International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 10 Issue 1 ǁ 2022 ǁ PP. 15-21

In Vitro Efficacy Evaluation of *Sarvonutra-Diva*™: The Herbal Pre-Mix for Women Wellbeing

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***Abstract**:*
Women, the feminine gender of human beings, play dynamic and diversifying roles in day-to-day activities. Women well-being doesn't restrict to good health and societal progress but also extends to 'quality of life (QOL) at individual level'. In comparison to men, among women synchronised, periodical action & regulation of various hormones are essential for wellbeing. However, modern lifestyle, stress, dietary habits etc. affected women's QOL, thereby copulation & infertility. Hence supplements must be natural origin with antioxidant, anti-inflammatory, libido-improving activity with no-toxicity.Sarvonutra-Diva™, the blend of herbal extract, developed as per Ayurvedic text for women wellbeing. Battery of in vitro tests viz. cytotoxicity, antioxidant & anti-inflammatory activities were evaluated in human ovarian cancer cell lines (SKOV3); in addition, the acute oral toxicity testing was evaluated as per OECD guideline – 423.In vitro results suggest minimal cytotoxicity was noted in SKOV3 cells as higher as 1000 µg/mL of Sarvonutra-Diva™, hence 250 & 500 µg/mL were used for antioxidant & anti-inflammatory activity. Significant dose dependence protection was noted in H_2O_2 induced oxidative stress and LPS induced inflammation by 250 & 500 µg/mL of Sarvonutra-Diva™ in SKOV3 cells. Acute single oral administration of Sarvonutra-Diva™ to female mice have shown no-morbidity & no-mortality. The results of current study conclude that has potential antioxidant & anti-inflammatory activities with no-toxic effects in the tested range.

***Key Words:** Women-wellbeing; libido, antioxidant activity, anti-inflammatory activity, cytotoxicity; human ovarian cancer cells – SKOV3.*

Date of Submission: 21-12-2021 Date of acceptance: 04-01-2022

I. Introduction:

Economic developments and the slogan of 'gender equality & equal opportunity' have enormously increased the employment opportunities for women, at the same time the happiness and wellbeing of women have decreased since the year 1970 (Stevenson, B., and Wolfers, J. 2009).One of the most considerable reasons could be 'lack of alternate workforce for household responsibilities'. In view of this, the term 'women wellbeing/wellness' coined and defined aswomen's health in terms of physical, emotional, and social aspects, along with quality of life (QoL), such as reproductive and hormonal issues, bone health, gastrointestinal (GI) stress, and urinary incontinence (Zender, R., Olshansky, E. 2009). Wellness of women interlinked with genetic composition of her parents, nutritional status as baby in womb, active growth period, pubertal stage, reproductive age and post-conceive stage. Hence, the progress towards women wellbeing is key for the progress of any nation;however, it is noteworthy to mention that region, race, religion, family structure, etc. are the sensitive & major influencing factors.

Continuity of human life on the globe is through motherhood, a complexphysical & physiological process initiates with copulation, conceive, development of embryo and birth toa young one.A variety of hormones acts in series and synergistic passion for ovulation, menstruation, fertilization, embryo development, delivery & lactation. It is well established fact that balanced action of reactive oxygenspecies (ROS) and antioxidant factors play critical role in regular process of body defence (immune) system, ovulation, menstruation & fertilization (Agarwal et al., 2012; Ruder et al., 2008).However, the imbalance either due to overwhelming action of ROS or insufficient antioxidant activity lead to devastating action of lipid peroxidation, protein carbonyl formation, DNA mutation and apoptosis (Tenkorang et al., 2018).Pertaining to female infertility various factors viz., ovulation process, fertilization & zygote formation, embryo development, the polycystic ovarian fibrosis (PCOS), preeclampsia, etc., influenced by the ROS, oxidative stress & inflammation

(Takagi., et al., 2004; Lagod., et al., 2001). Further, the *in vitro*, *in vivo*, animal and human studies suggests that inflammation interferes with female sexual desire and arousal by both direct (neural) and indirect (endocrine, endothelial, social/behavioral) mechanisms (Lorenz, 2019).

