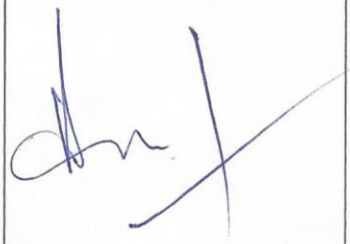
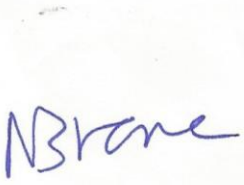
CLINICAL STUDY REPORT

ASSESS THE EFFICACY OF FUROSAP®, A TESTOSTERONE BOOSTER SUPPLEMENT, IN HUMAN VOLUNTEERS: *An Add-On Study*

Saroj Hospital & Maternity Centre, Lucknow

Investigational Product:	Furosap®
Indication:	Testosterone deficiency
Study design:	Open-labelled, single-armed, single-centric, observational study
Sponsor's Name:	Chemical Resources
Sponsor's Address:	3-A, Industrial Area Phase-II, Panchkula-134109, Haryana, India
Protocol Number:	CR-TEST-02/7-14
Study initiation date:	August 2014 (1st screening date)
Study completion date:	July, 2015 (last follow-up date)
Principal investigator:	Dr. Anuj Maheshwari, MD
Co-investigator:	Dr. Narsingh Verma, MD

SIGNATURE SHEET

For trial	Name & Qualifications	Address, Contact no. & Email ID	Signatures
Principal Investigator	Dr. Anuj Maheshwari, MD	**Address:** Saroj Hospital & Maternity Centre, Lucknow **Contact No.:** 09839133984 **Email:** dranuj.maheshwari@rediffmail.com	
Co-Investigator	Dr. Narsingh Verma, MD	**Address:** Saroj Hospital & Maternity Centre, Lucknow **Contact No.:** 09839064560 **Email:** narsinghverma@gmail.com	

1. SYNOPSIS/SUMMARY

Name of the company/ sponsor	Chemical Resources, 3-A, Industrial Area, Phase-II, Panchkula-134109, Haryana
Name of product	Furosap®
Name of the active ingredient	Protodioscin
Protocol number	CR-TEST-02/7-14
Title of study Assess the efficacy of Furosap®, A Testosterone booster supplement, in human volunteers: *An add-on study*	
Principal Investigator Dr. Anuj Maheshwari	**Co-Principal Investigator** Dr. Narsingh Verma
Study centre	Single centre
Study period	
Date of first enrolment	01-09-2014
Date of last enrolment	26-02-2015
Objectives	
Primary objective	• To assess the efficacy of Furosap®
Secondary objective	• To evaluate the percent of subjects responding to Furosap®. • To demonstrate the effect of Furosap® on safety parameters including cardiovascular functions. • To identify the effect on mood, mental alertness, reflex erection and overall performance.
Methodology	Open labelled, single arm, single centric, observational study
Number of subjects	50 subjects

Inclusion criteria	i. Agrees to written as well as audio-visual informed consent. ii. Ability to understand the risks/benefits of the protocol. iii. Male between 35-65 years of age. iv. Diagnosed with symptomatic hypogonadism.
Exclusion criteria	i. Uncooperative subjects ii. Impaired hepatic function indicated by SGOT/SGPT >2.5 times the upper limit of normal. iii. Patients suffering from CAD iv. Abnormal liver or kidney function tests (ALT or AST > 2 times the upper limit of normal; elevated creatinine, males > 125 µmol/L or 1.4mg/dl, females > 110 µmol/L or 1.2mg/dl) v. History of malignancy vi. History of hypersensitivity to any of the investigational drugs vii. Receiving any other testosterone booster therapy/medication/supplement within the last 2 months viii. History of coagulopathies ix. High alcohol intake (>2 standard drinks per day) x. History of psychiatric disorder that may impair the ability of subjects to provide written informed consent xi. Any medical condition, where the investigator feels participation in the study could be detrimental to the subjects overall well-being.
Duration of administration	12 weeks
Duration of study	6-8 months
Statistical methods	Data will be analyzed using appropriate parametric and non-parametric test.

Results

Efficacy Conclusions

1. Free testosterone levels were improved up to 46% in approx. 90% of the study population.
2. The frequency of sexual intercourse was also increased in the 98% of the study population.

3. 85.4% of the study population showed improvement in the sperm count.
4. The abnormal sperm morphology was also improved in the 14.6% of the study population.
5. All patients enrolled in the study showed improvement in mental alertness and their mood.
6. The reflex erection was also enhanced in the 95.2% of the study population.
7. The improvement in overall performance in all enrolled patients was observed.

Safety conclusions

On completion of study, following safety conclusions were made:

1. No significant change in serum liver function tests was observed.
2. No significant change in cholesterol levels was observed.
3. No significant change in hemogram was observed.

Conclusion

It can be concluded from the recorded results that the Furosap®, which is an herbal supplement prepared from fenugreek seeds extract, is effective and safe for testosterone deficient or hypogonadism patients.

Date of report:

2. TABLE OF CONTENTS

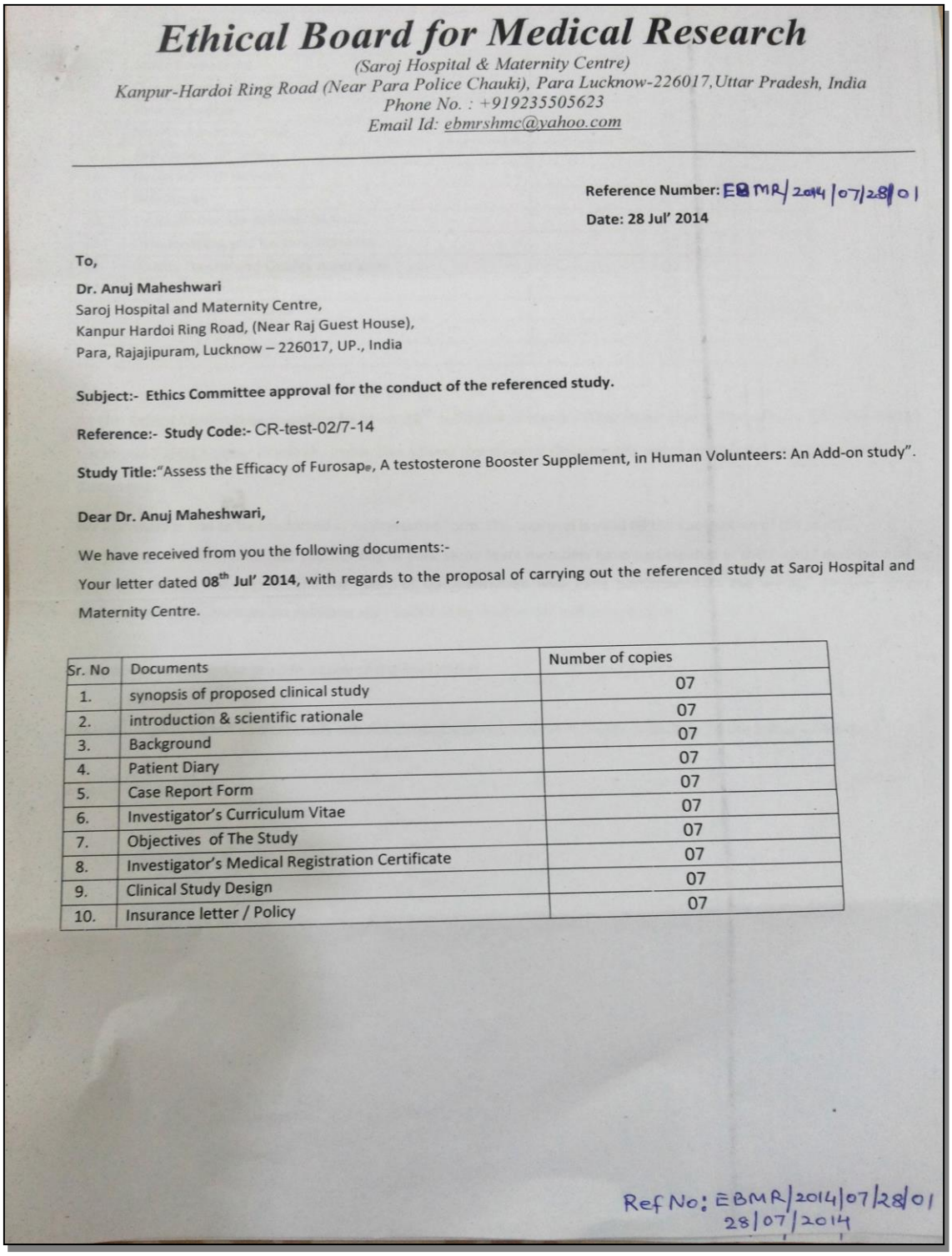
3. LIST OF ABBREVIATIONS

- **ADR** - Adverse Drug Reaction
- **AE** - Adverse Event
- **AST** - Aspartate Aminotransferase
- **ALP** - Alkaline Phosphatase
- **ALT** - Alanine Aminotransferase
- **BMI** - Body Mass Index
- **FBS** - Fasting Blood Sugar
- **CRC** - Clinical Research Coordinator
- **CO-PI** - Co-Principal Investigator
- **DBP** - Diastolic Blood Pressure
- **DCGI** - Drug Controller General of India
- **HDLC** - High-Density Lipoprotein Cholesterol
- **HDL** - High Density Lipoprotein
- **IEC** - Institutional Ethics Committee
- **IP** - Investigational Product
- **IRB** - Institutional Review Board
- **LDL** - Low Density Lipoprotein
- **PI** - Principal Investigator
- **SAE** - Serious Adverse Event
- **SBP** - Systolic Blood Pressure
- **TC** - Total Count
- **TG** - Triglycerides
- **TT** - Total Testosterone
- **VLDL** - Very Low Density Lipoprotein

4. ETHICS

- **Independent Ethics Committee (IEC) or Institutional Review Board (IRB)**

  The present study was duly approved by independent ethics committee named as Ethical Board for Medical Research, Lucknow.



Ethical Board for Medical Research

(Saroj Hospital & Maternity Centre)
Kanpur-Hardoi Ring Road (Near Para Police Chauki), Para Lucknow-226017, Uttar Pradesh, India
Phone No. : +919235505623
Email Id: ebmrshmc@yahoo.com

Reference Number: EBMR/2014/07/28/01
Date: 28 Jul' 2014

To,

Dr. Anuj Maheshwari
Saroj Hospital and Maternity Centre,
Kanpur Hardoi Ring Road, (Near Raj Guest House),
Para, Rajajipuram, Lucknow – 226017, UP., India

Subject:- Ethics Committee approval for the conduct of the referenced study.

Reference:- Study Code:- CR-test-02/7-14

Study Title:"Assess the Efficacy of Furosap®, A testosterone Booster Supplement, in Human Volunteers: An Add-on study".

Dear Dr. Anuj Maheshwari,

We have received from you the following documents:-

Your letter dated **08th Jul' 2014**, with regards to the proposal of carrying out the referenced study at Saroj Hospital and Maternity Centre.

Sr. No	Documents	Number of copies
1.	synopsis of proposed clinical study	07
2.	introduction & scientific rationale	07
3.	Background	07
4.	Patient Diary	07
5.	Case Report Form	07
6.	Investigator's Curriculum Vitae	07
7.	Objectives of The Study	07
8.	Investigator's Medical Registration Certificate	07
9.	Clinical Study Design	07
10.	Insurance letter / Policy	07

Ref No: EBMR/2014/07/28/01
28/07/2014

11.	Study Population	07
12.	Study Agent	07
13.	Study Evaluations	07
14.	Clinical Study Agreement (Draft Version)	07
15.	Study Schedule	07
16.	No of subjects And visit	07
17.	Assessment Of Safety	07
18.	Declaration of Helsinki	07
19.	References	07
20.	Ethics/Protection of Study Subjects	07
21.	Data Handling and Record retention	07
22.	Quality control and Quality Assurance	07
23.	Audit And Inspections	07
24.	Financing And Insurance	07
25.	Publications policy	07
26.	Scientific References	07

At the **Ethics Committee** meeting held on **22th Jul'2014** at Hardoi Ring Road (Near Para Police Chauki), Para Lucknow-226017,Uttar Pradesh, India, the above mentioned documents were examined, reviewed and discussed.

We approve the trial to be conducted in its presented form. The approval is valid till the completion of the study.

We hereby confirm that neither you nor any of your study team members have participated in the voting/ decision making procedure of the committee. The members of the committee who have participated in the voting/ decision making procedure of the committee do not have any conflict of interest in the referenced study.

Also you are requested to provide a copy of the final report.

Decision:-Approved but Clinical study can not started without Insurance Policy submitted to the Ethics Committee.

Yours sincerely,

Mr. R S Kanausia

(Member Secretary)

MEMBER SECRETARY

Ethical Board for Medical Research
Lucknow

Ref No: EBMR/2014/07/28/01
28/07/2014

DCGI Approved Ethics Committee

File No. ECR/437/Ethical/Inst/UP/2013
Directorate General of Health Services
Office of Drugs Controller General (India)

FDA Bhawan, Kotla Road,
New Delhi – 110 002
Dated: 7/2/2014

To,
The Chairman,
Ethics Committee,
Ethical Board for Medical Research,
Saroj Hospital & Maternity Centre,
Kanpur – Hardoi Ring Road (Near Para Police Chauki),
Para, Lucknow – 226 017,
Uttar Pradesh, India.

SUB: - Ethics Committee Registration No. **ECR/528/Inst/UP/2014** issued under Rule 122DD of the Drugs & Cosmetics Rules1945.

Dear Sir/ Madam,

Please refer to your application no. Nil dated 22.03.2013 submitted to this office for the Registration of Ethics Committee.

