1. SUMMARY AND CONCLUSION

Present investigation is to develop safe and effective formulation for aphrodisiac potentials in males which helps to improve aphrodisiac disability. Though a great amount of scientific research has been accompanied in India and worldwide on medicinal plants, traditionally proven herbal preparations/formulations do not yet fully conform to standards of drug testing, safety, and efficacy. Though opportunities for developing traditional preparations as drugs with international acceptance are enormous, is hardly any intensive effort in this direction at Government or industry level in India, which has resulted in a decline in enthusiasm for studying plant preparations for their the therapeutic value.

As herbal the therapeutic preparation are multifaceted blends, which initiate from natural sources, excessive efforts are essential to assure a continuous as well as satisfactory excellence. Through wisely selecting herbal material as well as a consistent manufacturing procedure, design and quantity of constituents must be reserved as persistent as possible, as this is a requirement for reproducible beneficial outcomes.

Improvement of CTD is an vital clue along with union consequently so far there is no agreement on usage of a solo unified method either organization wise or medicine wise. According to South East Asia, India is amongst principal countries in statement of advance of pharmacopeial criteria as well as alteration of current regulatory rules. Evidence-based proposals for regulatory authorization as well as linking of several pharmacopeial monographs would aid herbal producers increase better admittance to controlled markets across world.

Though there is a worldwide awareness in traditional herbal medications, there are worries about use of unapproved as well as unregulated medications. Scientific community is worried about excellence, standardization, clinical security and effectiveness of herbal medicines. Knowledge of allopathic business proposes that principles are essential to aid science as well as excellence of investigation. Time has come to agree similar for herbal medicines. Except investigation information on Indian herbal medicines encounter native
as well as global governing standards, our traditional methods will find it tough to contend with Chinese efforts. Golden statement for herbal remedies is “Effective Regulations Improve Research” also those countries with TM law take miscellaneous methods for permitting, providing, manufacturing as well as trading traditional remedies

Sensual disability, that is frequent incapability to attain normal sensual contact, that include several procedures like premature ejaculation, retarded, retrograded, erectile disability, or inhibited ejaculation, reduced libido, compulsive sensual behavior, failure of Detumescence, and orgasmic disorder are on increase worldwide mixture of elderly population also or growing etiological issues. Hunt for natural addition from the therapeutic plants is being increased perhaps, mixture of its decreased adverse effect, its ready accessibility as well as decreased cost. Therefore, cumulative hunt for the therapeutic plants with aphrodisiac abilities has demanded necessity to review approaches existing for screening the therapeutic plants with aphrodisiac potentials in males (Yakubu et al., 2007).

From literature review it was found that Crocus sativus, Piper betel, Symplocos racemosa and Myristica fragrans. Crocus sativus is reported to improve sensual behavior, possess anti-stress and antioxidant properties. Piper betel is proven aphrodisiac and improves spermatogenesis. Symplocos racemosa is phosphodiesterase inhibitor and may act to improve erection. Myristica fragrans is reported to possess aphrodisiac action and its anti-stress and immunomodulatory action may show effect to improve libido and no formulations was developed in combination of ingredients and hence plants were selected for development studies.

Besides, biochemical components in herb in HM preparations might vary liable on yield seasons, origins of plant, and procedures for dehydrating as well as additional aspects. Hence, it appears to be essential to control maximum of phytochemical elements of herbal products in consequences to guarantee consistency as well as repeatability of pharmacological as well as clinical investigation, to recognize their bioactivities also probable adverse effects of active constituents also to improve product excellence control.
Therefore, numerous chromatographic methods, such as high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and, gas chromatography (GC), thin layer chromatography (TLC), could be useful for this type of records. By this way, entire herbal preparation could be observed as active ‘compound’. Perception of phyto correspondence was advanced in Germany to confirm uniformity of herbal preparation. According to this perception, a chemical outline, such as a chromatographic pattern, for herbal preparation must be raised when matched with outline of a clinically established reference product.

Ash values are supportive in defining excellence as well purity of raw material in crushed form. Total ash typically involves of mineral radicals like carbonates, silica of sodium, and calcium phosphates, potassium, magnesium, silicates. Sulphated ash includes management of preparation by weak Sulphuric acid previously to ignition. In this, all oxides as well as carbonates are transformed to sulphates also it is done at a higher temperature (Mukharjee, 2002). Values for total ash were 3.0, 5.55, 0.95, 3.77 % w/w. for Nutmeg, Lodhra, Kesar, Tamulapatra respectively. Fromm results it could be concluded that inorganic radicals are present but are in limited amount as per specification.