Female sexual desire and arousal are two important factors for individual QoL, reduce the infertility, as the copulation process involves the hormonal, neuronal, and muscular functions. Literature suggests that, frequent and regular penile-vaginal intercourse was positively correlated with greater mental health wellbeing, less depressive symptoms, better heart rate variability, and lower blood pressure, among other outcomes (Brody., 2010; Hall et al., 2014).Regular sexual activity has advantages for women, including regulating the menstrual cycle, relieving dysmenorrhea, and reducing the risk of endometriosis (Meaddough, et al., 2002).

The existing therapeutic agents are synthetic in nature, and have been known adverse effects upon long-term use. In addition, the cost of such wellness products are higher and becoming burden to low & mid-level socioeconomic population. In view of the above given background, the *Sarvonutra-Diva*™, a herbal product for improving the wellness of women is developed.

II. Materials and Methods:

The *Sarvonutra-Diva*™, was tested using *in vitro* system for its cytotoxicity, anti-oxidant potential, anti-inflammatory function along with *in vivo*non-clinical acute oral toxicity/safety as per OECG 423 guidelines. The *in vitro* studies were conducted in 'Human Ovarian Cancer cells - SKOV3'. The non-clinical study was conducted in SD rats.

2.1 Cytotoxicity Evaluation:

The monolayer cell culture of SKOV3 was trypsinized and the cell count was adjusted to 100,000 cells/mL using RPMI 1640, containing 10% FBS. About 10,000 cells/well were added and incubated at 37°C in humidified chamber with 5% $CO_2$for 24 h. The partial monolayer formed was washed once with medium and different concentrations (7.5125 µg/ml to 1000 µg/ml)of *Sarvonutra-Diva*™, was added each well of96 well plates and then the plates were re-incubated as mentioned above. After 24 h, microscopic examination was carried out and observations were noted. The drug solutions in the wells were discarded and 50 µL of MTT in DPBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2atmosphere. The supernatant was removed and 100 µL of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated and the concentration of test drug needs to inhibit the cell growth by 50% (CTC_{50}) values were generated from the dose-responsevalues for each cell line.

2.2 Anti-Oxidant Potential Evaluation:

Trypsinized monolayer of SKOV3 and cells were adjusted to 1.0 x 10^5 cells/ml; then 0.1 ml was added per well of the 96 well microtitre plate and incubated at 37°C in humidified chamber with 5% CO_2 per 24 h. Post-incubation the partial monolayer was washed once with medium and cells were treated with 500 µM of H_2O_2in medium with 2% FBS per 3h. The non-toxic concentrations (250 or 500 µg/mL) of *Sarvonutra-Diva™* were added after 3h and then the plates werere-incubated at 37°C in humidified chamber with5% CO_2 per 24 h. Post-incubation the plates were examined for microscopic alterations and the solutions werediscarded and 50 µL of MTT in DPBS was added to each well. The similar process explained above was adopted for evaluation of cell viability and 50% of cell inhibition (CTC_{50}).

2.3 Anti-Inflammatory Potential Evaluation:

The anti-inflammatory potential of *Sarvonutra-Diva™* was evaluated in lipopolysaccharide (LPS) induced inflammation in SKOV3 cells.The cells(10,000 cells/mL per well) were seeded in 96 well plate using RPMI-1640 with 10% FBS and incubated at 37°C in humidified chamber with 5% CO_2 per 24 h. The non-toxicconcentration of *Sarvonutra-Diva™*along with 100 µg/mL of lipopolysaccharide (LPS) were added after 24 hr and re-incubated at 37°C with 5% CO2 for 24 h.The test solutions were discarded and cell viability was measured through MTT assay as explained above.