Based on the documents submitted by you, this office hereby registers **ETHICS COMMITTEE, ETHICAL BOARD FOR MEDICAL RESEARCH, SAROJ HOSPITAL & MATERNITY CENTRE** situated at **KANPUR – HARDOI RING ROAD (NEAR PARA POLICE CHAUKI), PARA, LUCKNOW – 226 017, UTTAR PRADESH, INDIA** with Registration number **ECR/528/Inst/UP/2014** as per the provisions of Rule 122DD of the Drugs and Cosmetics Rules, 1945 subject to the following conditions:

1. This Registration is subject to the conditions specified under Rule 122DD and Appendix VIII of Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945.
2. The Ethics Committee shall review and accord its approval to a clinical trial at appropriate intervals as specified in Schedule Y and the Good Clinical Practice Guidelines for Clinical Trials in India and other applicable regulatory requirements for safeguarding the rights, safety and well-being of the trial subjects.
3. In the case of any serious adverse event occurring to the clinical trial subjects during the clinical trial, the Ethics Committee shall analyze and forward its opinion as per procedures specified under APPENDIX XII of Schedule Y.
4. The Ethics Committee shall allow inspectors or officials authorized by the Central Drugs Standard Control Organization to enter its premises to inspect any record, data or any document related to clinical trial and provide adequate replies to any query raised by such inspectors or officials, as the case may be, in relation to the conduct of clinical trial.
5. The licensing authority shall be informed in writing in case of any change in the membership or the constitution of the ethics committee takes place.
6. All the records of the ethics committee shall be safely maintained after the completion or termination of the study for not less than five years from the date of completion or termination of the trial (Both in hard and soft copies).
7. If the Ethics Committee fails to comply with any of the conditions of registration, the Licensing Authority may, after giving an opportunity to show cause why such an order should not be passed, by an order in writing stating the reasons therefore, suspend or cancel the registration of the Ethics Committee for such period as considered necessary.

8. This registration shall be in force for a period of three years from the date of issue, unless it is sooner suspended or cancelled.

9. Ethics Committee shall consist of not less than seven members and is subject to a maximum of 15. One among its members, who is from outside the institute, shall be appointed as chairman, one member as a Member Secretary and rest of the members shall be from Medical, Scientific , Non-Medical and Non-scientific fields including lay public

10. The committee shall include at least one member whose primary area of interest or specialization is Non-scientific and at least one member who is independent of the institution besides; there should be appropriate gender representation on the Ethics Committee.

11. The Ethics committee can have as its members, individuals from other Institutions or Communities, if required.

12. Members should be conversant with the provisions of clinical trials under Schedule Y, Good Clinical Practice Guidelines for clinical trials in India and other regulatory requirements to safeguard the rights, safety and well-being of the trial subjects.

13. For review of each protocol the quorum of Ethics Committee shall be at least five members with the following representations:
    I. Basic medical scientist (preferably one pharmacologist)
    II. Clinician
    III. Legal expert
    IV. Social scientist or representative of non-governmental voluntary agency or philosopher or ethicist or theologian or a similar person.
    V. Lay person from community

14. The members representing medical scientist and clinicians should have Post graduate qualification and adequate experience in their respective fields and aware of their role and responsibilities as committee members.

15. As far as possible, based on the requirement of research area such as HIV, Genetic disorder, etc., specific patient group may also be represented in the Ethics Committee.

16. There should be no conflict of interest. The members shall voluntarily withdraw from the Ethics Committee meeting while making a decision on an application which evokes a conflict of interest which may be indicated in writing to the Chairman prior to the review and be recorded so in the minutes. All members shall sign a declaration on conflict of interest.

17. Subject experts or other experts may be invited to the meetings for their advice. But no such expert shall have voting rights.

18. This certificate is issued to you on the basis of declaration/submission by you that yours is an Institution and registration is sought for Institutional Ethics Committee.

7/2/2014

(A. Visala)
Deputy Drugs Controller (I) & Licensing Authority

A. Visala
Deputy Drugs Controller (I)
Dte. General of Health Services
Central Drugs Standard Control Organisation
FDA Bhawan, Kotla Road, New Delhi-110002

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- **Ethical conduct of the study**

The study was performed in compliance and accordance with ICH guidelines for Good Clinical Practices (GCP), including the archiving of essential documents, and per

international ethical standards guaranteed by the Declaration of Helsinki and its subsequent amendments.

Patient confidentiality was maintained throughout the study.

- **Patient information and consent**

  All subjects for the study were provided a consent form and provided sufficient information for subjects to make an informed decision about their participation in this study. This consent form was submitted with the protocol for review and approval by IEC for the study. The formal consent of a subject using the IEC-approved consent form was obtained before the subject is submitted to any study procedure. Consent form was signed by the subject or legally accepted representative and the investigator-designated research professional obtained the consent.

5. INTRODUCTION

Testosterone is a hormone produced by the testicles and is responsible for the proper development of male sexual characteristics. Testosterone is also important for maintaining muscle bulk, adequate levels of red blood cells, bone growth, sense of well being and sexual function. An estimated 25% of men have low levels of testosterone. Besides involving in the development and maintenance of male characteristics and male sexual organs, testosterone has also effects on other body organs such as brain, muscle, kidney, bone, liver and skin [1].

According to the recommendations of the International Society of Andrology (ISA), the International Society for the Study of Aging Male (ISSAM), the European Association of Urology (EAU), the European Academy of Andrology (EAA) and the American Society of Andrology (ASA), testosterone deficiency syndrome (TDS) is a clinical and biochemical syndrome which is associated with advancing age and it is characterised by symptoms such as low libido, increased fat mass, decreased muscle mass, loss of concentration, erectile dysfunction (ED), depression, decreased bone mineral density and by the deficiency in serum testosterone levels [2] [3].

Testosterone circulates in plasma non-specifically bound to albumin and specifically bound to sex hormone-binding globulin (SHBG) and rest of the testosterone remains in unbound form known as free testosterone. Binding proteins in the body fluids can act as a storage form for steroids that have high rate of metabolism during passage of blood through the liver. Although the free and non-specifically bound hormone fraction in plasma (commonly referred to as the bio-available fraction) is the hormone available at the cellular level in specific tissues where partial dissociation of the specific steroid-protein complex may occur and inactive pro-hormones may be converted intra-cellularly to active hormones. There is good evidence that this fraction reflects more accurately the clinical situation than the total hormone levels in plasma. Thus, during evaluation of testosterone levels in the body, free testosterone levels are more preferred. It has also been observed in testosterone replacement therapy in elderly males that replacement with very low free testosterone levels not only improves the mental well-being and social interactions but more importantly significant improvement of cerebral perfusion in selected areas of the CNS involved in emotional behaviour, general arousal reaction and wakefulness also takes place which could be caused by selective responsiveness of these areas to androgen. In healthy males, serum levels of free testosterone and of non SHBG-bound or so called “bioavailable” testosterone (*i.e.* the sum of the free fraction and the

fraction loosely bound to albumin) decrease by as much as 50% between the age of 25 and 75 years. The sharper decline of these fractions in comparison with total testosterone is explained by an age-associated increase of sex hormone binding globulin (SHBG) concentrations. The levels of total and free testosterone are correlated with other parameters of the body too. Total and free testosterone concentrations are negatively correlated with waist/hip circumference ratio and visceral fat area and negatively associated with increased glucose, insulin, and C-peptide concentrations also. Free testosterone levels are also found to have inverse association with coronary artery disease [4, 5, and 6].

Prevalence of testosterone deficiency or hypogonadism

The overall prevalence of hypogonadism or testosterone deficiency is high and differs according to the following given criteria in Table 1 [7, 8, 9, and 10]:

Table 1: Prevalence of hypogonadism or testosterone deficiency (Criteria-wise)

S. No.	Criteria	Prevalence
i.	Hypogonadism in males (HIM) study	Crude prevalence 38.7% in men ≥45 years (Total testosterone <300ng/mL)
ii.	Baltimore Longitudinal study of Aging (BLSA)	Prevalence of 12%, 19%, 28% and 49% in men in their 50s, 60s, 70s and 80s respectively (Total testosterone <325 ng/dL)
iii.	Boston Area Community Health (BACH) study	Prevalence of Androgen deficiency of 5.6% among men aged 30-79 years
iv.	Massachusetts Male Aging Study (MMAS)	Prevalence of hypogonadism of 12.3% among men aged 40-70 years (Total testosterone <200 ng/dL)

According to the demographic data it has been clearly demonstrated that the percentage of older men with testosterone deficiency is increasing progressively, supporting the concept that serum testosterone levels decline regularly and gradually with age. There is a significant percentage of men aged more than 60 years with testosterone levels below the lower limits for young adult men [11, 12].

Country-wise prevalence of hypogonadism

It has been seen that the prevalence of hypogonadism varies country-wise along with the parameter of age. The data given below in Table 2 represents the prevalence of this disease in USA, European countries and other Asian countries [13].

Table 2: Prevalence of hypogonadism or testosterone deficiency (country-wise)

S. No.	Country/Region	Prevalence (Total testosterone value)
i.	Germany	12.8% (TT< 300 ng/dL)
ii.	USA	5.6% (TT < 200 ng/dL)
iii.	European Countries	2.1% (TT < 317 ng/dL)
iv.	Australia	31.2% (TT < 350 ng/dL)
v.	Malaysia	19.1% (TT < 317 ng/dL)
vi.	China	9.52% (TT 200–400 ng/dL)
vii.	Brazil	19.8% (TT < 300 ng/dL)
viii.	India	24.2% (TT < 300 ng/dL)
ix.	Chile	28.1% (TT <198.4 ng/dL)

Associated Diseases

Untreated hypogonadal middle-aged men exhibit a high prevalence of cardio-metabolic risk factors that are correlated to total testosterone levels. This suggests that testosterone deficiency is associated with adverse medical conditions that create serious health risks, especially in younger age. A cross-sectional cohort study among 434 consecutive male patients aged 50–86 year demonstrated that the prevalence of psychosomatic symptoms and metabolic risk factors accumulated with decreasing testosterone levels. Testosterone deficiency is more common in men with certain diseased states including obesity, diabetes, hypertension, hyperlipidemia, asthma, chronic obstructive pulmonary disease (COPD) and prostatic disease. The study conducted under TIMES2 (Testosterone replacement in hypogonadal men with either metabolic syndrome or type 2 diabetes) showed that testosterone replacement therapy (TRT) improved glycemic control, lipid levels, sexual function and libido in men with type 2 diabetes and metabolic syndrome with no corresponding increase in adverse events [14] [15] [16].

Testosterone and diabetes

Hyperinsulinemia is shown to suppress serum testosterone levels. Testosterone levels are reduced in men with type 2 diabetes mellitus with an inverse association between testosterone levels and glycosylated hemoglobin (HbA_{1c}). In men with low plasma testosterone, the possibility of type 2 diabetes mellitus is increased and prospective studies have shown that low testosterone levels causes development of type 2 diabetes mellitus in men. Low levels of testosterone are associated with a decreased lean body mass and relative muscle mass is inversely associated with insulin resistance and prediabetes. Epidemiological evidence from several longitudinal population studies shows that low testosterone is an independent risk factor for the development of both metabolic syndrome and type 2 diabetes mellitus. Prospective studies have shown that men with higher testosterone levels (range, 449.6-605.2 ng/dL) have 42% lower risk of type 2 diabetes mellitus (RR, 0.58, 95% CI, and 0.39 to 0.87). Reduced levels of total testosterone and sex hormone binding globulin (SHBG) (associated with IR) are both independent risk factors in middle-aged men who later develop type 2 diabetes mellitus [17, 18].

Testosterone and obesity

Testosterone is an important signalling molecule in regulating energy utilization and multiple cellular metabolic pathways, including nitrogen retention and regulation of adipogenesis. Considerable evidence exists with regard to the role of testosterones in increasing and maintaining muscle mass and reducing fat mass and therefore regulation of body composition. This suggests that testosterone deficiency may contribute to the etiology of obesity and, in case of hypogonadism, testosterone treatment may turn out to be beneficial in managing obesity, also in combination with exercise and diet. Hyperinsulinism is associated with adiposity which suppresses synthesis of SHBG and thus, levels of circulating testosterone. In addition, insulin and leptin exert suppressive effects on testicular steroidogenesis and may contribute to further disruption of pulse amplitude of luteinizing hormone (LH) diminishing stimulation of the testicular steroidogenesis. Further, conversion of testosterone to estradiol in adipose tissue resulting in elevated serum estradiol, may contribute to inhibition of androgen biosynthesis via central feedback mechanism involving the hypothalamus-pituitary gonadal axis. It is well recognized that adipocytes secrete a host of adipokines that regulate a variety of metabolic processes in endocrine, paracrine and autocrine fashion. Thus, adipocytokines, secreted by visceral fat modulate the hypothalamo-pituitary-testicular axis and inhibit

testosterone production. Modulation of GnRH secretion by Kisspeptins produced by adipose tissue causes significant lowering circulating levels of testosterone. Thus, this circle results in which metabolic syndrome suppresses testosterone biosynthesis and conversely, reduced testosterone concentrations predispose and contribute to the onset of development of metabolic syndrome and in turn results in obesity [19].

Testosterone and anaemia

A clinical study showed that hypogonadism may be an additional cause of anaemia and reduced ESA (erythropoiesis-stimulated agents) responsiveness in men with chronic kidney disease (CKD). This study was conducted in ESA-naive male patients with anaemia who presented lower testosterone values. According to their study, testosterone deficient patients are 5.3 times more likely to be anaemic than testosterone sufficient patients. It has been hypothesized that testosterone stimulates erythropoiesis via production of hematopoietic growth factors and possible improvement of iron bioavailability. Testosterone deficiency predisposes to anaemia and reduces responsiveness to erythropoiesis-stimulating agents (ESA) [20].

Testosterone and cardiovascular disease (CVD)

Low testosterone predicts mortality from CVD but is not associated with death from other causes. Low free testosterone and higher SHBG & LH levels are associated with all-cause mortality. In cause specific analysis, lower free testosterone and higher LH predicts CVD mortality while higher SHBG predicts non-CVD mortality. Prevention of androgen deficiency might improve cardiovascular outcomes but is unlikely to affect longevity otherwise [21].