Occasionally, mineral variables like calcium oxalate, carbonate amount of raw material silica, changes total ash standards, such variables are then detached by giving acid (as these are solvable by hydrochloric acid) treatment and then acid-insoluble ash value is quantified. Outcomes for acid insoluble ash were 1.64, 2.91, 0.14, and 1.59 % w/w for Nutmeg, Lodhra, Kesar, and Tamulapatra respectively which are far within limits as per specifications.

Extractive values are valuable for assessment of crude drugs as well as provide an indication about outline of chemical components existing in them. Quantity of extractive drug produced to a specified solvent is frequently an estimated amount of a convinced component or collection of associated components medicine comprises. In some cases quantity of drug solvable in an assumed solvent is a guide for its concentration. Solvent
utilized for abstraction must be in a situation to dissolve considerable amounts of constituents wanted (Indian Pharmacopoeia, 1996).

Water soluble extractive value found to be 10.40, 45.54, 70.26 and 20.42 for Nutmeg, Lodhra, Kesar, and Tamulapatra respectively which are far within limits as per specifications.

Alcohol soluble extractive value found to be 75.98, 51.16, 16.67 and 69.35 for Nutmeg, Lodhra, Kesar, and Tamulapatra respectively which are far within limits as per specifications. From results it can be concluded that components present in nutmeg and Tamulapatra are more alcohol soluble than water and components present in Kesar and Lodhra are more water soluble. LOD indicates amount of moisture present in sample. Moisture is important factor as it is responsible for microbial growth and may degrade quality of product due to hydrolysis of water sensitive components. Values for moisture from results are within specification limit.

Thin layer chromatography unique factor utilized for identifying contamination as well as judging excellence of drug. RF values were calculated using different visualizing tools like 254nm light, 366 nm light and derivatization agent.

It is tough to establish limitations to regulate these provisions. If formulation comprises one or more extract, every extract could be considered and evaluated before mixing as well as after blending to achieve uniqueness of preparations. Simple bioassay for biological regulation of herbal medications must be integrated to progress animal models for toxicity as well as safety assessment. Some of blocks in regulation of herbals are
1.1. Unavailability of library of standard compounds quarantined from herbs in their pure form for utilization in investigative use. It is monotonous work to separate every standard marker component in its purest form for estimating use by examining in laboratories itself.

1.2. Poor accessibility of public examination houses to assist industry in regular analysis

1.3. Non obtainability of a method where typical or correctly recognized herbs are obtainable for production.

1.4. High cost comprised in obtaining of sophisticated introduced devices like HPTLC, HPLC, LCMS etc.

One of greatest approaches of regulating herbs as well as herbal preparations based on current methodical implements is chromatography. It not only aids in forming precise plant individuality but also aids in regulating chemical purity of herbals. One such method is standard compound evaluating as well as fingerprint analysis. HPTLC is greatest regularly utilized wherever merely fingerprinting of plant is essential deprived of measuring composite however similar could also be computed with aid of a densitometer.

In present work extracts was sourced from a good supplier who is proficient of providing suitable quality extracts from technically feasible process in that complete monitoring specifications were set jointly by our RandD and supplier of RandD. After mutual agreement specs and STP of all raw materials were transferred to supplier RandD. During preformulation study all anticipated concerns were addressed, actives and excipients were checked for any incompatibility.

HPTLC method development for Myristicin and Safrole which are active components from nutmeg was done. Composition of mobile phase for HPTLC examination was improved through analysis of dissimilar solvent systems of variable polarity. Final solvent system
selected was 16 mL toluene, 2 mL ethyl acetate, and 0.4 mL glacial acetic acid in which Myristicin and Safrole showed prominent and distinct band at Rf 0.84 and 0.64 respectively hence from this study quality for nutmeg extract can be judged. This method can also be used for further analysis and standardization of nutmeg from this poly herbal formulation using Myristicin and Safrole as standard.

HPTLC method development for Crocin one of active component from Kesar (Crocus sativus) extract was done. Composition of solvent system was optimized to 9.3 mL toluene, 0.7 mL Ethyl Acetate which gave good separation and one black colored band of Crocin in found at Rf 0.74.

HPTLC method development was done for Gallic acid from Lodhra extract. Composition mobile phase was improved to 8 mL toluene, 8 mL ethyl acetate, and 4 mL Formic acid which gave good separation and one black colored band of Gallic acid in found at Rf 0.70.