2.4 Acute Oral Toxicity Testing:

The health hazards that are likely to arise from a single oral administration of *Sarvonutra-Diva™*were assessed in Sprague Dawley (SD) rats. Such data could be used to classify the test item to one of a series of toxicity classes defined by the LD_{50} cut-off values as per the Acute Toxic Class Method (OECD 423). A stepwise testing approach was adopted by using 3 female SD rats per every step, up to four steps. Handling of animals, test procedures, bleeding & euthanization, if necessary, etc., were performed as per the 'Institutional Animal Ethical Guidelines' with approval from IAEC.

2.4.1 Animals & Housing:

A total of 12 SD female rats; of which 6 rats aged 8 – 9 weeks with body weights ranging from 179.05 to 191.06 g for low dose (Step I & II) and another 6 rats aged 9 – 10 weeks weighing about 197.94 to 210.95 g were selected for high dose (Step III & IV) studies. One animal per cage washoused in solid bottom 'Individually Ventilated Cages' (IVC) [Citizen # V4/V30/10].Steam sterilized 'clean corn cob' was used as bedding material with periodic replacement on every 7th day. The temperature and relative humidity range of the experimental room was 20.1°C to 21.9°C and 50 to 64% respectively. Light and dark cycles of 12 hours each were maintained throughout the experimental phase. Rats have free access to purified water (Reverse Osmosis) and pelleted diet as *ad libitum.*

2.4.2 Test Compound Administration:

Intended clinical route of *Sarvonutra-Diva^{TM}*administration is oral route, hence the compound was dissolved in purified water (18 MΩ). A maximum dose volume of 10 mL/kg of test substance solution was administered to animals using *gavage*, based on body weight. Animals were fasted overnight, and administered the designated doses of *Sarvonutra-Diva^{TM}*. In addition, post-administration the feed was with-held for another 3 h with free access for drinking water.

In view of the lack of the information/data about the safety/toxicity of *Sarvonutra-Diva^{TM}*; a dose of 300 mg of *Sarvonutra-Diva^{TM}* per kg body weight of animal was administered in Step-I. The test compound related effects, if any were further confirmed with same dose (300mg/kg b.wt)in Step-II. Similarly, a dose of 2000 mg/kg of *Sarvonutra-Diva^{TM}* were administered to animals of Step-III and confirmed in Step-IV.

2.4.3 Mortality and General Clinical Signs:

All the animals were monitored for toxic signs related to test compound, if any, post-administration of*Sarvonutra-Diva^{TM}* at 30 min and at 1, 2, 3 and 4 h., there after once daily. Similarly, the mortality and morbidity was monitored twice a day throughout the experimental period. Food intake & water consumption were also observed during the experimental period. The body weight of the animals were recorded on 0th day (before test compound administration), 7th and 14th day using weighing balance (Sartoriuos # GPA3202).

2.4.4 Necropsy and Gross Pathology:

In case any pre-terminal mortality noticed during the experimental period, animals were subjected for gross necropsy to unveil the cause of death. Whereas, all surviving animals till the end of 14th day were euthanized by CO_2 asphyxiation. The necropsy procedures such as gross examination of the animals, collection of organs were performed and recorded as per the guidelines.

III. Results:

3.1 Cytotoxicity:

The following were the results of cytotoxicity evaluated in SKOV3 cell lines at a concentration range of as low as 7.5125 µg/ml to as high as 1000 µg/ml toof *Sarvonutra-Diva^{TM}* (Fig. 1).The results suggest that no-cytotoxicity at tested concentrations up to 500 µg/ml of *Sarvonutra-Diva^{TM}* and a minimal cytotoxicity (12.95±0.88%) was noted even at 1000 µg/ml to of *Sarvonutra-Diva^{TM}* (Fig. 1). In view of this, the CTC_{50} of *Sarvonutra-Diva^{TM}* is > 1000 µg/ml, hence further *in vitro* efficacy testing of anti-oxidant and anti-inflammatory activities were evaluated at a concentration of 250 and 500 µg/ml to of *Sarvonutra-Diva^{TM}*.