Treatment of testosterone deficiency

The metabolic syndrome is a cluster of co-morbidities and is associated with an increased cardiovascular risk. It is often found in obese patients and insulin resistance plays a key role in its pathogenesis. A number of testosterone replacement therapy (TRT) preparations are currently available in the US market. Intramuscular injections of short-acting testosterone derivatives achieve good serum concentrations within 2-3 days with levels returning to baseline in most men by 2 weeks, resulting in an injection schedule of 1-2 weeks. Topical gels or patches provide a more stable serum-testosterone concentration over time than injections. Patches currently available in the US are associated with a high rate of skin reaction and their use has been largely replaced by T gels (Testosterone gels). The main disadvantages of T gels are cost and a black box warning concerning transfer potential to women and children. Thus, larger long-term

studies are needed to evaluate the advantages of testosterone treatment in hypogonadal men with diabetes or metabolic syndrome. Testosterone administration should favour formulations that are capable of maintaining stable physiological levels of testosterone over time [22].

The herbal formulation used in the present study named as "Furosap®" has been examined for its effectiveness in testosterone deficient subjects. It has been chosen because of the components comprising this herbal formulation extracted from herb known as *Trigonella foenum-graecum* which is also known as Fenugreek.

Rationale for choosing FUROSAP® in the study

FUROSAP® is an innovative product made through a novel patented process, involving physical separation of active ingredients from the seeds of Fenugreek herb (*Trigonella foenum-graecum*) without affecting the chemical properties of the active fractions. It is a natural and promising dietary supplement. Its dietary supplement comprises Protodioscin as the major fraction which has been isolated from fenugreek seeds. This component helps to boost the testosterone levels via stimulating pituitary gland and improves the medical state of patients suffering from hypogonadism or testosterone deficiency. This was the underline reason to choose Fenugreek seed extract *i.e.* Furosap® for evaluating the objectives of present study.

6. AIMS AND OBJECTIVES

The aim of the study was to evaluate the effect of a testosterone booster supplement *i.e.* Furosap® in humans suffering from hypogonadism which was achieved by considering following given objectives:

Objectives

- To assess the efficacy of FUROSAP®
- To evaluate the percent of subjects responding to FUROSAP®
- To demonstrate the effect of FUROSAP® on safety parameters including cardiovascular functions
- To identify the effect on mood, mental alertness, reflex erection and overall performance

End Points

- Improvement in testosterone levels.
- Improvement in sperm profile.
- Improvement in mood, mental alertness, reflex erection and overall performance.
- No abnormal changes in lipid profile.
- No abnormal changes in liver function test.

7. INVESTIGATIONAL PLAN

Study Design

This was an observational, open labelled and single armed study. This study was conducted at Saroj Hospital & Maternity Centre situated on Kanpur Hardoi Ring Road, Para, Rajajipuram, Lucknow – 226017, UP, India.

Study Population

1. Eligible age for study: Between 35 to 65 years
2. Gender eligible for study: Male
3. Accepts Healthy Volunteers: No

Inclusion Criteria

1. Agrees to written as well as audio-visual informed consent.
2. Ability to understand the risks/benefits of the protocol
3. Male between 35-65 years of age.
4. Diagnosed with Symptomatic hypogonadism

Exclusion Criteria

1. Uncooperative Subjects
2. Impaired hepatic function indicated by SGOT/SGPT >2.5 times the upper limit of normal.
3. Abnormal liver or kidney function tests (ALT or AST > 2 times the upper limit of normal; elevated creatinine, males > 125 µmol/L or 1.4mg/dL, females > 110 µmol/L or 1.2mg/dL)
4. Patients suffering from CAD
5. History of malignancy
6. History of hypersensitivity to any of the investigational drugs
7. Receiving any other testosterone booster therapy/medication/supplement within the last 2 months
8. History of coagulopathies
9. High alcohol intake (>2 standard drinks per day)
10. History of psychiatric disorder that may impair the ability of subjects to provide written informed consent
11. Any medical condition, where the investigator feels participation in the study could be detrimental to the subjects overall well-being.

Stopping Rules

The criteria for the "stopping" of trial or "discontinuation criteria" was only in the case of Serious Adverse Event (as defined in safety assessments clause).

8. TREATMENTS

Screening and Treatment of the Subjects

The subjects were screened for the clinical study on the basis of above given inclusion/exclusion criteria. Allocation of the product was done after screening and enrolment of study subjects. The subjects were followed up after 4 weeks, 8 weeks and 12 weeks. Safety was assessed at each follow-up visit. Subjects complaining of significant symptoms following administration of investigational product were planned to be evaluated for objective parameters of adverse drug reactions. Investigational product was considered to be discontinued in case of any serious adverse drug reaction and subjects were pre-planned to be managed according to the clinical condition.

Investigational Product

- **Product name**

  Furosap®

- **Formulation**

  Each capsule contained 500mg of investigational product to be taken orally per day

- **Packaging**

  Each pack contained 30 capsules

- **Storage**

  The investigational product was instructed to be stored at room temperature in cool and dark place and should be protected from direct sunlight.

- **Accountability procedure**

  Allocation of the product was done by the site staff only. Distribution of the product was maintained in the IP accountability log provided by the sponsor to the site staff. Each entry was maintained separately with the date/signature of the principal investigator & study coordinator. The person responsible for the distribution of the product had also signed on the IP accountability log. The accountability log must be produced by principal investigator or study coordinator at the time of audit.

- **Concomitant medication**

  All concomitant prescription medications taken during study participation were recorded on the case report forms (CRFs). Medications to be reported in the CRF were concomitant prescription medications, over-the-counter medications (OTC) and non-prescription medications taken at the time of adverse events (all grades) too.

9. EFFICACY EVALUATION

Assessment of Efficacy

- Scoring chart evaluation (Evaluation of mental alertness, mood, reflex erection & overall performance) (Annexure I)
- Frequency of sexual intercourse evaluation

The following laboratory investigations were also done after intervals along with the above given evaluations:

i. At Baseline

- BMI
- Free testosterone
- Total testosterone
- DHEA-S levels
- Fasting blood sugar
- Fasting lipid profile (TC, LDL, HDL, TG, VLDL)
- Liver function test (AST, ALT, ALP)
- Haemogram (Using routine methods)
- Semen examination (Sperm count, sperm mobility, sperm morphology)

ii. Follow-up Month (1st and 2nd)

- BMI
- Semen examination (Sperm count, sperm mobility, sperm morphology)
- Fasting lipid profile (TC, LDL, HDL, TG, VLDL)

iii. End of study (3rd Month)

- BMI
- Free testosterone
- Total testosterone
- DHEA-S levels
- Fasting blood sugar
- Fasting lipid profile (TC, LDL, HDL, TG, VLDL)
- Liver function test (AST, ALT, ALP)
- Haemogram (Using routine methods)
- Semen examination (Sperm count, sperm mobility, sperm morphology)

10. SAFETY EVALUATION

Assessment of Safety

Safety was assessed at each follow-up visit. Investigational product was planned to be discontinued in case of any serious adverse reaction followed by management of patient according to the clinical condition.

Definitions

Adverse Reaction

WHO technical report no 498 (1972) "a response to a drug which is noxious and unintended, and which occurs at normal doses normally used in man for prophylaxis, diagnosis, or therapy of a diseases, or for the modification of physiological function"

"All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions"

Adverse Events / Adverse Experience

Any untoward medical occurrence that may present during the clinical study with the product at the same time does not necessarily have a causal relationship with this treatment.

"Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment"

Serious Adverse Event or Reaction

A serious adverse event or reaction is any untoward medical occurrence that at any dose:

- Results in death
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is life-threatening
- Congenital anomaly/birth defect.

Reporting of Adverse Events

At the time of screening of subjects any clinical abnormality should be noted as All Ready Exist (ARE) condition in the CRF's. The adverse event will be considered only if it matches the above defined criteria or any new clinical finding in the subjects or abnormal laboratory values, not to ARE conditions.

All the adverse events should be reported as mentioned in the schedule Y of the drug and cosmetics act, and rules 1940.

Reporting to Sponsor

All the serious adverse events will be reported by the principle investigator to the sponsor via phone call with in 24hrs and details of clinical findings at the time of SAE's will be sent to sponsor via fax within 24hrs.

Reporting to EC

All the serious adverse events will be reported by the principle investigator to the EC within 7 working Days.

Ethical Justification

The investigational product to be used in this study is already available in the market and has been used by Indian consumers for several years. The Investigators do not foresee any safety issues related to the study intervention as the same amount of investigational product will be replaced. Moreover there was no additional financial burden on the study participants as the cost of the study and procedures will be borne by the sponsor. In case of development of untoward medical incident related to the investigational product the investigators and the sponsor will take responsibility for the management.

11. RESULTS

A. Demography

Table 3: Patient demography data

	Mean ± Standard Deviation	Minimum	Maximum
Age (Years)	43.08±7.35	35.00	61.00
Height (cm)	166.16±4.93	151.00	176.00
Weight (Kg)	70.38±12.18	27.40	91.60
BMI	25.46±4.13	10.98	31.90
SBP (mmHg)	124.00±9.40	104.00	160.00
DBP (mmHg)	79.65±6.53	64.00	90.00
Pulse (/Min)	77.53±6.40	56.00	96.00

a. Age

The study population consisted of subjects ranging from 35-65 years of age. The minimum age of the patient enrolled was 35 years and maximum age of the patient was 61 years. The mean age of the study population was 43.08 years as shown below in Fig. 1.

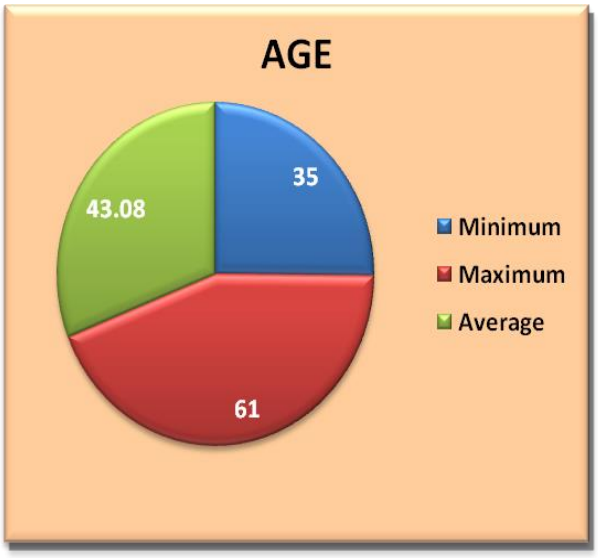


Fig. 1: Patient Age

b. Height, Weight & BMI

The average height and weight of the study subjects were 166.16 cm and 70.38 Kg respectively as shown below in Fig. 2 & Fig. 3. The average BMI of the study population

was recorded to be 25.46kg/m^2. The minimum BMI was 10.98 kg/m^2 and maximum was 31.9 kg/m^2 in the study subjects as shown in Fig. 4.

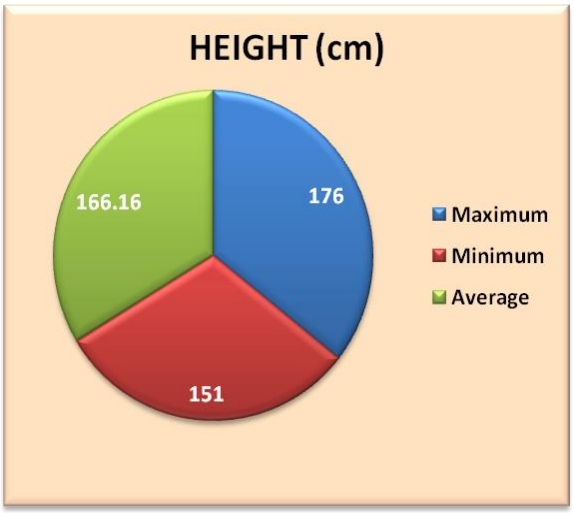


Fig. 2: Patient Height

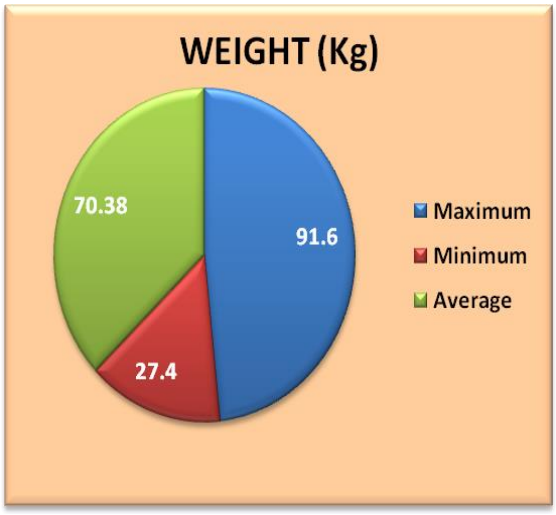


Fig. 3: Patient Weight

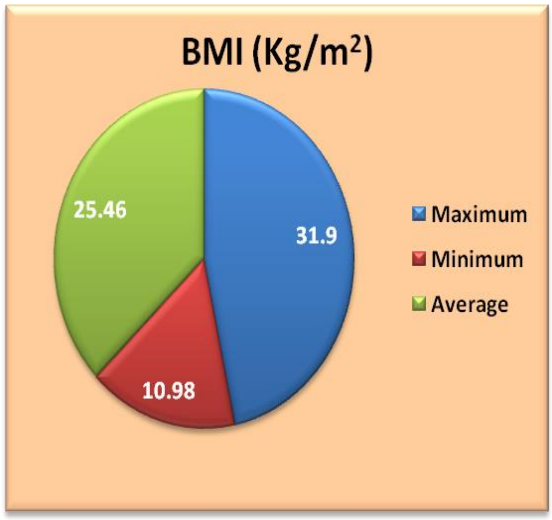


Fig. 4: Patient BMI

c. Blood Pressure & Pulse Rate

The mean systolic blood pressure and diastolic blood pressure was 116.78 mmHg and 75.32 mmHg, respectively in the study subjects as shown in Fig. 5 & 6. The mean pulse rate of the study subjects was 77.53/min. The minimum pulse rate was 56/min and maximum was 96/min in study subjects as shown in Fig. 7.