HPTLC method development was done for Loturine acid from Lodhra extract. Composition of solvent system was optimized to 7 mL chloroform, 5 mL Acetonitrile and 2 mL Triethylamine. Loturine acid gave a quenching effect and shows a blue color band at RF 0.7

HPTLC method development was done for Eugenol from Tamulapatra extract. Composition of solvent system was optimized to 9.7 mL toluene, 0.3 mL ethyl acetate. One black colored band of Eugenol in found at Rf 0.72.

From above results quality of extracts can be judged. Sample can be standardized and potency of extracts can be determined using se methods. HPTLC method developed can also be used to standardize final formulation.

Extract is composed of hundreds of components which are present in very low amount. Variability also occurs within se components and hence it is necessary to detect these
active components to standardize extract as well as formulation by using modern
techniques. HPLC compromises very influential separation capacity, so that complex
chemical constituents in extracts can be detached into numerous comparatively simple sub
fractions. With aid of spectral data, retention time change, selectivity, instrumental
interferences, chromatographic parting capabilities also measurement accuracy could
deliver valuable data necessary in structure clarification.

HPLC method was developed for Myristicin and Safrole from nutmeg, Crocin from Kesar,
Loturine acid from Lodhra extract, and Eugenol from Tamulapatra extract.

Composition of mobile phase for HPLC examination were improved by evaluating
dissimilar mobile of variable polarity as well as mobile phase that provided greatest
outcomes was designated for standardization purpose.

Retention time for Crocin is 13.2 minute, retention time for Myristicin and Safrole is 19.3
and 21.2 minute respectively, retention time for Loturine acid is 5.3 minute, retention time
for Gallic acid from Lodhra extract is 8.5 minute. Retention time Eugenol from Tamulapatra extract was found to be 7.0 min. This information is further used along with
wavelength that gives maximum absorbance as shown in results to standardize extract and
this standardized extract is further used for standardizing final formulation.

Formulation designed, developed and standardized in present sis is an Ayurvedic
proprietary medicine in capsule form containing four Ayurvedic herbs named Nutmeg, Kesar, Lodhra and Tamulapatra. Safety of formulation has been established in animal
toxicity study.

All extract was standardized by analyzing physicochemical parameter along with analytical
determination by using sophisticated techniques like HPTLC, UV spectrophotometer and
HPLC. After incompatibility development study only compatible excipients which were
found to be non-reactive with extract were used for MSD formulation. Four different
formulae using two different strategies like mixing and granulation were developed for MSD formulation.

Present strategies on excellence as well as constancy for herbal must be revised to render them suitable for multi element traditional herbal preparation. Product issue condition of finished product must be grounded on TLC in contradiction of fingerprint patterns of standards. Likewise, constancy of formulation must be measured by possible variations in TLC outline regularly as essential components are frequently not measurable also constancy of indicators is not essentially suggestive of constancy of standard. Along with this detailed batch documentation and in process quality control tests will help in maintaining batch to batch consistency of finished product.

Further in India are no guidelines for testing quality of herbal products from any government agency or association of Ayurvedic product manufacturers which is acceptable internationally. It is high time that government and industry shared inputs to clear confusion and ambiguity, drafted new regulations for nutritional products and formulated policy document to steer herbal exports. It needs political will to improve herbal industry and someone has to decide and do it usher peaking of herbal industry. Issues of occurrence of heavy metals similar to lead, cadmium, mercury and arsenic, presence of bacteria and toxins in herbal formulations should be first addressed blend adverse publicity affects industry as a whole. Finally, it is strongly felt that herbal industry has a strong future if only government, regulatory authorities, industry and medical profession think positive about patient’s health. Let us not forget that humanity has survived and prospered using herbals for generations. Let us all strive to make sincere efforts to bring back glory of our herbals.

Herbal extracts are more hygroscopic and having tendency to form lumps hence For such type of raw materials wet granulation may be only feasible means of producing capsule, and for low dose drugs granulation process is seen as being capable of “locking” drug into granules and ready minimizing potential for segregation and poor content uniformity.
Capsule dosage form was selected as a formulation blend it is Easy to manufacture, avoids direct exposer of content towards outer conditions like moisture, temperature, humidity and light, More batches can be taken in less time, Less manufacturing steps are involved in manufacturing process, Sometime only mixing of raw materials will be sufficient to formulate capsule, Easy to swallow for patients blend of slippery nature of capsule shell. ingredients added in formulation is as shown in which is developed by various trials taken an based on literature data and studies ingredients were selected to prepare stable formulation.

Four formulations are selected for further studies for compatibility and stability. As per results MSD/CA/001 contains Microcrystalline cellulose, Colloidal anhydrous silica. MSD/CA/002 contains DCP, Colloidal anhydrous silica. MSD/CA/003 contains Lactose, Colloidal anhydrous silica. MSD/CA/004 contains microcrystalline cellulose, DCP, Colloidal anhydrous silica.

