Therapeutic potential of Seabuckthorn (*Hippophae rhamnoides* L.) in medical sciences

Hamid Ahani1,*, Soroush Attaran2,*

**ABSTRACT**

Seabuckthorn has multiple-use properties. This review explores the medicinal applications of *Hippophae rhamnoides* in healing ailments. The plant is being used in different parts of the world for its nutritional and medicinal properties. Sea buckthorn-based preparations have been extensively exploited in folklore treatment of slow digestion, stomach malfunctioning, cardiovascular problems, liver injury, tendon and ligament injuries, skin diseases and ulcers. In recent years, the medicinal and pharmacological activities of Seabuckthorn have been well investigated using limited clinical trials. Homeopathy is a well-respected modality to assist wellness. Traditional and modern medicinal experts have been applied this plant to treat various diseases. Seabuckthorn is an important plant because of its immense medicinal and therapeutic potential. However, several knowledge gaps identified in this paper would give impetus to new academic and R&D activities, especially for the development of Sea buckthorn-based herbal medicine and nutraceuticals. Its full application in dermatology may be attributed to the presence of a variety of flavonoids, vitamins, and unsaturated fatty acids. Great use of the plant in the traditional system for dermatological aspects, demands further comprehensive phytochemical work based on its actual use by the traditional population. Anti-inflammation is the most important applicable ingredient of this miracle berry.

**1. Introduction**

Sea Buckthorn (*H. rhamnoides* L.) from Elaeagnaceae family has become a crop of interest for the food processing industry. The accepted name in the plant list org of this species is *Elaeagnus rhamnoides* (L.)[1]. The exact number of species in the genus *Hippophae* is still unclear however, there are considered to be seven species and *H. rhamnoides* has nine subspecies [2].

*H. rhamnoides*, also known as common sea buckthorn is a species of flowering plant, native to the cold-temperate regions of Europe and Asia. It is a spiny deciduous shrub. The plant is used in the cosmetic industry, in traditional medicine (useful for the treatment of skin disorders resulting from bed confinement, stomach and duodenal ulcers cardiovascular diseases and perhaps the growth of some tumors), as animal fodder and for ecological purposes. The plants have a very developed and extensive root system, and the roots live in symbiosis with nitrogen-fixing Frankia bacteria. The roots also transform insoluble organic and mineral matters from the soil into more soluble states[3].

Vegetative reproduction of the plants occurs rapidly via root suckers. *E. rhamnoides* has a strong ability to maintain leaf water and

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can increase chlorophyll content, reduce photosynthesis and water relations during drought stress [4]. Seed germination at its lowest point of origin China with 32% and the most was in East Azerbaijan with 95% [5].

Means of germination percent in seed pretreatments (control, cold, ice water, hot water, lime juice and Gibberellic acid) were 7.5, 23.75, 21.25, 0, 15, and 42.5 in the field (Ahani et al., 2014) and 3.75, 43.75, 17.5, 1.25, 15 and 37.5 in the greenhouse (Ahani et al, 2014) and in the laboratory were 33, 12, 41, 4, 9 and 32, respectively[1, 6].

The DNA weight and the mean A260 and A280 values of the samples from China were found to be statistically significantly higher than those of the samples from Iran, whereas the mean A260/280 ratio of the samples from Iran was higher than that of the sample/isolate from China albeit by a non-significant difference [5, 6].

Despite the decrease in morphological characteristics encountered to drought, this species has been able to tolerate this amount of salt and no died that showed comparative tolerance for this species. Therefore offers for Elaeagnus rhamnoides species resistance threshold electrical conductivity (EC= 12.03dS m⁻¹) of the lower levels were used to determine which however need to do other tests because the morphological indicators studied here were not able to clearly explain the differences in salt tolerance and Physiology parameter (i.e. Water Use Efficiency (WUE), Relative Water Content (RWC), Water Potential (WP), Water Saturation Deficit (WSD), Chlorophyll content and Photosynthetic of leaves[7].