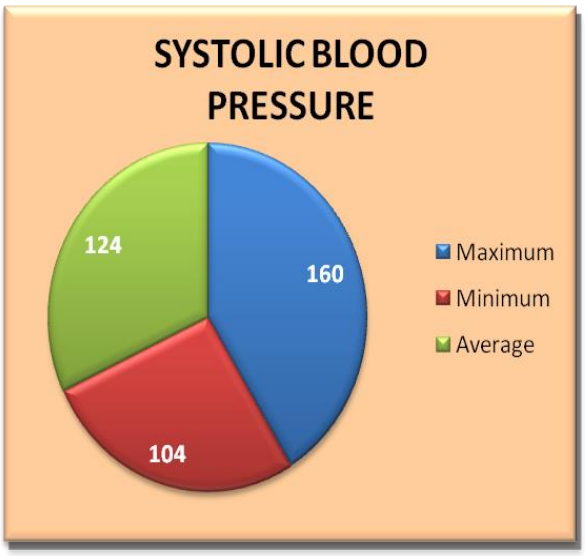


Fig. 5: Systolic Blood Pressure

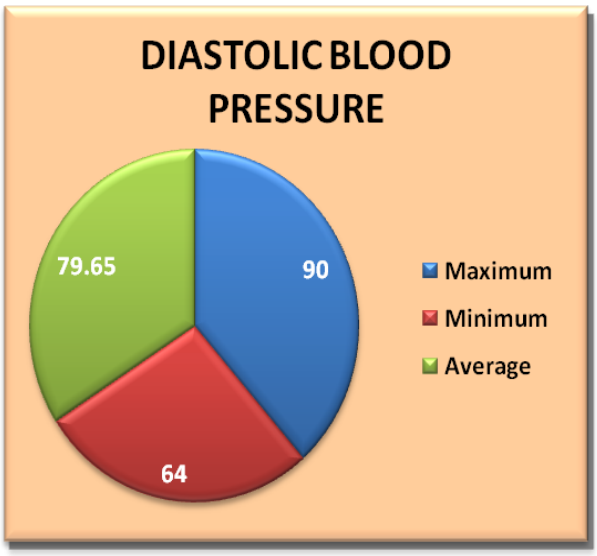


Fig. 6: Diastolic Blood Pressure

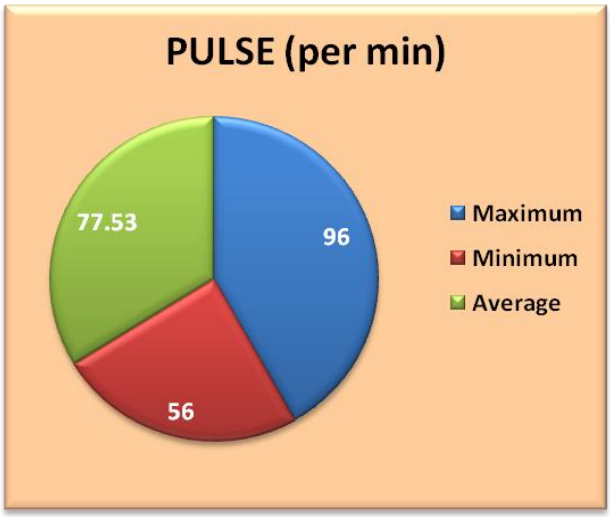


Fig. 7: Pulse Rate

B. Efficacy data

a. Free testosterone

The study subjects have shown improvements in the levels of free testosterone till 12th week of the study as compared to baseline data as shown in Fig. 8. The mean free testosterone level at baseline was 8.171pg/mL and the level was raised to 11.97pg/mL on the completion of study after 12 weeks. Approx. 46% increase in the free testosterone levels was observed in the study subjects as compared to baseline levels. The increase in free testosterone levels was statistically significant (p-value 0.000) (Table 4).

Approx. 90% of the study population showed improvements in free testosterone levels on completion of treatment.

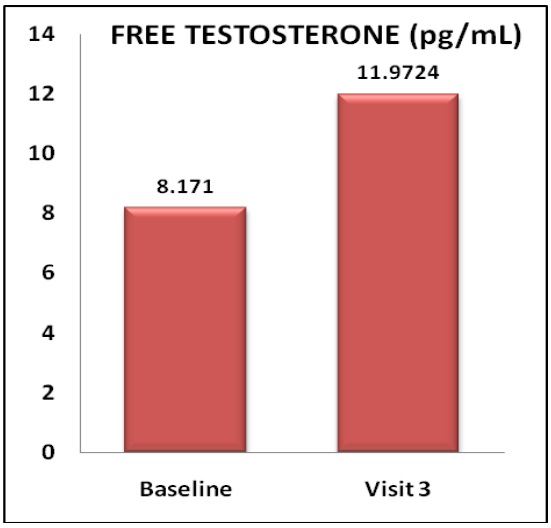


Fig. 8: Free testosterone data

Table 4: Statistical data of free testosterone levels

FREE TESTOSTERONE		Mean ± Std. Deviation	p-value
Pair 1	Baseline	8.17±5.044	.000**
	On Completion	11.97±5.653	

b. Total testosterone

The total testosterone levels were shown to be improved in the study subjects till 12th week of the study as compared to baseline levels. The initial mean total testosterone level was 405.19mg/dL which was raised to 436.33mg/dL on completion of the study as shown in Fig. 9. These observations were statistically non-significant (p-value 0.164).

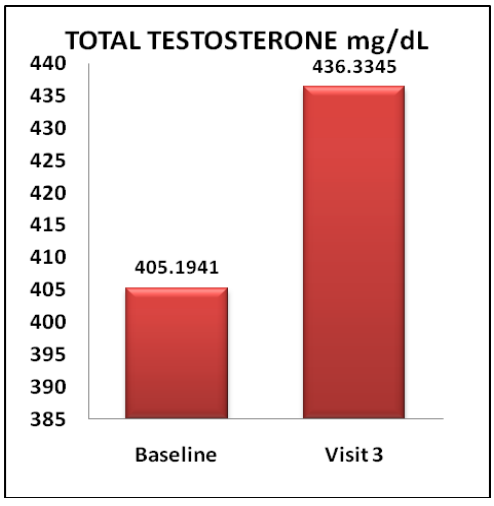
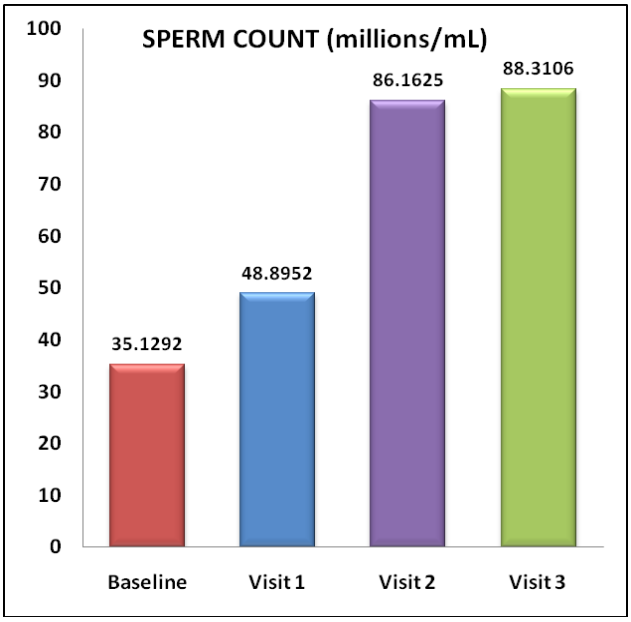


Fig. 9: Total Testosterone

c. Sperm count

The study population has shown increase in sperm count till the completion of the study as compared to baseline levels as shown in Fig. 10. The increase in sperm count was statistically significant (p-value 0.001) (Table 5).

The percentage of subjects with improvement in sperm count was 81.2% on 4th week, 85.4% on 8th week and 85.4% on 12th week as shown in Fig. 11.



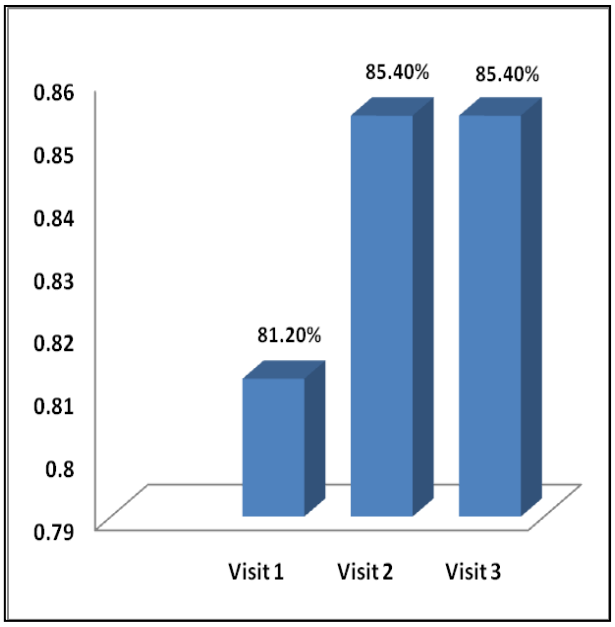


Fig. 10: Sperm count

Fig. 11: Percent Study subjects improved

Table 5: Statistical data of sperm count

SPERM COUNT (millions/ml)		Mean ± Std. Deviation	p-value
Pair 1	Baseline	35.12±19.300	**.001****
	Visit 1 (4th week)	48.89±23.185	
Pair 2	Baseline	35.12±19.300	**.001****
	Visit 2 (8th week)	86.16±94.935	
Pair 3	Baseline	35.34±19.450	**.000****
	Visit 3 (12th week)	88.31±21.800	

d. Sperm mobility

The sperm mobility was improved significantly in study population on completion of the treatment. At baseline, percent mean sperm mobility was 35.79% which was improved to

74% on completion of treatment as shown in Fig. 12. The improvement in sperm mobility was statistically significant (p-value 0.000) (Table 6).

On Visit 1, 68.8% of study subjects were shown to have improvements in sperm mobility. Similarly, on Visit 2 & Visit 3, study subjects who have improvements in sperm mobility were 83.3% and 81.2% respectively (Fig. 13).

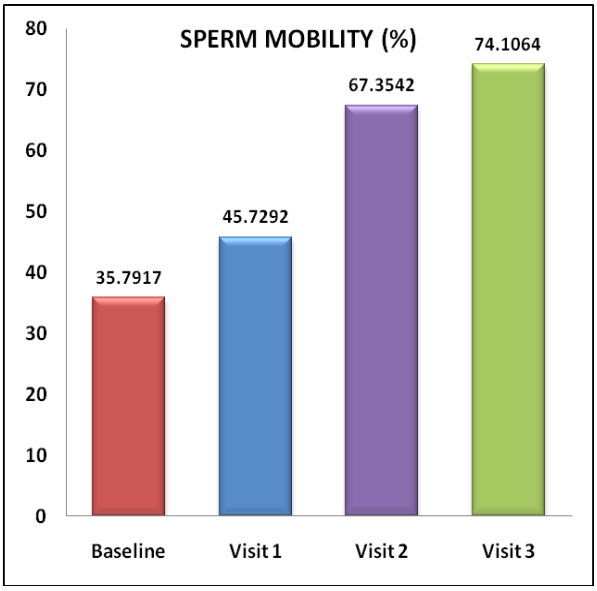


Fig. 12: Sperm mobility

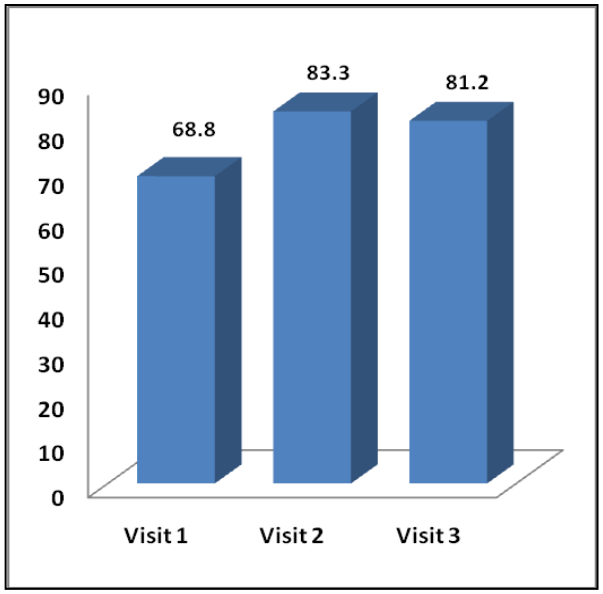


Fig. 13: Percent study subjects improved

Table 6: Statistical data of sperm mobility

SPERM MOBILITY (%)		Mean ± Std. Deviation	p-value
Pair 1	Baseline	35.79±19.154	.022*
	Visit 1 (4th week)	45.72±22.097	
Pair 2	Baseline	35.79±19.154	**.000****
	Visit 2 (8th week)	67.35±17.967	
Pair 3	Baseline	35.91±19.342	**.000****
	Visit 3 (12th week)	74.10±14.593	

e. Abnormal sperm morphology

The abnormal sperm morphology decreased with the continuation of treatment. At baseline, 42% of the sperms have abnormal morphology and this percentage was reduced to 15% on the completion of treatment (Fig. 14). This decrease in abnormal sperm morphology was statistically significant (p-value 0.000) (Table 7).

