Basic Medical Sciences

Research in basic medical sciences is carried out both in Council’s permanent institutes/centres as well as at various research institutions, medical colleges and universities in the country. The findings of studies carried out in the area of biochemistry, pharmacology, physiology and traditional medicine are highlighted.

BIOCHEMISTRY

Systemic lupus erythematosus (SLE) is an auto-immune disorder characterized by the production of anti-DNA antibodies. The origin of antibodies and the etiology of SLE is not yet known. There are many compounds (polyamines, histones and nuclear matrix elements) in the vicinity of DNA which may react upon irradiation. These polybasic molecules found in the cell have a great affinity for acidic constituents such as DNA and their interaction might play a role in the pathogenesis of SLE. A study has been sponsored by the Council at Aligarh Muslim University, Aligarh to characterize the photoconjugates between positively charged amino acids, DNA fragments and polynucleotides respectively. Calf thymus DNA fragments of about 200 base pair (bp) and poly-nucleotides were covalently crosslinked with lysine or arginine under UV light. Both lysine and arginine were found covalently photoconjugated to DNA and poly(dC) respectively, and resulted in the formation of crosslinks. The photoadducts were characterized by various physico-chemical methods. Nearly five lysine residues were found to be in the photobound state between one helical turn of DNA, while one arginine residue was present between four helical turns. Photoaddition of lysine and arginine to 200 bp DNA and to poly (dc) rendered them thermodynamically more stable than their native analogues. After systemic characterization of the 200 bp photoadducts, they were used as antigens for generation of antibodies in experimental animals. Both the lysine and arginine photoadducts of 200 bp were highly immunogenic in rabbits, inducing high titer antibodies. The induced antibodies were found to be non-precipitating in nature. A strong recognition of DNA - lysine photoadduct was observed with anti-DNA - autoantibodies found in the sera of patients with SLE. Poly(dC) - lysine was recognised more strongly than the DNA-lysine photoadduct. Poly(dC) - lysine appears to provide an immuno-dominant epitope(s) for SLE autoantibody recognition. The result suggests possible involvement of DNA-lysine photoadduct or similar modified structure(s) as a potential trigger for anti-DNA autoantibody production.

PHARMACOLOGY

Nicotine, a major component of cigarette smoke plays an important role in the development of cardiovascular disease and lung cancer in smokers. A study was conducted at University of Kerala, Thiruvananthapuram on the effects of antioxidants isolated from lemons and Allium species as compared to α-tocopherol on the damages induced by nicotine in rats. The results showed that the administration of nicotine in rats causes increase in lipid levels as well as lipid peroxidation products in serum and tissues. Simultaneous administration of garlic or onion oil or SACS (S-allylcysteine sulfoxide) from garlic, or SMCS (S-methylcystine sulfoxide) from onion, or hesperidine (from lemon) effectively counteracted the deleterious effects of nicotine administration. The antioxidant effects of these natural products are comparable to those of α-tocopherol. Administration of these antioxidants improved the natural antioxidant defence mechanisms of the body.

A project aimed to develop novel uterine relaxants by modification of isoxsuprine structure (by structure hybridisation of isoxsuprine with atenolol, metoprolol and acebutol), which would reduce tachycardia was undertaken at IRR, Mumbai. The physico-chemical properties necessary to achieve potent activity were also taken into consideration during the design of the compounds. Thus a series comprising eight compounds
was synthesized. The compounds were evaluated for uterine relaxant activity first in isolated rat uterus \textit{(in vitro)} and then in pregnant rats \textit{(in vivo)}. In pregnant rats, delay in onset of labour was used as a measure of uterine relaxant activity. The mechanism of action of these compounds was established by cAMP (3H) assay using homogenized uterine strips. Test compounds exhibited potent uterine relaxant activity both in \textit{in vitro} and \textit{in vivo} methods. No adverse effect on the pregnant rats or their pups was noted. They relaxed the uterus by stimulating \( \beta \)-adrenergic receptors which led to increased production of cAMP. When the compounds were evaluated for cardiac activity in isolated guinea pig atrium and on dog heart, they did not produce tachycardia and thus they were found safe when compared to isoxsuprine hydrochloride.

**PRE-CLINICAL TOXICOLOGY**

The Council has sanctioned a Pre-Clinical Toxicology Unit at NIN, Hyderabad. The Unit has carried out pre-clinical toxicology studies on alpha interferon of Shanta Biotech and initiated studies on DNA vaccine for rabies developed by IISC, Bangalore.

**CLINICAL PHARMACOLOGY**

Clinical Pharmacology Unit was set up at TRC, Chennai in collaboration with Institute of Pharmacology, Madras Medical College, Chennai. The Unit carried out dose related pharmacokinetic studies on rifampicin and ofloxacin. HPLC methodology for estimating rifampicin was standardized. Bioavailability studies of rifampicin and ofloxacin in plasma and ofloxacin in saliva are now being undertaken. The methods have been standardized and the Centre is recognised by Drug Controller of India for such studies. The protocols have been approved and already 5 companies have registered. Safety and efficacy studies and adverse drug reaction monitoring when rifampicin, isoniazid and pyrazinamide are administered alone or in combination would be undertaken besides pharmacokinetics of anti-TB drugs in diabetic patients and interactions of anti-TB drugs in children.