2. Ecological review

Seabuckthorn is multiple-use properties. It is very rich in its biodiversity. The exact number of species in the genus Hippophae is still unclear however, there are considered to be seven species H. rhamnoides L. has 9 subspecies. According to the latest study, there are 15 species and subspecies in Hippophae (Table 1). H. rhamnoides subspecies has been widely used for ecological restoration and producing a series of products. In Europe, H. rhamnoides subsp. rhamnoides is used in many countries like Germany, Italy, Switzerland, Sweden, Finland etc. Several improved varieties have been cultivated in these counties [8, 9].

In the Central Asia and South Asia, a widely distributed subspecies is H. rhamnoides subsp. turkestanica. Due to its rich wild resources, this subspecies is being used for producing a lot of products in India, Pakistan, Turkmenistan, Kirghizstan, etc. Since seabuckthorn is rich in wild resources, so they are directly being used for various purposes. But wild seabuckthorn has only disadvantages like small berries, many thorns for processing. Few studies have been done on these species and subspecies. Since many countries are aware of seabuckthorn is a very important plant in economy and ecology, it is believed that more attention and more studies will be given to those genetic resources of Hippophae, including those that have been used and that have not been touched but very promised. It is estimated that more than 20 countries have their breeding programs on seabuckthorn[10, 11].

Each country has its localization of genetic resources so it is needed to make international cooperation on the exchange of genetic resources of Hippophae. It is believed that the favourite varieties will be produced through wide international cooperation [12].

3. Medicinal advantages

Valuable substances contained in seabuckthorn oil play an important role in the proper functioning of the human body and give skin a beautiful and healthy appearance. A balanced composition of fatty acids gives the number of vitamins or their range in this oil and explains its frequent use in cosmetic products for the care of dry, flaky or rapidly aging skin. Moreover, its unique unsaturated fatty acids, such as palmitoleic acid (omega-7) and gamma-linolenic acid (omega-6), give seabuckthorn oil skin regeneration and repair properties. Sea-buckthorn oil also improves blood circulation, facilitates oxygenation of the skin, removes excess toxins from the body and easily penetrates through the epidermis. Because inside the skin the gamma-linolenic acid is converted to prostaglandins, seabuckthorn oil protects against infections,
prevents allergies, eliminates inflammation and inhibits the aging process (Figure 1; Table 2) [13-15].

Table 1. The Distribution and the Status of Utilization of Hippophae [12]