On Visit 1, 29.8% of the study subjects had abnormal sperm morphology but with the progress in treatment, the percentage of subjects with abnormal sperm morphology reduced to 17% on Visit 2 and 15.2% on completion of the treatment (Fig. 15).

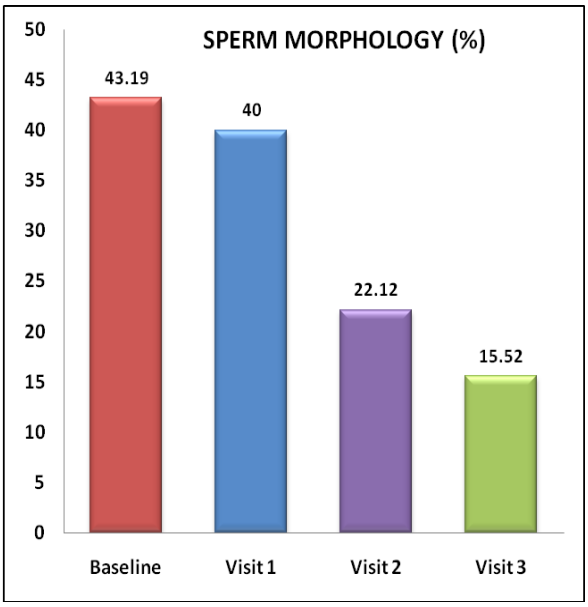


Fig. 14: Abnormal sperm morphology

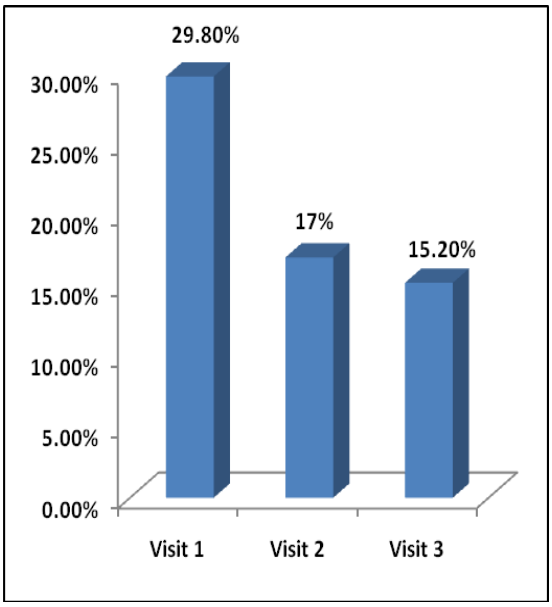


Fig. 15: Percent study subjects improved (%)

Table 6: Statistical data of abnormal sperm morphology

ABNORMAL SPERM MORPHOLOGY (%)		Mean ± Std. Deviation	p-value
Pair 1	Baseline	43.19±19.122	.465ns
	Visit 1 (4th week)	40.00±20.216	
Pair 2	Baseline	43.19±19.588	**.000****
	Visit 2 (8th week)	22.12±15.026	
Pair 3	Baseline	43.19±19.626	**.000****
	Visit 3 (12th week)	15.52±11.139	

f. Frequency of sexual intercourse

The study population showed increase in the frequency of sexual intercourse per month. Average frequency of sexual intercourse per month at baseline was 4 which was doubled to 8 on completion of treatment (Fig. 16). The change was statistically significant (p-value 0.000) (Table 7).

On Visit 1 (after 4 weeks) of the study, 65.3% of the study subjects showed improvements and on Visit 2 (after 8 weeks), the percentage of the study subjects was

raised to 95.9%. On the completion of treatment (after 12 weeks), total of 98% population showed increase in the frequency of sexual intercourse per month. It has been shown graphically in Fig. 17.

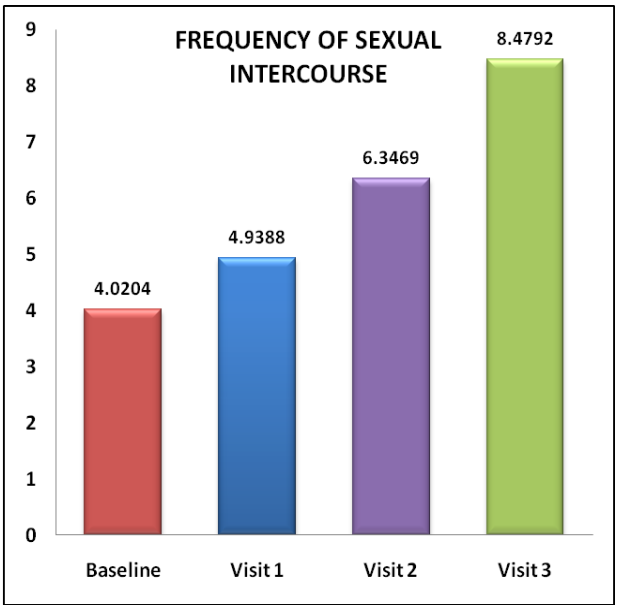


Fig. 16: Frequency of sexual intercourse

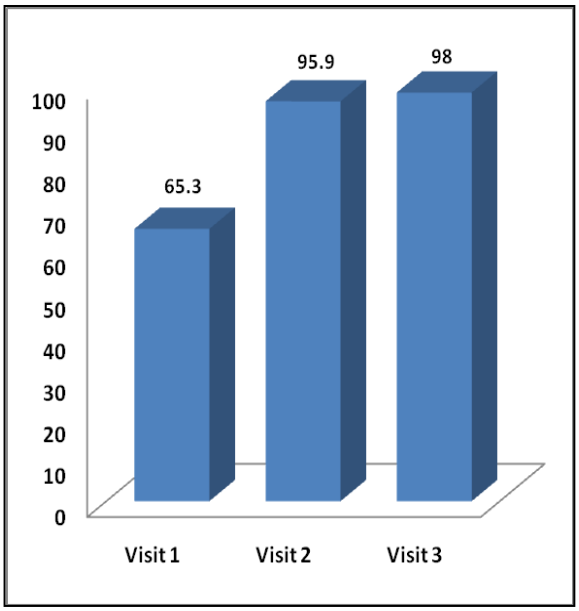


Fig. 17: Percent study subjects improved

Table 7: Statistical data of frequency of sexual intercourse/month

FREQUENCY OF SEXUAL INTERCOURSE/MONTH		Mean ± Std. Deviation	p-value
Pair 1	Baseline	4.02 ± 1.435	.000**
	Visit 1 (4th week)	4.93 ± 1.248	
Pair 2	Baseline	4.02 ± 1.435	.000**
	Visit 2 (8th week)	6.34 ± 1.315	
Pair 3	Baseline	4.04 ± 1.443	.000**
	Visit 3 (12th week)	8.47 ± 2.153	

g. Effect on Mental alertness, Mood, Reflex Erection and Overall performance

The study subjects showed 40% improvement in mental alertness. On completion of treatment, improvement in the mental alertness was increased as compared to baseline levels (Fig. 18). The results were statistically significant (p-value 0.000) (Table 8).

After 4 weeks of treatment, 50% of the study subjects have improvement in the mental alertness which was increased with the treatment period. On Visit 2 (after 8 weeks) the number of subjects with improved mental alertness raised to 87% and on Visit 3, the number of subjects with improvement in the mental alertness was 100% (Fig. 19).

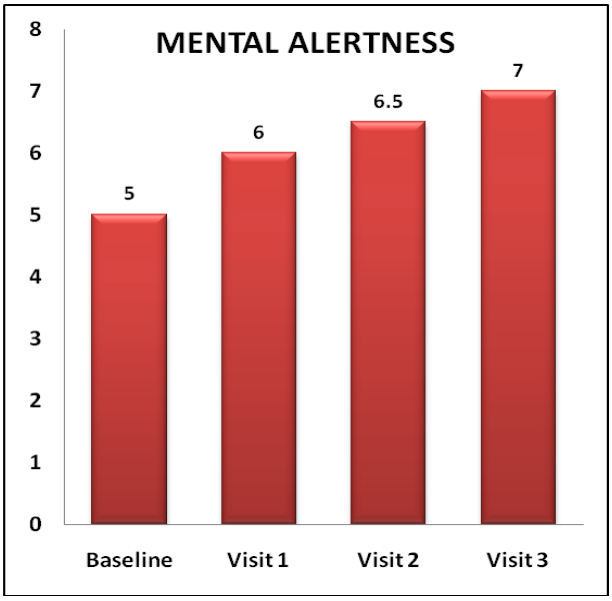


Fig. 18: Mental alertness

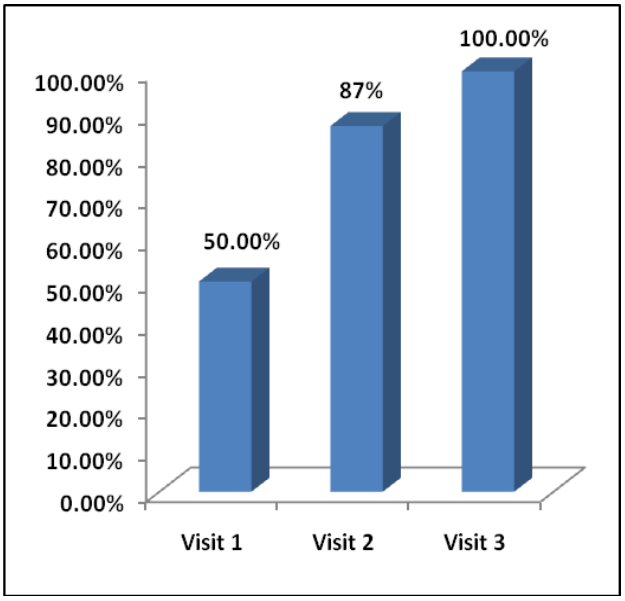


Fig. 19: Percent subjects improved (%)

Table 8: Statistical data of mental alertness

MENTAL ALERTNESS	Median	Minimum	Maximum	p-value
Baseline	5.00	4.00	7.00	.000**
Visit 1 (4th week)	6.00	4.00	7.00	
Baseline	5.00	4.00	7.00	.000**
Visit 2 (8th week)	6.50	5.00	8.00	
Baseline	5.00	4.00	7.00	.000**
Visit 3 (12th week)	7.00	6.00	8.00	

Similarly, the study subjects showed 60% improvement in mood. On completion of treatment, the mood was improved as compared to baseline conditions (Fig. 20). The results were statistically significant (p-value 0.000) (Table 9).

After 4 weeks of treatment, 60.9% of the study subjects have improvements in mood and it was improved with the treatment period. On Visit 2 (after 8 weeks) the number of subjects

with improved mood raised to 89.1% and on Visit 3, the number of subjects with improved mood was 100% (Fig. 21).

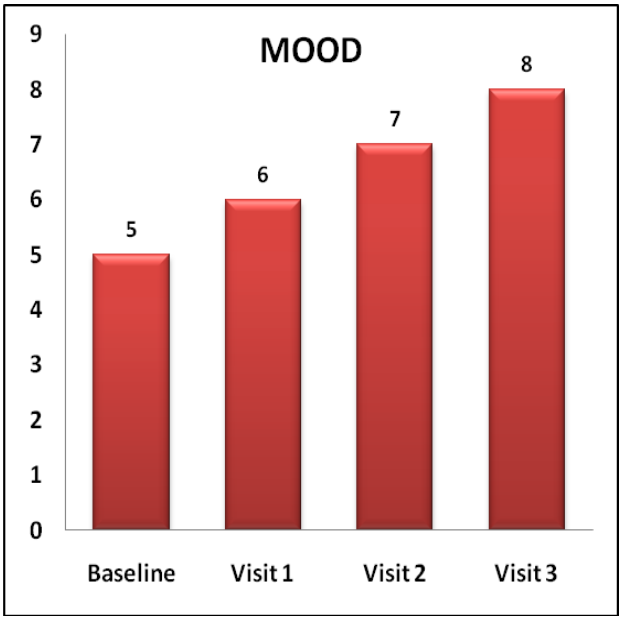


Fig. 20: Mood

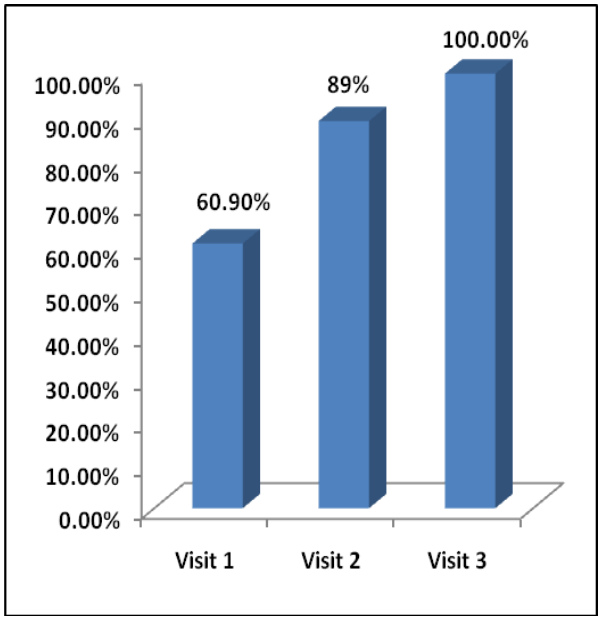


Fig. 21: Percent subjects improved (%)

Table 9: Statistical data of mood

MOOD	Median	Minimum	Maximum	p-value
Baseline	5.00	3.00	7.00	.000**
Visit 1 (4th week)	6.00	4.00	7.00	
Baseline	5.00	3.00	7.00	.000**
Visit 2 (8th week)	7.00	5.00	8.00	
Baseline	5.00	3.00	7.00	.000**
Visit 3 (12th week)	8.00	6.00	9.00	

In the same way, the study subjects showed 60% improvement in reflex erection. On completion of treatment, the reflex erection was improved as compared to baseline conditions (Fig. 22). The results were statistically significant (p-value 0.000) (Table 10).