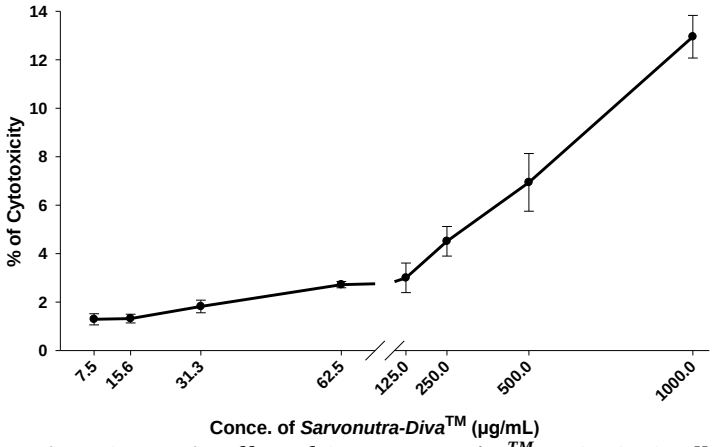

Fig.1. Cytotoxic Effect of *Sarvonutra-Diva^{TM}* on SKOV3 cells

3.2 Anti-oxidant Activity:

The anti-oxidant abilityof *Sarvonutra-Diva^{TM}*was assessed in SKOV3 cell lines exposed to as radical generating source. Subsequently, the cells exposed to H_2O_2 were incubated with250 and 500 µg/mlof *Sarvonutra-Diva^{TM}*, followed by assessed for cell viability through MTT assay. The data suggests that a higher level of protection was noted in cells (53.31±6.33%) incubated with 500 µg/mlof *Sarvonutra-Diva^{TM}*, in comparison to cells (28.41±5.52%) treated with 250 µg/mlof *Sarvonutra-Diva^{TM}* (Fig. 2).The results suggest a dose dependent protection with *Sarvonutra-Diva^{TM}*.

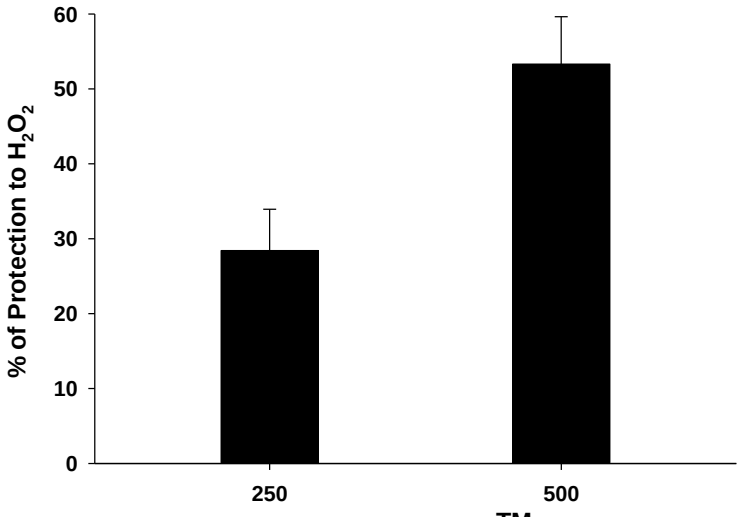

Fig. 2. Antioxidant Potential of *Sarvonutra-Diva^{TM}*

3.3 Anti-Inflammatory Potential:

The anti-inflammatory potential of *Sarvonutra-Diva^{TM}* was evaluated using SKOV3 cell lines treated with LPS as source of inflammatory agent and subsequently the protection was assessed with incubation of *Sarvonutra-Diva^{TM}*. The percentage protection was evaluated through MTT assay. Two concentrations of of *Sarvonutra-Diva^{TM}* viz. 500 µg/mland 250 µg/ml have shown the protection of 46.76± 5.43 % and 33.95 ± 4.52%, respectively over LPS control (Fig. 3). The results of anti-inflammatory potential suggest that a dose dependent response.

