these drugs will be of immense help in reducing the incidence of ischaemic heart disease and thereby indirectly would be extremely useful for postponement of ageing process.

The result of present experiment clearly show that administration of both Asparagus racemosus root extract and Mucuna pruriens seed extract greatly reduces the serum triglyceride level in both forty to fifty nine years and in sixty to seventy five years of male individuals (Tables 7 and 21 and Histograms 7 and 21). It is also to be noted that the triglyceride level was higher to a great extent in the sixty to seventy five years of ge group in comparison to the group of forty to fifty nine years of age. Further it could be found that although the treatment was only for thirty days the significantly lowered level of triglyceride were maintained even after two months. The result indicates that both root extracts of Asparagus racemosus and seed extracts of Mucuna pruriens were related to the reduced synthesis of triglyceride in both age groups that have been studied.
A similar reduction in serum cholesterol was found in forty to fifty nine years and sixty to seventy five years age groups and the reduction of cholesterol induced by the above extracts in both age groups were statistically significant. Maintenance of low level of serum cholesterol even after two months indicate that the effect could be maintained for a long time.

It was also interesting to note that individuals belonging to sixty to seventy five years age group had higher serum cholesterol concentration in comparison to forty to fifty nine years of age (Tables 6 and 22 and Histograms 6 and 22).

In view of the protecting effect of HDL cholesterol against the development of atherosclerosis it appears all the more interesting to note that HDL cholesterol level had increased in both age groups of male individuals by treatment of Asparagus racemosus root extract and Mucuna pruriens seed extracts. The potency of the extracts is proved in view of maintenance of increased level of High density lipoprotein in both groups of individuals.

It appears therefore that risk of coronary disease in people belonging to both groups is reduced greatly since there occurs a lowering of serum triglyceride and serum cholesterol levels along with a rise in HDL cholesterol by both Asparagus racemosus root and Mucuna pruriens seed extracts.
In the present study serum creatinine level was found to be significantly low in *Asparagus racemosus* treated individuals in both age groups whereas *Mucuna pruriens* treated individuals show a significant rise in serum creatinine level in both forty to fifty nine and sixty to seventy five years age group of male individuals.

It is well known that immobility causes muscle weakness. Muscle strength and ability to maintain coordinate muscle work decrease with advancement of age in comparison to a maximum in young adult of thirty years of age (Norris and Shock 1960). McLennan et al (1980) established values for handgrip pressure in healthy adults aged sixty to eighty years showing that males had a stronger handgrip than females and there was a linear decline in strength with advancement of age. It has therefore been postulated that decrease in mobility and a gradual change to a sedentary life is likely to be responsible for the weakness associated with old age. It is true that reduction in activity is universal with advancement of age and the hypothesis has not been contested but the fact that muscle strength in the elderly increase with training does support argument that weakness in old age is due to immobility only. There is little if any evidence that the physiological decrease in strength is primarily due to changes within the muscle themselves. There is no evidence that fibrous tissue replacement of muscle tissue (McLennan et al 1980) is the cause of muscle weakness in old age. When we concentrate our attention to muscle weakness associated with myopathies of different origin it is noted
that in steroid myopathy, CPK level is normal but there is creatinuria (Kissel and Mendell 1992). Steroid myopathy has been attributed to decrease muscle protein synthesis and increased protein catabolism due to increased skeletal muscle protease activity. On the other hand in myopathy with aldosterone excess, CPK levels may be elevated and creatinine level does not alter much and the muscle weakness in these cases may reveal necrotic and regenerating fibres, and the changes are likely to be due to hypokalemia. Again in myopathy with hyperparathyroidism (Kissel and Mendell 1992) it has been reported that several muscle enzymes are normal in these conditions. It has been suggested that under this situation moderate to severe hypocalcemia may be responsible for muscle weakness.

In the present observation it appears that *Asparagus racemosus* treatment in both age groups had a similar effect as that of steroid which was associated with creatinuria and this might be the reason, why serum creatinine level had gone down to a significant extent in both groups. On the other hand rise in serum creatinine in *Mucuna pruriens* treated cases in both age groups might be due to the effect as that of hyperparathyroidism or aldosterone excess.

In the result presented here in connection with the status of different polyamine fraction like serum Putrescine, Cadaverine, Spermine and Spermidine with advancement of age as analysed in two age groups as
above, it can be seen that all these parameters declined significantly in both the age groups Table.

It is well known that the naturally occurring polyamines like Putrescine, Cadaverine, Spermine and Spermidine are distributed throughout the eukaryotes. It is also known that cell proliferation and differentiation require their biosynthesis. For the more, it was well documented that generation of these polyamines is tightly regulated (Pegg and McCaun 1982; Tabor and Tabor 1984). The metabolic reactions responsible for the formation of polyamines and their biotransformation are well known. Ornithine has been shown to be mother substance of polyamine, which is catalysed by Ornithine decarboxylase, the rate limiting enzyme to produce Putrescine, and hence other polyamines. Decarboxylated S-Adenosyl methionine catalyses decarboxylated substrate and helps in the formation of spermidine in presence of spermidine synthase. The formation of spermidine from spermine also needs an amino propyl group derived from decarboxylated S-Adenosyl methionine and presence of spermidine synthase. An interplay of both positive and negative feedback control with Putrescine serving as an activator and spermidine as a repressor.

We know that during normal embryogenesis cell grow and divide without restraining forces operating on them. These are the undifferentiated cells but gradually they become differentiated and they undergo a particular line of development as ectodermal, endodermal and
mesodermal tissues, the cells of which grow and divide with normal restraining forces operating on them. Normal cells can reach a steady state of growth which provides a balanced economy for the body as a whole. A tight control system in manifested over the growth factor and cell loss. Physiologic stimuli can increase in tissue growth but this growth ceases when the stimulus is taken off or a new steady state is achieved (Fakuda et al 1990, Teodori 1990).

It is well established that polyamines are required for the formation of nucleolus, oocyte maturation, rodent embryogenesis and for appropriate embryonic development (Fozard et al 1980). Polyamines have been found to be increased in renal and cardiac hypertrophy as well as in regenerating liver (Pegg 1981), different types of leukaemia (Banerjee et al 1998), Skin, breast and rectal carcinoma (Chanda and Ganguly, 1988), change in protein, DNA and RNA content in human breast, rectal carcinomatous tissue (Chanda and Ganguly, 1990), in human brain tumor (Chanda and Ganguly 1991), in human ovarian and cervical carcinoma (Chanda and Ganguly 1995), Putrescine and GABA profile in human breasts, rectal, cervical and ovarian carcinoma (Bandopadhyay and Ganguly, 1999, 2000 a,b; Bandopadhyay et al 1999). Phorbol diesters and other agents which help differentiation in human myelocytic leukaemia cells have been found to cause an increase in Putrescine and Spermidine levels suggesting that polyamines, play an important role in the process (Huberman et al 1981). Thus it is more or less established
that polyamines are essentially important in growing and multiplying cells and that they play an important role in prokaryotic cells and certainly that they are involved in growth stimulation in eukaryotic cells. Polyamines could be traced in important organelles such as ribosomes and it has been observed that these stimulate a number of processes involved in replication, transcription and translation which are essential for growth and multiplication. With a view to assess whether *Asparagus racemosus* & *Mucuna pruriens* treatment could cause growth and multiplication of cells in advanced ages estimation of these parameters in both the age groups studied under the experiment Table.

It is obvious therefore that the postponement of ageing following *Asparagus racemosus* and *Mucuna pruriens* treatment is possibly related to improvement of thyroid status, gonadal activities and reduction in coronary risks by controlling lipid profile.