After 4 weeks of treatment, 37.0% of the study subjects have improvements in reflex erection. On Visit 2 (after 8 weeks) the number of subjects with improved reflex erection raised to 71.7% and on Visit 3, the number of subjects with improved reflex erection was 95.6% (Fig. 23).

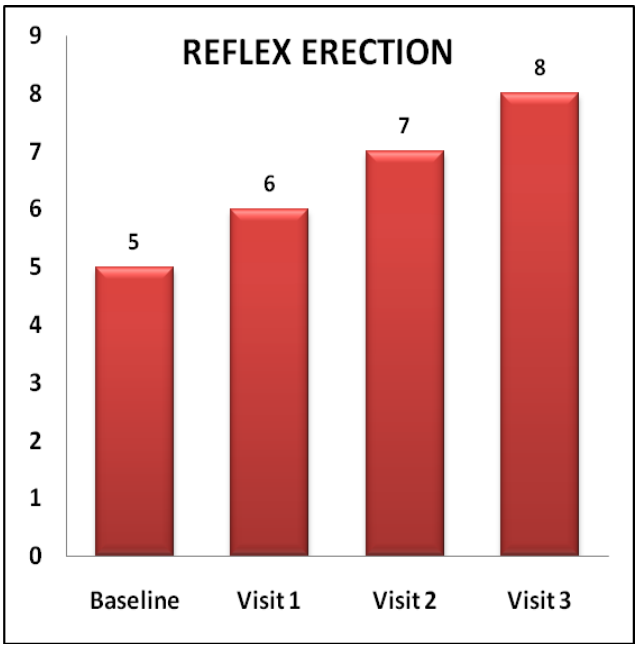


Fig. 22: Reflex Erection

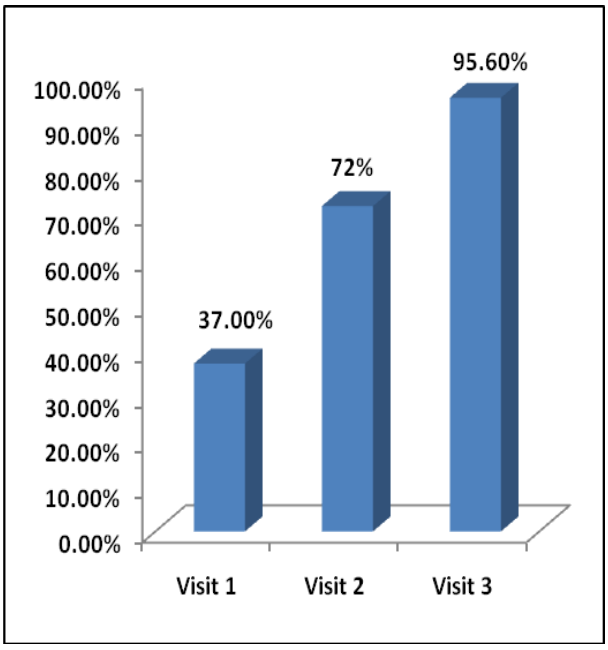


Fig. 23: Percent subjects improved (%)

Table 10: Statistical data of reflex erection

REFLEX ERECTION	Median	Minimum	Maximum	p-value
Baseline	5.00	4.00	7.00	.001**
Visit 1 (4th week)	6.00	4.00	7.00	
Baseline	5.00	4.00	7.00	.000**
Visit 2 (8th week)	7.00	5.00	8.00	
Baseline	5.00	4.00	7.00	.000**
Visit 3 (12th week)	8.00	6.00	9.00	

Similarly, the study subjects showed 60% improvement in overall performance. On completion of the treatment, the overall performance was improved as compared to baseline situations (Fig. 24). The results were statistically significant (p-value 0.000) (Table 11).

After 4 weeks of treatment, 41.3% of the study subjects have improvements in overall performance. On Visit 2 (after 8 weeks) the number of subjects with improvement was raised to 82.6% and on Visit 3, the number of subjects was 97.8% (Fig. 25).

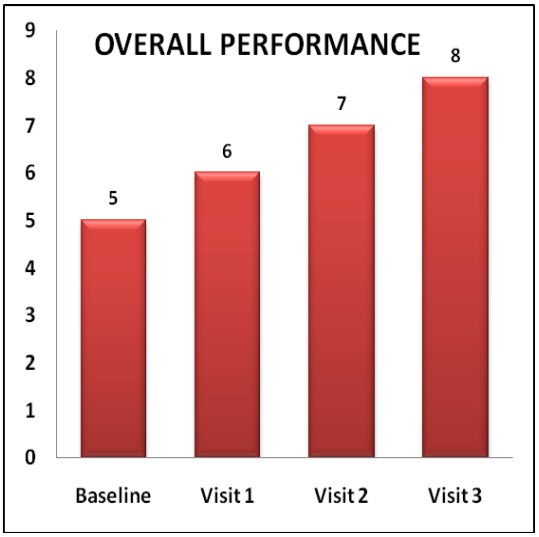


Fig. 24: Overall performance

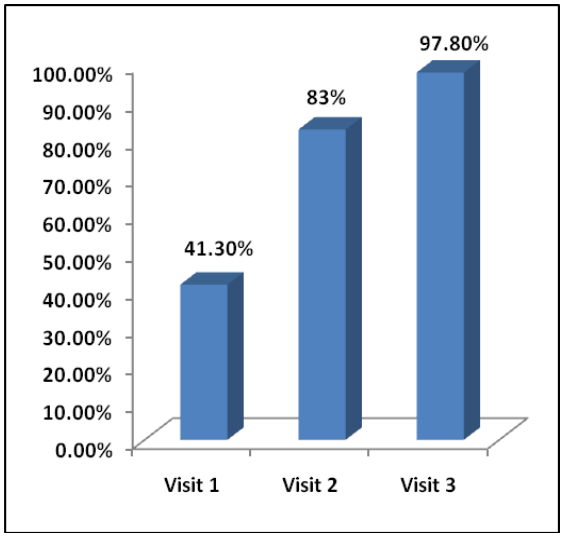


Fig. 25: Percent subjects improved (%)

Table 11: Statistical data of overall performance

OVERALL PERFORMANCE	Median	Minimum	Maximum	p-value
Baseline	5.00	4.00	7.00	**.002****
Visit 1 (4th week)	6.00	5.00	8.00	
Baseline	5.00	4.00	7.00	**.000****
Visit 2 (8th week)	7.00	5.00	8.00	
Baseline	5.00	4.00	7.00	**.000****
Visit 3 (12th week)	8.00	6.00	9.00	

C. Safety Evaluation

a. Hemoglobin levels

No significant change in haemoglobin levels were observed on completion of the study as compared to baseline values (Fig. 26) (Table 12).

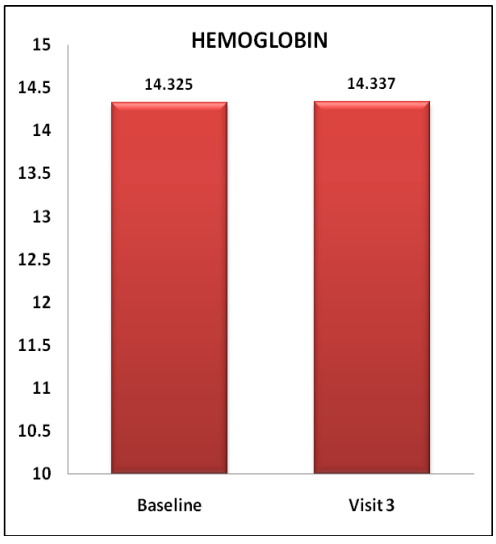


Fig. 26: Hemoglobin levels

Table 12: Statistical data of hemoglobin levels

HEMOGLOBIN		Mean ± Std. Deviation	p-value
Pair 1	Baseline	14.325 ± 1.56	0.958
	Visit 3 (12th week)	14.337 ± 1.25	

b. DHEA-S levels

No significant change in DHEA-S levels was observed on completion of the study as compared to baseline values (Fig. 27) (Table 13).

c. Fasting Blood Sugar (FBS) level

No significant change in FBS levels was observed on completion of the study as compared to baseline values (Fig. 28) (Table 14).

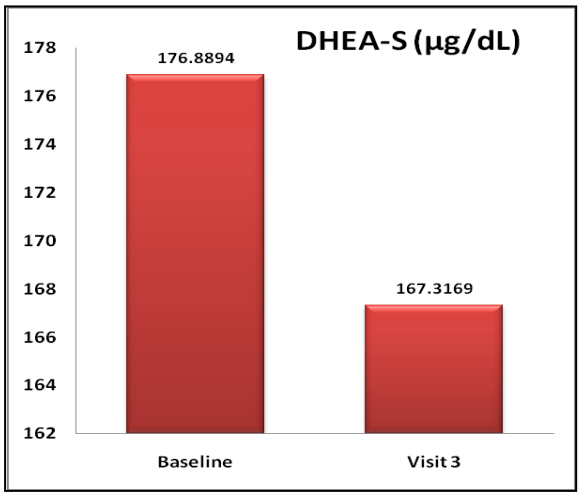


Fig. 27: DHEA-S levels

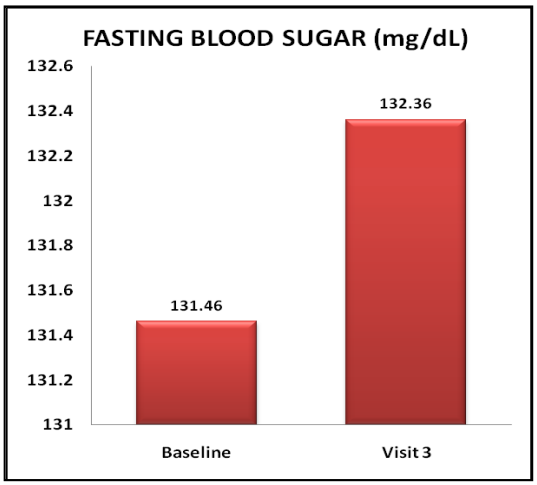


Fig. 28: Fasting Blood Sugar

Table 13: Statistical data of DHEA-S levels

Parameter		Mean ± Std. Deviation	p-value
DHEA-S	Baseline	176.88±93.116	**.279ns**
	Visit 3 (12th week)	167.31±88.411	

Table 14: Statistical data of fasting blood sugar levels

Parameter		Mean ± Std. Deviation	p-value
FBS	Baseline	131.46±78.695	**.945ns**
	Visit 3 (12th week)	132.36±52.874	

d. AST/GOT activity

No significant change in SGOT activities were observed on completion of the treatment (Fig. 29) (Table 15).

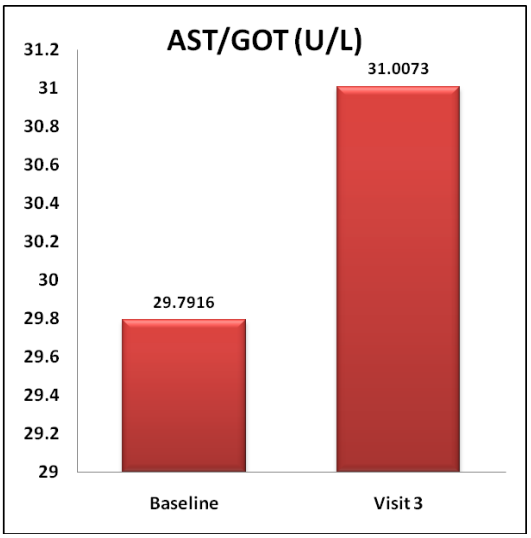


Fig. 29: AST/GOT activity

Table 15: Statistical data of AST/GOT activity

Parameter		Mean ± Std. Deviation	p-value
AST/GOT	Baseline	29.79±9.960	**.593ns**
	Visit 3 (12th week)	31.00±13.792	

e. ALT/GPT activity

No significant change in ALT/GPT activities was observed on completion of the study as compared to baseline values (Fig. 30) (Table 16).

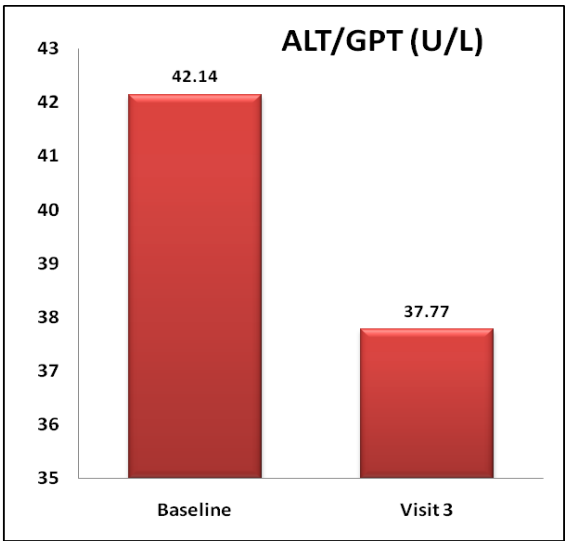


Fig. 30: ALT/GPT activity

Table 16: Statistical data of ALT/GPT activity

Parameter		Mean ± Std. Deviation	p-value
ALT/GPT	Baseline	42.14±22.614	**.198ns**
	Visit 3 (12th week)	37.77±24.825	

f. ALP activity

No significant change in ALP activity was observed on completion of the study as compared to baseline values (Fig. 31) (Table 17).

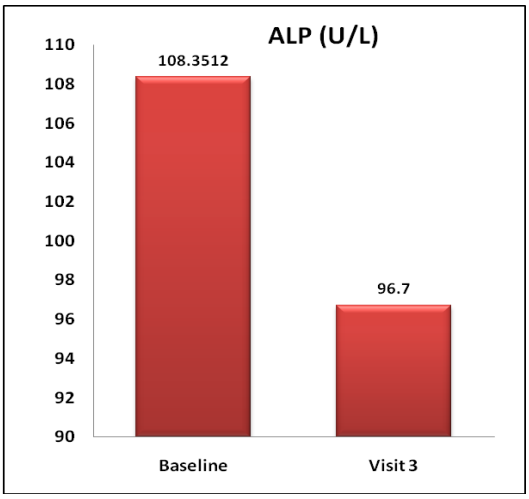


Fig. 31: ALP activity

Table 17: Statistical data of ALP activity

Parameter		Mean ± Std. Deviation	p-value
ALP	Baseline	108.35±43.006	**.093***
	Visit 3 (12th week)	96.70±31.483	

g. BUN levels

No significant change in Blood urea nitrogen levels were observed on completion of treatment as compared to baseline values (Fig. 32) (Table 18).