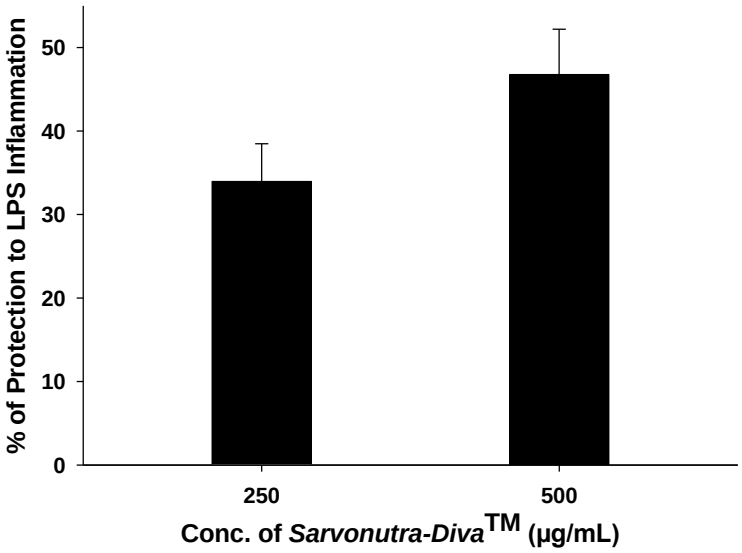

Fig. 3. Anti-inflammatory Potential of *Sarvonutra-Diva^{TM}*

3.4 *In Vivo* Acute Oral Toxicity:

The *Sarvonutra-Diva^{TM}* oral administration effect were assessed through step-wise evaluation of 'acute oral toxicity' as per OECD guidelines 423. Two different concentrations viz. 300 mg/kg and 2000 mg/kg were orally administered to SD rats. Results of the study suggest no-morbidity and no pre-terminal mortality was noted during the observation period up to 14 days. The food and water intake of all the animals administered with *Sarvonutra-Diva^{TM}* (300 mg/kg and 2000 mg/kg b.wt) were normal throughout the experimental period.

A proportionate increase in body weight was noted among the rats administered with *Sarvonutra-Diva^{TM}*(Fig. 4). The mean body weight gain by the Step I group rats on 7th and 14th day are 28.3±5.26 g and 24.2±4.65 g, respectively. Similarly, the body weight gain was noted in rats of 'Step II group' on day-7 (16.4±1.99 g) and day-14 (15.9±0.34 g). The body weight gain of ratsadministered with 2000 mg/kg of *Sarvonutra-Diva^{TM}*was 28.0±8.61 g (Step III) and 16.2±0.70 g (Step IV) on day-7. Similarly, on day-14 the gain in body weight was 15.7±1.31 g and 15.4±0.15 g among the Step III and Step IV group rats, respectively. Further, the gross necropsy and pathological examination of animals sacrificed at the end of experiment have shown 'no abnormality'. All the cage side observations were normal among the rats received 300 and 2000 mg/kg b.wt of *Sarvonutra-Diva^{TM}*.