In all four combinations Magnesium stearate was used for lubricant effect, Propyl and Methyl paraben were used as preservatives.

Incompatibility studies were carried out for each extract comparing individually with or extract and excipients individually. Only physical parameters like color, lump formation and water contain were observed in preformulation study. From results it was concluded that extracts and ingredients used in formulations were stable and compatible with each or. At initial stage disintegration time of all combinations was 15 minutes, but after 3 month it reaches to around 27 minutes. However in MSD/CA/002 disintegration time was reached to 16 minute which is not considerable. Disintegration time of or combination was increase blend of increasing moisture content which lead to formation of lumps and n capsule shell becomes more insoluble in water which results in to increasing disintegration time. Even in chemical analysis i.e. total polyphenol was remained unaltered in MSD/CA/002 as compare to or combinations.
Preclinical assessment, Clinical involvements, remarks or recognized and documented is an initial point for historical information based medication advancement.

Though clinical presence of traditional medication durations for numerous generations, it could be problematic to include such practice in present therapeutic use as preparation dissimilarity might arise owing to questions like excellence, effectiveness, safety, bioavailability as well as mechanisms of activity of greatest extensively used plants.

There is necessity for methodical authentication excellence, effectiveness, safety, bioavailability as well as mechanisms of activity of these drugs which are being utilized in since centuries.

Acute toxicity experiments were done to investigate consequence of formulation in specified dose. No lethality was observed in any group after 48 hrs. There were no symptoms of CNS toxicity and behavior of all animals was normal. Hence LD 50 of MSDES is more than 2000 mg/ kg p.o. Hence dose of 200 mg/ kg for all formulae were selected for further studies.

Chronic toxicity was studied to evaluate effect of drug is used chronically. These studies give idea for unwanted symptoms if drug is used for long term.

At termination of experiment i.e. on 90th day of treatment, every animal was in good physical condition as revealed by regular presence of breathing pattern, tint of body surfaces, occurrence as well as type of movement, noticeable involuntary shrinkage or confiscations of shrinkage of voluntary muscle, also the harm of impulse etc.

Following necropsy and histopathological examination it was found that MSDES01 patches and hence proved to be toxic for chronic treatment. All or formulae i.e. MSDES02-04 did not show any signs of toxicity and exhibited normal histology of liver. This concluded that formulation MSDES01 cannot be used for long whereas formulations MSDES0204 can be used for long term in chronic condition without any toxic effect.
Which was confirmed by histopathological studies?

It has been detected that in animals medicated with Sildenafil citrate at dosage of 30 mg/kg, there was substantial (p<0.01) upsurge in mount incidence when matched with animals in control group.

Due to its mechanism of action, sildenafil citrate dilates cardiac tone as well as or smooth muscles. During clinical trials it was found that administration of sildenafil citrate leads to penile erection. Hence later on use of sildenafil was used on patients suffering from erectile disability.

In animals treated with testosterone at dosage of 0.5 mg/kg, at dosage of 30 mg/kg, there was substantial (p<0.01) upsurge in mount incidence when matched with animals in control group. This is probably due to mechanism of achievement by testosterone which leads to upsurge in protein development in target cells. Testosterone is transformed to DTH (droteosterone) with aid of enzyme 5α reductase. DTH gets bind to cytoplasm receptor protein migrates to nucleus. After migrating, DTH stimulates DNA and RNA synthesis. It leads to stimulation of RNA polymerase and lastly protein production improved with an upsurge in body weight also weight of ancillary sensual organs. Complete effect observed in all groups seems to be increase when compared to groups treated with control.

In animals medicated by formulations MSDE 01 to 04 at 200mg/kg per day there was substantial (p<0.01) upsurge in mount incidence when matched to animals in control set. Mount incidence is closely equivalent to standard utilized. This action may be due to nutmeg which stimulates mounting behavior of male mice. (Ahmad et al., 2003).

It has been observed that in animals medicated by Sildenafil citrate at dosage of 30 mg/kg, as well as testosterone at dosage of 0.5 mg/kg individually in dissimilar set, there was substantial (p<0.01) growth in mount latency, Intromission incidence, Intromission dormancy when matched with animals by control group.
While in animals treated with formulations, there was substantial (p<0.01) growth in mount latency, Intromission incidence, Intromission dormancy when matched with animals by control group.

*M. fragrans* suggestively improves growing Incidence, Intromission Occurrence, as well as Intromission Dormancy along with initiated significant decreasing in Mounting Dormancy also Post Ejaculatory Intermission (*Tajuddin et al.*, 2003).