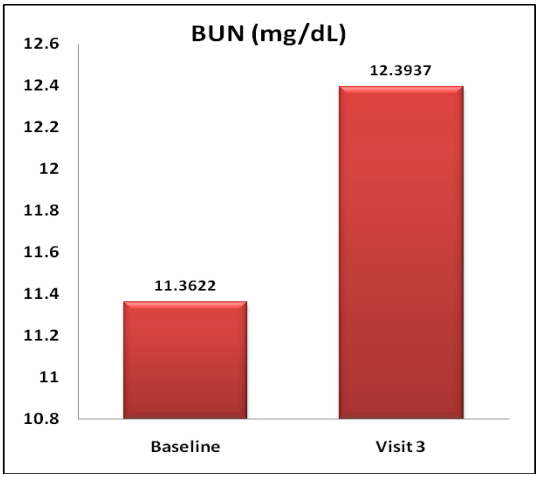


Fig. 32: BUN levels

Table 18: Statistical data of BUN levels

Parameter		Mean ± Std. Deviation	p-value
BUN	Baseline	11.36±3.908	**.082ns**
	Visit 3 (12th week)	12.39±4.321	

h. Cholesterol levels

The initial levels on baseline were 168.20mg% and these were minutely reduced to 167.35mg% after 12 weeks (Fig. 33) (Table 19).

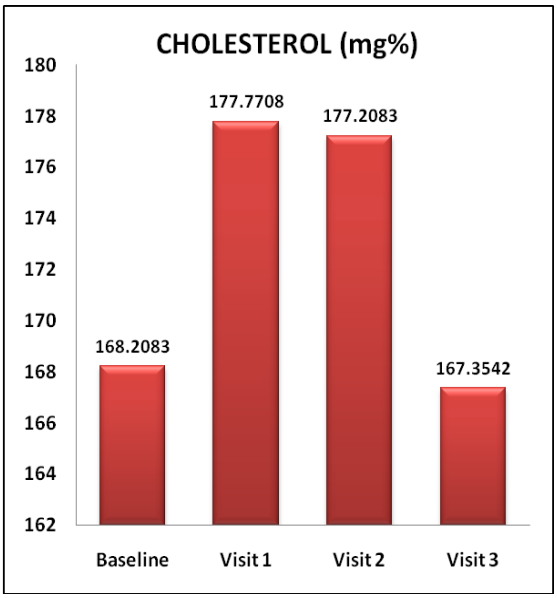


Fig. 33: Cholesterol levels

Table 19: Statistical data of cholesterol levels

Parameter		Mean ± Std. Deviation	p-value
Cholesterol	Baseline	168.20±33.858	**.546ns**
	Visit 1 (4th week)	170.77±40.219	
	Baseline	168.20±33.858	**.210ns**
	Visit 2 (8th week)	177.20±44.291	
	Baseline	168.20±33.858	**.872ns**
	Visit 3 (12th week)	167.35±9.222	

i. Triglyceride levels

No significant change in serum triglyceride levels were observed on completion of treatment as compared to baseline values (Fig. 34) (Table 20).

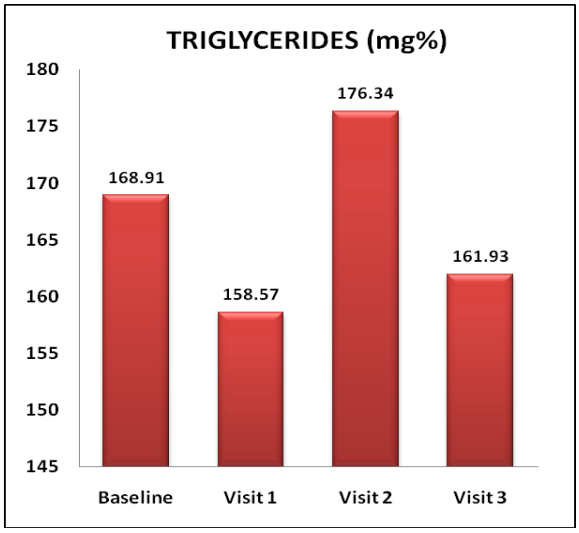


Fig. 34: Triglyceride levels

Table 20: Statistical data of triglyceride levels

Parameter		Mean ± Std. Deviation	p-value
Triglycerides	Baseline	168.91±89.724	**.538ns**
	Visit 1 (4th week)	158.57±78.257	
	Baseline	168.91±89.724	**.682ns**
	Visit 2 (8th week)	176.34±80.558	
	Baseline	168.91±89.724	**.704ns**
	Visit 3 (12th week)	161.93±93.847	

j. HDL cholesterol

These HDL levels were statistically non-significant (p-value 0.755) (Table 21). The levels of HDL cholesterol were increased to a small extent in study subjects on completion of treatment as compared to baseline levels (Fig. 35). At baseline, HDL levels were 40.69mg% and they were increased to 41.18mg% after 12 weeks.

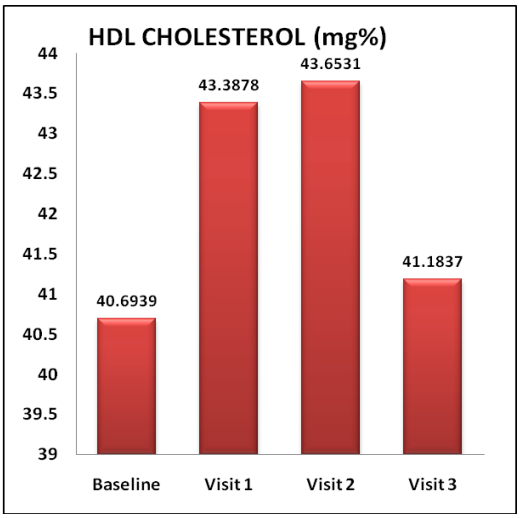


Fig. 35: HDL cholesterol levels

Table 21: Statistical data of HDL cholesterol levels

Parameter		Mean ± Std. Deviation	p-value
HDL Cholesterol	Baseline	40.69±11.372	**.102ns**
	Visit 1 (4th week)	43.38±12.982	
	Baseline	40.69±11.372	**.089ns**
	Visit 2 (8th week)	43.65±10.991	
	Baseline	40.69±11.372	**.755ns**
	Visit 3 (12th week)	41.18±10.347	

k. LDL cholesterol

These LDL levels were statistically non-significant (p-value 0.161) (Table 22).The LDL levels were decreased in study subjects after 12 weeks as compared to baseline levels. The mean baseline levels of LDL cholesterol were 106.87 mg% and decreased to 100.20 mg% in study subjects on completion of treatment.

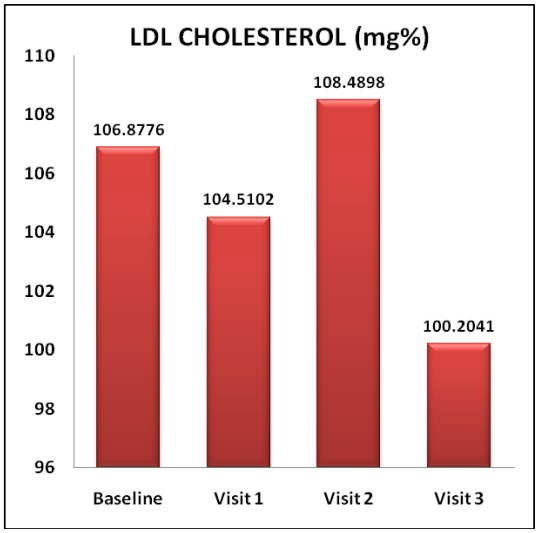


Fig. 36: LDL cholesterol levels

Table 22: Statistical data of LDL cholesterol levels

Parameter		Mean ± Std. Deviation	p-value
LDL Cholesterol	Baseline	106.87±32.800	**.564ns**
	Visit 1 (4th week)	104.51±36.826	
	Baseline	106.87±32.800	**.825ns**
	Visit 2 (8th week)	108.48±43.051	
	Baseline	106.87±32.800	**.161ns**
	Visit 3 (12th week)	100.20±29.164	

l. VLDL cholesterol

Reduction in VLDL levels was observed on completion of study treatment as compared to baseline values in study subjects (Fig. 37). The mean baseline levels of VLDL cholesterol were 33.77 mg% and these levels were decreased slightly to 32.37 mg% after 12 weeks of the treatment. The VLDL cholesterol levels were statistically non-significant (p-value 0.704) (Table 23).

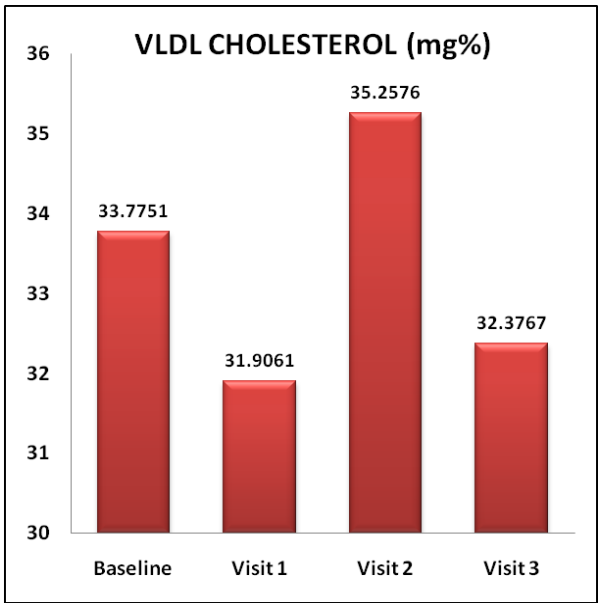


Fig. 37: VLDL cholesterol levels

Table 23: Statistical data of VLDL cholesterol levels

Parameter		Mean ± Std. Deviation	p-value
VLDL Cholesterol	Baseline	33.77±25.321	**.583ns**
	Visit 1 (4^{th} week)	31.90±15.754	
	Baseline	33.77±25.321	**.683ns**
	Visit 2 (8^{th} week)	35.25±16.116	
	Baseline	33.77±25.321	**.704ns**
	Visit 3 (12^{th} week)	32.37±18.770	

m. Total leukocyte count (TLC)

No significant change in TLC was observed on completion of treatment as compared to baseline values which has been depicted by Fig. 38 (Table 24).

Table 24: Statistical data of total leukocyte count

Parameter		Mean ± Std. Deviation	p-value
TLC (× 10^3)	Baseline	7.52±1.86629	**.454ns**
	Visit 3 (12^{th} week)	7.32±1.74877	

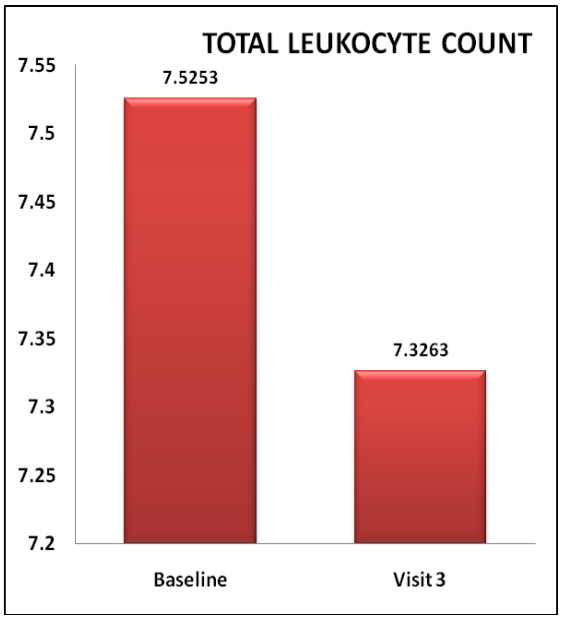


Figure 38: Total leukocyte count

n. Differential leukocyte count

No significant change in differential leukocyte count was observed on completion of treatment as shown in in Fig. 39 (Table 25).