**PHYSIOLOGY**

A study was completed at AIIMS, New Delhi during the year to study the role of angiotensin converting enzyme (ACE) inhibitors in myocardial stunning. In open-chest pentobarbitone anaesthetised cats, the left anterior descending coronary artery was occluded for a period of 15 min followed by 60 min of reperfusion to produce myocardial stunning (MS). Saline/drug (Captopril, BK, receptor antagonist Hoe and Losartan) was administered 5 min before reperfusion. In the vehicle-treated group, the plasma renin activity was enhanced, following ischemia as well as reperfusion, signifying the involvement of renin angiotensin system in MS. Global hemodynamic function (mean arterial pressure, left ventricular-end-diastolic-pressure, peak (+) and (-) dP/dt was also depressed along with depletion of myocardial high energy phosphate compounds, rise in both plasma thiobarbituric acid reactive substances and creatine phosphokinase and depletion of endogenous myocardial antioxidants (glutathione, superoxide dismutase and catalase), strongly suggesting the involvement of oxidant stress in MS. The results showed that both Hoe-140 (200 mg/kg/over 2 min. \textit{iv}) and losartan (5 \( \mu \)g/kg/over 15 min. \textit{iv}) when administered alone effected a complete recovery of hemodynamic functions along with significant improvement in the metabolic status. The results of the present study indicated significant role played by the ACE independent pathway in the etiopathogenesis of MS.

To assess the impact of specific nutritional and psychological interventions on the performance of young sports persons, a community based controlled intervention study was undertaken at Sports Medicine Centre, West Bengal on a sample of 65 adolescent (10-14 yr) male, residential trainee footballers selected on the basis of physical, nutritional and sports performance levels and exposed to uniform training in soccer. Random allocation to different predesigned nutritional intervention schedules of high carbohydrate, high-protein, high-fat and balanced diet regimes was done for 17 weeks continuously except in control group. The existing diet was modified and supplemented as required. Half of the subjects in each group also received specific psychotherapeutic interventions. Pre and post-intervention assessment of performance level was done through field tests in physical fitness, motor performance and specific sports skills, along with selected laboratory parameters. Balanced diet had generalized improvement in agility. 35% fat-rich diet in late phase improved motor performance and sports skills. Carbohydrate-rich diet improved pulse rate and cardio-respiratory endurance along with other changes. Psychotherapeutic
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interventions caused improvement in psycho-laboratory and performance parameters, sports skill and motor performance.

**TRADITIONAL MEDICINE AND MEDICINAL PLANTS RESEARCH**

The multidisciplinary networking for the traditional medicine research programmes continued with central co-ordination. The national task force trials were continued on traditional remedies for diabetes. A Centre on Drug Development from Natural Products at CDRI, Lucknow and the Centre for Clinical Pharmacology in Traditional Medicine at Seth G.S. Medical College and K.E.M. Hospital, Mumbai were continued during the year under report. The Centre for Standardisation, Quality Control and Formulation of Natural Products/Selected Herbal Remedies at RRL, Jammu and Central Biostatistical Monitoring Unit at NIE, Chennai continued to give their support to the Programme. A Unit for Standardisation and Quality Control of Selected Herbal Remedies/Natural Products at National Institute of Pharmaceutical Education and Research, Chandigarh has been initiated.

During the year, a Clinical Pharmacology Unit was established at TRC, Chennai to study herbal immunomodulators and hepatoprotective drugs as adjunct to anti-tuberculosis drugs. A second Centre for Clinical Pharmacology has also been set up for studies on pharmacokinetics, bioavailability and herb-drug interactions at the BYL Nair Hospital, Mumbai. Cultivation of selected medicinal plants and agro-technology for endangered species of *Picrorhiza kurroa* has been taken up at the Institute of Himalayan Bioresource Technology, Palampur. The programme has an effective networking between institutions and other agencies like CSIR, DBT and Department of Indian Systems of Medicine and Homoeopathy to avoid duplication, strengthen infrastructure and improve expertise.

**Traditional Remedies for Diabetes Mellitus**

Multicentric, randomised, double blind clinical trials on *Vijaysar* (*Pterocarpus marsupium*) were completed at SCB Medical College, Cuttack, Madras Medical College, Chennai and Medical College, Kottayam. The study validated the findings that *Vijaysar*, although a safe alternative, required a higher dosage as compared to allopathic drug. A new study was initiated at the same centres to see if it was equally effective in treating patients with uncontrolled diabetics.