Crocin and extract upsurges mounting incidence, erection occurrence as well as intromission occurrence behaviors also decreases intromission dormancy, mount dormancy along with ejaculation dormancy factors. (*Hosseinzadeha et al.*, 2008).

Karimi et al. resoluted antioxidant potential of Stigma Phenolic and Flavonoid Composites. Free radical scavenging and ferric decreasing power actions may be responsible for action.

Lodhra has effective antioxidant action beside ABTS test method (*Vijaya baskaran et al.*, 2010). *Ravichandran et al.* also revealed that standard constituents of *Symlocos racemosa* i.e. salireposide and benzoyl salireposide have effective antioxidant action (*Ravichandran et al.*, 2005).

*Piper betel*. Initiates decrease in propagative organ masses, number of litters, circulating level of *estrogen*, serum glucose concentration, fertility, enzyme action of acid phosphatase as related to control value (*Sharma et al.*, 2007).

From preclinical studies it could be confirmed that formulation containing mixture of extracts used has a potent aphrodisiac action.

From all above data and experimental studies it could be concluded that extracts used that is Lodhra, nutmeg, Kesar and Tamulapatra has aphrodisiac action and is used in formulation.
From physicochemical and physical testing data it is concluded that raw material used was of standard quality. Final formulation MSD/CA/002, was found to be physically, chemically stable, and formulation prepared is standardized using HPLC and HPTLC method. Therefore product is standardized, stable and with potent aphrodisiac action.

For future same formulation can be applied for patent. Along with proper CTD dossier, MSD/CA/002 can be registered anywhere in world as safe, efficacious and fully standardized Herbal product for male sensual disability.

1.5. Future scope and Recommendations:

Research and development of a new formulation in herbal sector for such a complicated indication like Aphrodisiac is like drawing on a huge canvas and hence there is a great future prospect to the present work.

In the present investigation there is setting up of a research problem and objectifying the goals towards achieving betterment in the Aphrodisiac activity is intended. As a research candidate I can see the things achieved through my research.

The present works define problem and GAP for new formulation, selection of potential herbs, analysis and validation of a well-developed dosage form like capsule. As per the preview of product following future scope of study can be predicted at present scenario.

The formulation has to undergo intensive preclinical studies to reach up to the insight for the best possible mode of action of formulation. Herbal formulation has beauty of multiple targeted actions for any condition. In agreement to that the present formula needs to be screened against androgenic activity action on testosterone levels, phosphodiesterase activity etc. the formula may possess special activity in diabetes/depression induced ED the same should be screened in animals. There should be same models higher than rat to get
more insight into behavioral changes. There should be acute, sub-acute and chronic efficiency studies in animals.

Present work extends possibility of performing toxicity and safety animal models in acute, sub-acute and chronic dosing pattern to make sure in vivo safety pattern of formula with biopsies of vital organs like liver, lungs, heart, muscles and kidney for toxic actions. Stability data for the period of 3 years has to be retrieved in order to get clearer picture of the chemical stability of the active contents in the formulation which gives clinical efficacy over shelf life of 2 years. The same will help physical to correlate clinical utility and clinical efficacy over shelf life.

Clinical assessments of formulation on human volunteers present a huge scope to actually document clinical outcomes. Healthy volunteers and patients are to be used in different models of clinical studies. The studies will involve selling up of behavioral and clinical parameters to access effect of product. Different levels of body enzymes and screening of semen will also help in reporting exact utility of product to help patients.

Clinical studies taking sildenafil like drug as a standard needs to be done in order to get to know exact position of clinical efficacy of product against widely used alternative. Towards seeking a complete solution towards problem an extension of formulation can be thought to opt for symptomatic as well as chronic or root cause treatment.

There could be development of a different formulation like massage oil to support clinical effectiveness of product. The same formulation should be developed based on the product activities to be amplified. The formula needs to be developed and standardized. The same product together with capsule form should be clinically as well as preclinical tested for safety and efficacy.

As sexual dysfunctioning are becoming predominant in the present time due to several reasons there should be more and more research carried out so that there would be betterment in sexuality of mankind.
As not only India but many developed countries are facing sexual dysfunctioning as a major ailment in society, there are many possibilities to get collaborated with foreign research partners to carry out further studies.

1.6. Limitations:

The researchers involved in the study are in agreement that there is limitation to the study because of several reasons like insufficient funding support from organizations involved in extensive research protocols.

The study limits itself to formulation and development with a brief insight an activity. The major portion of clinical assessment of product needs to be followed. There should be extensive research an activity, safety as well as marketing GAPS of product for the product to come into market.