Table 25: Statistical data of differential leukocyte count

Parameter		Mean ± Std. Deviation	p-value
Neutrophils	Baseline	61.2510±6.86322	**.043***
	Visit 3 (12^{th} week)	63.6510±7.69548	
Lymphocytes	Baseline	30.5694±6.35611	**.092ns**
	Visit 3 (12^{th} week)	28.6980±6.16946	
Monocytes	Baseline	3.1184±.83857	**.815ns**
	Visit 3 (12^{th} week)	3.0816±.73900	
Eosinophils	Baseline	4.5776±3.50293	**.451ns**
	Visit 3 (12^{th} week)	4.0857±4.30416	

Basophils	Baseline	.1714±.08660	**.030***
	Visit 3 (12th week)	.2143±.11365	

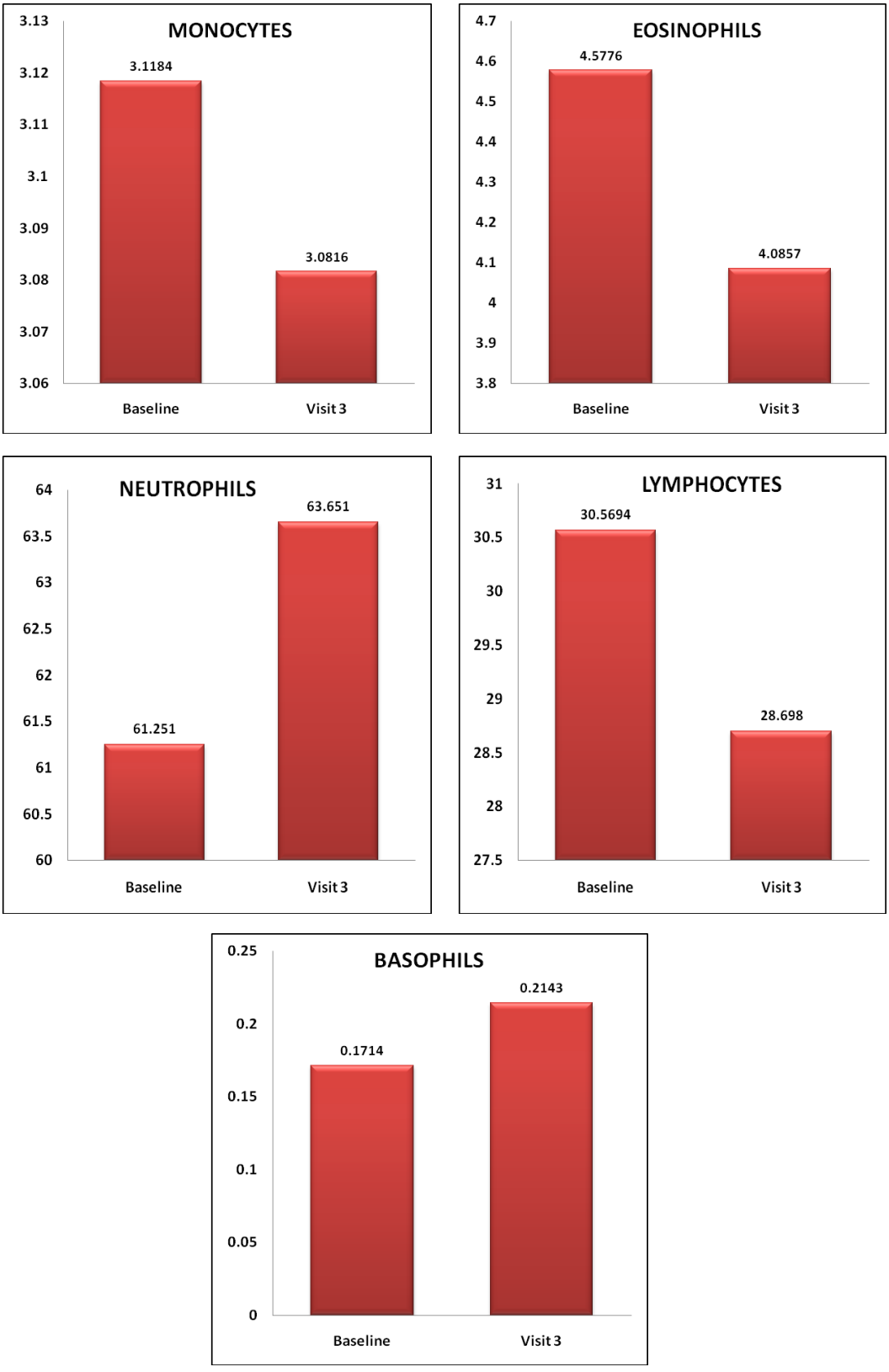


Fig. 39: Differential Leukocyte Counts

Table 26: Statistical data

Parameter		Mean ± Std. Deviation	p-value
DHEA-S	Baseline	176.88±93.116	**.279ns**
	Visit 3	167.31±88.411	
FBS	Baseline	131.46±78.695	**.945ns**
	Visit 3	132.36±52.874	
AST/GOT	Baseline	29.79±9.960	**.593ns**
	Visit 3	31.00±13.792	
ALT/GPT	Baseline	42.14±22.614	**.487ns**
	Visit 3	37.77±24.825	
ALP	Baseline	108.35±43.006	**.093***
	Visit 3	96.70±31.483	
BUN	Baseline	11.36±3.908	**.082ns**
	Visit 3	12.39±4.321	
Cholesterol	Baseline	168.20±33.858	**.546ns**
	Visit 1	170.77±40.219	
	Baseline	168.20±33.858	**.210ns**
	Visit 2	177.20±44.291	
	Baseline	168.20±33.858	**.872ns**
	Visit 3	167.35±39.222	
Triglycerides	Baseline	168.91±89.724	**.538ns**
	Visit 1	158.57±77.496	
	Baseline	168.91±89.724	**.682ns**
	Visit 2	176.34±80.558	
	Baseline	168.91±89.724	**.704ns**
	Visit 3	161.93±93.847	
HDL Cholesterol	Baseline	40.69±11.372	**.102ns**
	Visit 1	43.38±12.982	
	Baseline	40.69±11.372	**.089ns**
	Visit 2	43.65±10.991	
	Baseline	40.69±11.372	**.755ns**

	Visit 3	41.18±10.347	
LDL Cholesterol	Baseline	106.87±32.800	**.564ns**
	Visit 1	104.51±36.826	
	Baseline	106.87±32.800	**.825ns**
	Visit 2	108.48±43.051	
	Baseline	106.87±32.800	**.161ns**
	Visit 3	100.20±29.164	
VLDL Cholesterol	Baseline	33.77±25.321	**.583ns**
	Visit 1	31.90±15.754	
	Baseline	33.77±25.321	**.683ns**
	Visit 2	35.25±16.116	
	Baseline	33.77±25.321	**.704ns**
	Visit 3	32.37±18.770	
TLC (× 10^3)	Baseline	7.52±1.866	**.454ns**
	Visit 3	7.32±1.748	
Neutrophils	Baseline	61.25±6.863	**.043***
	Visit 3	63.65±7.695	
Lymphocytes	Baseline	30.56±6.356	**.092ns**
	Visit 3	28.69±6.169	
Monocytes	Baseline	3.15±.731	**.586ns**
	Visit 3	3.08±.739	
Eosinophils	Baseline	4.57±3.502	**.451ns**
	Visit 3	4.08±4.304	
Basophil	Baseline	.17±.086	**.030***
	Visit 3	.21±.113	

Efficacy conclusions

On completion of study, following efficacy conclusions were made from Furosap® treatment form testosterone deficient subjects under study:

1. Free testosterone levels were improved up to 46% in approx. 90% of the study population.
2. The frequency of sexual intercourse was also increased in the 98% of the study population.
3. 85.4% of the study population showed improvement in the sperm count.
4. The abnormal sperm morphology was also improved in the 14.6% of the study population.
5. All patients enrolled in the study showed improvement in mental alertness and their mood.
6. The reflex erection was also enhanced in the 95.2% of the study population.
7. The improvement in overall performance in all enrolled patients was observed.

Safety conclusions

On completion of study, following safety conclusions were made:

1. No significant change in serum liver function tests was observed.
2. No significant change in cholesterol levels was observed.
3. No significant change in hemogram was observed.

8. DISCUSSION

Medicinal plants play a substantial role in the life support systems. With increasing acceptance and use of medicinal plants in traditional therapies and with increasing commercial demands over the years, the consumption and collection of medicinal plants is accelerating. Plants have been the major source of drugs in Indian system of medicines and other ancient systems in the world. Fenugreek is one of the oldest medicinal plants, originating in India and Northern Africa. There are numerous medicinal uses of fenugreek including treatment of indigestion, baldness, hypoglycaemia, anti-hyperlipidemia, etc which makes it beneficial for consumption by humans.

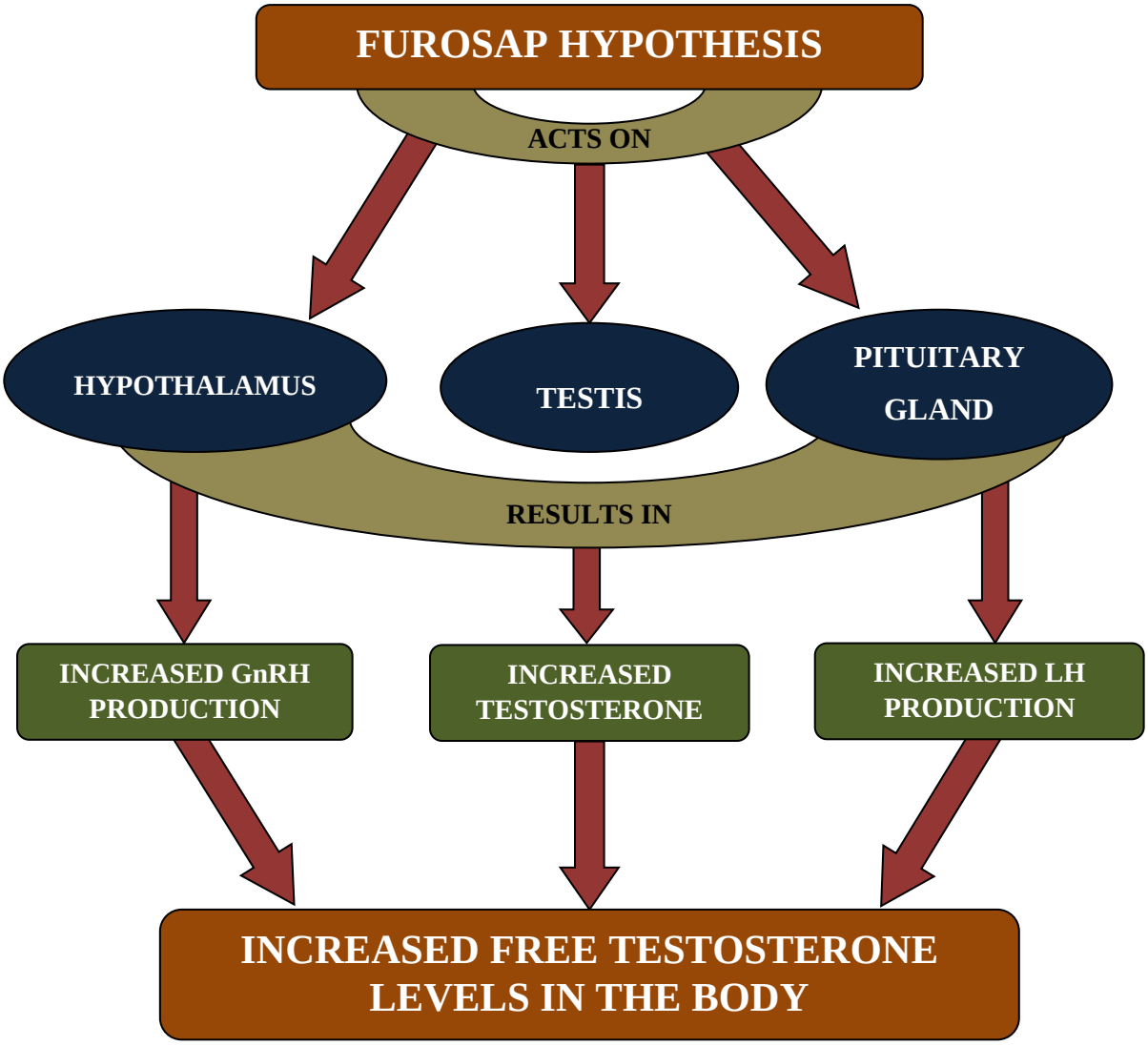
Fenugreek seeds extract branded as **FUROSAP®** has been used in the present clinical study. Fenugreek seeds have been chosen to prepare the formulation because seeds are the major source of furostanol-type steroid saponins called as trigoneosides and seeds also contain other bioactive components such as protodioscin, diosgenin, yamogenin, etc. Protodioscin is the major bioactive component in Furosap® which is generally responsible for the property of bitterness of fenugreek seeds.

It has been seen in Fig. 8 & Fig. 9 of the present report that the testosterone (Free testosterone and total testosterone) levels increase with the regular consumption of Furosap® which clearly depicts that the herbal formulation is effective to treat testosterone deficiency. In testosterone deficient subjects, Protodioscin present in Furosap® increases the levels of testosterone hormone. It boosts testosterone levels via stimulating pituitary gland. Protodioscin acts by stimulating 5-α-reductase enzyme, which plays a role in the conversion of testosterone into dihydrotestosterone. Dihydrotestosterone, in turn enhances erythropoiesis and muscle development.

This increase in free testosterone hormone level in the body has also shown to be helpful in increasing sperm count and sperm mobility. Normal levels of testosterone are necessary for normal sperm development because testosterone activates the genes in Sertoli cells which promote the differentiation of spermatogonia. The Fig. 10 for sperm count and Fig. 12 for sperm mobility indicates that the sperm activity increased with the regular consumption of Furosap® and the subjects thus, shown improvement in the state of hypogonadism.

Increased production of testosterone also contributes to the increase in sexual functions and it also increases the amount of unbound free testosterone which improves muscle mass, fat loss, strength and endurance. It has been illustrated in Fig. 16 for the frequency of sexual

intercourse that the sexual functions increased as the testosterone deficient subjects consumed Furosap® regularly.



This has also been observed by the Scoring chart of each diseased subject. The study subjects have shown improvements in mood, mental alertness, reflex erection and overall performance with the regular consumption of Furosap®. The baseline level of testosterone, sperm profile and frequency of sexual intercourse is much lower as compared to the levels made after the consumption of investigational product (At Visit 1, Visit 2 & Visit 3).

In addition to the stimulation of pituitary gland, protodioscin also stimulates the hypothalamus secretion of luteinizing hormone (LH) which also helps to increase the level of testosterone.

Thus, it can be concluded that fenugreek seeds extract – Furosap® is effective and safe to treat testosterone deficient or hypogonadism suffering subjects.

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Annexure I

SCORING CHART

Screening No. **Date:**

Enrolment No.

	Baseline	Visit 1	Visit 2	Visit 3
Mental alertness / /मानसिक सतर्कता				
Mood / मनःस्थिति				
Reflex erection / प्रतिवाह खड़ा होना				
Overall performance / कुल मिलाकर प्रदर्शन				
Any other clinical observation / कोई अन्य क्लिनिकल अवलोकन				

Signature of Co-Investigator **Signature of Principal Investigator**