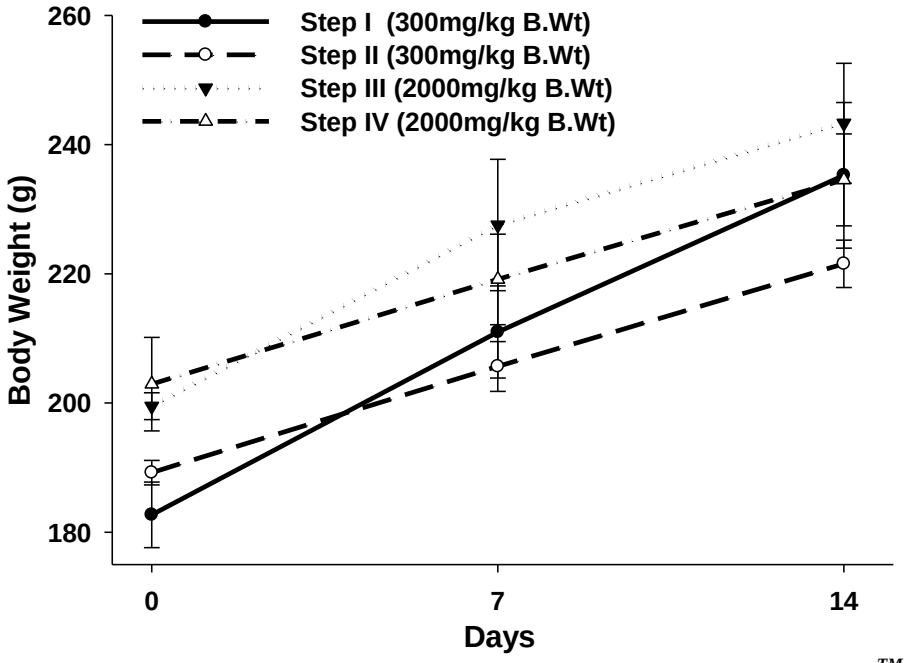

Fig. 4. Body weights of Rats Orally Administered with *Sarvonutra-Diva^{TM}*

IV. Discussion:

The *Sarvonutra-Diva^{TM}* is the herbal blend of *Asparagus racemosus*, *Saraka ashoka*, *Myristica fragrance*, *Symplocos racemose*, *Woodfordia fruticosa*, *Boerhavia diffusa*, *Centella asiatica*, *Citrullus lanatus*, *Areca catechu*, *Nelumbo nucifera,Cocus nucifera*. The test compound intended for wellbeing of women in terms of improving the mood swing, menstrual cycle regularities, libido thereby reducing the consequences of stress, anxiety, sleeplessness, infertility. It is well known fact that different types of hormones exert their action as individual and/or synergistically throughout the life span of women. As the term 'women wellbeing' includes the 'Quality of Life' (QoL) at individual level, it is essential to include the factors associated with sex, conceive, embryo-development, birth & nourishing of the baby.

The development in terms of education, socialization, urbanization, economic & industrialization helps in catering the equal opportunities to women. Thereby women are performing multi-faceted roles as employee at workplace, wife & mother at home. Hence the level of stress and anxiety have been mounted enormously. The 'work-burden' and stress together have negative impact on hormones and sexual desire of women. Hence, to improve the libido & arousal of women various synthetic and herbal products are available, however, there are their own limitations.

As per *Ayurvedic* literature *Asparagus racemosus* have been designated as female tonic, with main emphasis of alleviating female infertility, through improved libido, and reduction of inflammation especially in sexual organs, along with moistening of dry tissues of the sexual organs. *A. recemosus* enhances folliculogenesis

and ovulation process and prepares the womb for conception, prevents miscarriages. During postpartum phase it enhances the lactation, along with normalization of uterus and shift in hormones (Sharma & Bhatnagar, 2011).

Use of *Saraka ashoka* has ancient history, for menorrhagia (to stop excessive uterine bleeding), uterine disorders (menstrual pain in abdomen) etc. In addition, it has the proven anti-oxidant and anti-inflammatory activities (Baranwal, V.; 2014). The *Myristica fragrance* have been known for its anti-depressant, anti-inflammatory, anti-oxidant and analgesic activities (Kuete, V.; 2017). Ayurvedic literature suggest that *Symplocos racemose* bark extract has promising effect in treating female reproductive dysfunctions induced by cold restraint stress, along with positive effect on serum LH and FSH levels (Saraswathi, et al., 2012). The *Woodfordia fruticosa* flower extracts and *Boerhavia diffusa*root extracts were known to have the anti-inflammatory and analgesic activities. Few studies have also reported the antifertility activity of *W. fruticosa* flower and *B. diffusa*administered to laboratory animal models (Kumar et al., 2016; Jain, et al., 2016).