**Standardisation, Quality Control and Formulation of Natural Products/Selected Herbal Remedies**

The Centre for Advanced Research at the Regional Research Laboratory, Jammu continued studies on *Vijaysar*. Stability studies on *Vijaysar* capsules under natural weather conditions were continued. Samples of heartwood and aerial parts, mainly leaves of *P. marsupium* collected from different locations are being studied for active constituents. The LD50 data are being generated in two animal species (rat and mice). Studies on its effect on the cardiovascular system by *in vitro* and intraduodenal routes in anaesthetized animals and isolated tissues are also being undertaken. Modernization, optimisation and upscaling of the process of extraction of *Vijaysar* has commenced. Hypoglycaemic activity of VS-04 and VS-09 was confirmed in fasted and glucose loaded rats and experiments were carried out in the streptozotocin model. An activity patent application on VS-04 has been submitted and work on synthesis of VS-04 initiated. The water insoluble residue remaining at the end of the isolation process is being tested for hypoglycaemic activity.

*Varun* (*Crataeva nurvala*) is used for the treatment of urolithiasis. General pharmacological, acute and sub-acute toxicity and stability studies and chemical standardisation of its dried aqueous extract are being carried out. Pharmacological activity of marker compounds of aqueous extract against experimentally induced urolithiasis in rats is being studied.

A new mechanical device was developed to manufacture standardised *Kshaarasootra* (medicated) threads to facilitate technology transfer to the pharmaceutical industry.

**Drug Development From Natural Products**

The work carried out at the Advanced Centre at CDRI, Lucknow pertained to *Picrorhiza kurroa, Terminalia chebula* and *Centella asiatica*.

Dossier on Phase II multicentric trial on Picroliv, a product from *P. kurroa* was submitted to Drug Controller of India for approval to initiate Phase II trial. Stability studies on Sorensen buffer have also been carried out. HPLC fingerprint was developed and 27 compounds were
resolved. Pharmacokinetics with *in situ* absorption studies in recirculation model using rat intestinal loop and intraduodenal infusion were carried out.

Accelerated stability study of alcoholic extract (D003) and pure active marker of *T.chebula* showed that both the samples were stable for more than 2 years. Long-term stability study and chemical fingerprinting of D003 is being done. When compared to *Panax ginseng* it appeared to be superior. Effects on the cardiovascular and gastrointestinal systems and immunomodulatory functions were also studied.

Chemical fingerprinting and long-term stability study of *C.asiatica* is under progress. Wound healing activity was tested by observing the angiogenic effect, antioxidant profile, efficacy in immunocompromised wound and effect on DNA and protein content. Effect on learning and memory and anti-stress activity is being studied. Regulatory pharmacology studies are also being carried out.

**Clinical Pharmacology in Traditional Medicine**

At the Advanced Centre at Seth G.S. Medical College and K.E.M. Hospital, Mumbai, studies on neem oil for wound healing, mechanism of action of *Pterocarpus marsupium* and on a medicated oral rehydration solution were continued.

An in-house neem oil (Vranaropak) preparation based on Charaka’s methodology was standardised and its efficacy was compared to glycerine ichthammol and a commercial neem oil preparation. A randomised, observer blind, parallel group clinical trial was designed. Of the 7 patients recruited, 2 have completed the study. The study on angiogenesis using subcutaneous sponge model for blood flow measurement of technetium clearance in Wistar rats has continued.

The ingredients of oral rehydration solution viz. Ginger (*Zingiber officinalis*), Cumin (*Cuminum cyminum*) and Nagarmotha (*Cyperus rotundus*) strengthen the intestinal mucosal barrier. Standardization of ginger extract is being carried out. Experimental studies were done using *C.rotundus*. Red kidney beans were used as control which was compared to raw red kidney beans alone and in combination with *C. rotundus*. Bacterial colony count in the intestine and mesenteric lymph nodes came down with the latter. Difference in activity was seen between whole seed and powdered red kidney beans.

**Development of Standards of Therapeutically Important Indian Medicinal Plants**

Lack of documented evidence about the efficacy, toxicity and non-availability or inadequacy of standards for checking the quality of medicinal plants has been the major lacuna in the wider acceptance of herbal drugs. Despite there being a large number of single drugs and formulations in therapeutic use, very few concerted efforts have been made to develop standards. This has prevented modernization or modification of the methods of their preparation or production, as there is no way to establish the equivalence of the product made by the modified method with the original product. The standardized drugs of well defined consistent quality are needed for reliable clinical trials and therapeutic use.

A programme has been initiated to develop standards for 160 therapeutically important medicinal plants, in a period of 3 years employing sophisticated analytical techniques. It is planned to prepare monographs on them which would focus on phytochemistry, markers, their structure, pharmacology, toxicology, clinical aspects, safety, side effects/contraindications, if any, references and illustrations, besides covering other aspects like general description, synonyms, geographical sources and diagnostic description, *etc*.

The programme has been initiated at five institutions viz. Tropical Botanic Garden and Research Institute, Thiruvananthapuram; National Botanical Research Institute, Lucknow; RRL, Jammu; BV Patel Pharmaceutical Education and Research Development Centre, Ahmedabad and National Institute of Pharmaceutical Education and Research, Mohali.