Centella asiatica has nutritional value and rich source of protein, vitamins and minerals. In addition, it has been used in various commercial products in improving the mental abilities, to enhance vascular support, as an anti-stress agent, to enhance immunity, as a lipid-lowering agent, and for many other valuable properties (Chandrika, et al., 2015). *Citrullus lanatus* seed has essential oils helps in improving the anti-oxidant and anti-inflammatory functions upon consumption. Citrulline, major phytoconstituent of *C. lanatus* has smooth muscle relaxation activity thus attributed for prevention of preterm deliveries through action myometrium (Munglue, et al., 2012).

The *in vitro* anti-oxidant and anti-inflammatory activities of *Sarvonutra-Diva*™, evaluated in SKOV3 cells exposed to H_2O_2 and LPS, respectively demonstrated substantial protective potential.Such activities of compound is due to the presence of various phytoconstituents of the selected herbs. In addition, the *in vitro* cytotoxicity assessed in SKOV3 cells, suggest minimal cytotoxicity at 1000 µg/mL of *Sarvonutra-Diva*™, hence the CTC_{50} is also above 1000 µg/mL. These observations were corroborated with 'acute oral administration' testing at a concentrations of 300 mg/kg and 2000 mg/kg body weight in SD rats. No morbidity and mortality was noted among the rats up to 14 days. Measurement of body weight and food consumption are being considered as two important and sensitive parameters for *In Vivo* nonclinical toxicity/ safety evaluation studies, to assess the 'illness', if any, related to test compound administration (WHO, 2005; Reddy et al., 2021). It is noteworthy to mention that the results of the current study have shown a proportionateincrease in body weight gain and food intake by theanimals during the course of experiment suggests no evidence oftoxicity.

V. Conclusion:

The results of the study concludes the compound has antioxidant and anti-inflammatory activities with no toxicity. The CTC_{50} of *Sarvonutra-Diva*™could be >1000 µg/mL, as there was minimal cytotoxicity noted in SKOV3 cells. Further, the LD_{50} cut-off value is 5000 mg/kg b.wt., as no-morbidity & -mortality was noted even at 2000 mg/kg single oral administration. In view of the selected herbs, it would be an ideal agent for improving the women wellness.

Acknowledgements:
The product - *Sarvonutra-Diva*™was developed with intramural grants from M/s Sarvotham Group, Hyderabad, India. The authors thank Mr. Mohan Krishna, Managing Director, Sarvotham Group, for his constant support.

Conflict of Interest:
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References:

[1]. Agarwal, A., Aponte-Mellado, A., Premkumar, B.J., Shaman, A., and Gupta, S. (2012). The effects of oxidative stress on female reproduction: a review. Reproductive Biology and Endocrinology, 10:49; 1-31.
[2]. Baranwal, V. (2014). Asoka: Herbal Boon to Gynecological Problems: An Overview of Current Research. Ayurveda Journal of Health. 12(4), 58 – 62.
[3]. Brody, S. (2010), The Relative Health Benefits of Different Sexual Activities. The Journal of Sexual Medicine, 7: 1336-1361.
[4]. Chandrika, Udumalagala Gamage, and Peramune A.A.S. Prasad Kumara. "Gotu Kola (*Centella Asiatica*): Nutritional Properties and Plausible Health Benefits." Advances in Food and Nutrition Research, October 1, 2015, 125–57.
[5]. Hall, K.S., Kusunoki, Y., Gatny, H., and Barber, J. (2014). Stress symptoms and the frequency of sexual intercourse among young women. J Sex Med. 11(8): 1982–1990.
[6]. Jain, N.K., Jain, S., Mehta, S.C., Tonpay, S.D. (2016). Antiimplantation and Antiestrogenic Activity of *Boerhaavia diffusa* Root Extract in Female Albino Rats. American Journal of Pharmacological Sciences. 4(2), 15-19
[7]. Kuete, V. (Ed.) Myristica fragrans: A Review. In Medicinal Spices and Vegetables from Africa; Elsevier Inc.: Amsterdam, The Netherlands, 2017; pp. 497–512, doi:10.1016/B978-0-12-809286-6.00023-6.
[8]. Kumar, D., Sharma, M., Sorout, A., Saroha, K., Verma, S. (2016). *Woodfordia fruticosa* Kurz.: A Review on its Botany, Chemistry and Biological activities. Journal of Pharmacognosy and Phytochemistry. 5(3): 293-298
[9]. Lagod L, Paszkowski T, Sikorski R, Rola R: The antioxidant-prooxidant balance in pregnancy complicated by spontaneous abortion. Ginekol Pol 2001, 72:1073-1078.

[10]. Lorenz, T.K. (2019). Interactions between inflammation and female sexual desire and arousal function. Curr Sex Health Rep. 1(4): 287–299.
[11]. Meaddough E, L, Olive D, L, Gallup P, Perlin M, Kliman H, J. (2002). Sexual Activity, Orgasm and Tampon Use Are Associated with a Decreased Risk for Endometriosis. Gynecol Obstet Invest. 53:163-169.
[12]. Munglue, P., Eumkep, G., Wray, S., Kupittayanant, S. Uterine relaxant effects of watermelon (*Citrullus lanatus*) extracts. Physiology 2012 (Edinburgh) (2012) Proc Physiol Soc 27, PC364
[13]. Reddy, Y.S., Babu, K.N., Kumar, P.U., Harishankar, N., Qadri, S., Surekha, M.V., Hemalatha, R., Kumar, B.D. (2021) Nonclinical safety evaluation of oral recombinant anti-human papilloma virus vaccine (RHPV 16 & 18): Regulatory toxicology studies in mice, rats and rabbits–An innovative approach. Vaccine. 39 (5), 853-863.
[14]. Ruder, E.H., Hartman, T.J., Blumberg, J., and Goldman, M.B. (2008). Oxidative stress and antioxidants: exposure and impact on female fertility. Human Reproduction Update.14:4; 345–357.
[15]. Saraswathi, C.D., S.K. Gupta and S. Sreemantula, (2012). Protective effect of Symplocos racemosa bark on cold restraint stress induced reproductive changes in female rats. J. Nat. Prod., 5: 251-258.
[16]. Sharma, K., Bhatnagar, M. (2011). *Asparagus racemosus* (Shatavari): A Versatile Female Tonic. International Journal of Pharmaceutical & Biological Archives. 2. 855-863.
[17]. Stevenson, B., and Wolfers, J. (2009). The paradox of declining female happiness. Am. Econ. J. 1, 190–225. doi: 10.1257/pol.1.2.190
[18]. Takagi Y, Nikaido T, Toki T, Kita N, Kanai M, Ashida T, Ohira S, Konishi I: Levels of oxidative stress and redox-related molecules in the placenta in preeclampsia and fetal growth restriction. Virchows Arch 2004, 444:49-55.
[19]. Tenkorang, M.A., Snyder, B., and Cunningham, R.L. (2018). Sex-related differences in oxidative stress and neurodegeneration. Steroids. 133: 21–27.
[20]. WHO guidelines on nonclinical evaluation of vaccines; 2005. Available at <http://www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/ANNEX%201Nonclinical.P31-63.pdf>.
[21]. Zender, R., Olshansky, E. (2009). Promoting Wellness in Women Across the Life Span. Nurs Clin N Am 44; 281–291.