<table>
<thead>
<tr>
<th>Taxons</th>
<th>The Areas of Distribution</th>
<th>The Status of Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. rhamnoides. subsp. rhamnoides</td>
<td>Scandinavian countries, Baltic Sea countries, Germany, Belgium, Netherlands, Ireland, Poland, U.K., France, Russia</td>
<td>Many varieties are cultivated in some European countries and Canada</td>
</tr>
<tr>
<td>H. rham. subsp. sinensis</td>
<td>The North, Northwest, Southwest of China</td>
<td>Wild resources are used for ecological restoration and berries are processed for products. Some new varieties are in tests.</td>
</tr>
<tr>
<td>H. rham. subsp. yunnanensis</td>
<td>Sichuan, Yunnan, Tibet of China</td>
<td>Wild resources are used for ecological restoration only.</td>
</tr>
<tr>
<td>H. rham. subsp. mongolica</td>
<td>Siberia of Russia, Mongolia, Xinjiang of China</td>
<td>More than 60 varieties are cultivated in Russia, Mongolia, many East European counties. Many West European counties, Canada and China introduced the varieties for test</td>
</tr>
<tr>
<td>H. rham. subsp. turkestanica</td>
<td>India, Pakistan, Afghanistan, Turkmenistan, Kirghizstan, Uzbekistan, Kazakhstn, Iran, Turkey, Xinjiang, Tibet of China</td>
<td>Wild resources are used for ecological restoration and berries are processed for various products</td>
</tr>
<tr>
<td>H. rham. subsp. fluviatilis</td>
<td>Around Alps Mountains: Germany, France, Switzerland, Austria, Czech, Slovakia, Italy, Transylvanian Alps, the valley and the mouths of the Donube and its tributary.</td>
<td>Most of wild resources are protected as forest species. Some berries are collected for processing products</td>
</tr>
<tr>
<td>H. rham. subsp. carpathica</td>
<td>The Carpathian Mountains, Transylvanian Alps, the valley and the mouths of the Donube and its tributary.</td>
<td>Most of wild resources are protected as forest species. Some varieties are cultivated for the test.</td>
</tr>
<tr>
<td>H. goniocarpa</td>
<td>Sichuan, Qinghai of China</td>
<td>Most wild resources are protected as forest species. Very few studies have been done on it.</td>
</tr>
<tr>
<td>H. goniocarpa subsp. litangensis</td>
<td>Sichuan, Qinghai of China</td>
<td>Most wild resources are protected as forest species. Very few studies have been done on it.</td>
</tr>
<tr>
<td>H. neurocarpa</td>
<td>Sichuan, Qinghai, Gansu of China</td>
<td>Most wild resources are protected as forest species. Very few studies have been done on it.</td>
</tr>
<tr>
<td>H. neurocarpa subsp. stellatopilosa</td>
<td>Sichuan, Qinghai, Tibet of China</td>
<td>Most wild resources are protected as forest species. Very few studies have been done on it.</td>
</tr>
<tr>
<td>H. tibetana</td>
<td>Sichuan, Qinghai, Gansu, Tibet of China, Nepal, India</td>
<td>Most wild resources are protected as grassland species. Very few studies have been done on it.</td>
</tr>
<tr>
<td>H. gyantsensis</td>
<td>Tibet of China</td>
<td>Most wild resources are protected as forest species. Some berries are collected for producing Tibetan medicine.</td>
</tr>
<tr>
<td>H. salicifolia</td>
<td>The southern slope of Himalayan Mt. Tibet of China, Bhutan, Nepal, India</td>
<td>Most wild resources are protected as forest species. Some berries are collected for producing products.</td>
</tr>
</tbody>
</table>
The immune system of this plant can prevent some virus spread. Modern cosmetic and pharmaceutical companies search for natural substances which display unique properties such as sea-buckthorn oil, which added to a product even in a small quantity will undoubtedly ensure its uniqueness [13]. 14-Noreudesmanes and a phenylpropane heterodimer from seabuckthorn berry inhibit Herpes simplex type 2-virus replication, therefore; these bioactive exhibited an antiviral effect [16]. Seabuckthorn extract may make restrictions for the Dunge virus [17]. Seabuckthorn decreases fever of some diseases [18]. Extraction of leaves can limit lung cancer. In addition, seabuckthorn prevents some viruses such as victoria and influenza viruses [19]. Seabuckthorn and several plants could limit the spreading of HIV [20]. Adeno, HIV, HPV viruses can limit by seabuckthorn [21].

Sea buckthorn has also shown unique biological properties against viral diseases, anti-viral activity against the influenza virus and herpes virus. The suppressing effect on the influenza virus is provided by the inhibition of viral neuraminidase present in the virus. Seabuckthorn properties were evaluated with the help of humoral immune reaction against NCD (Newcastle disease virus). Hexane extract from sea buckthorn acts positively against indomethacin, stress, and ethanol which contribute to the development of gastric ulcers [10]. Treatment with SBT bud extract reduced the virus titer to 2.0 TCID50/ml at 50 μg/ml, while the HA titer was reduced from 1431 (control) to 178. Concentrations lower than 50μg/ml displayed an inhibitory effect in the HA assay, but not in the TCID50 virus titration [22].

In traditional Chinese medicine and the former Soviet Union inflammation of the mouth, stomach ulcers, radiation injuries and burns have been used [23]. Anti-bacterial and antioxidant support and protection of the natural seeds of this plant species is recommended. Methanol fruit and leaves of the plant are also antioxidants and help prevent cell necrosis [24].

The oil extracted from berries is used for the treatment of gastritis, stomach ulcers, erosion of the uterus and inflammation of genital organs. Seabuckthorn leaves contain nutrients and bioactive substances which mainly include flavonoids, carotenoids, free and esterified sterols, triterpenols, and isoprenols. The leaves are an equally rich source of important antioxidants including carotene, vitamin E, catechins, elagic acid, ferulic acid, folic acid and significant values of calcium, magnesium and potassium [11]. Bone-breaking fever virus in the blood of substances extracted from sea-buckthorn leaves is inhibited [17]. The total phenolic content of root and seed extracts was significantly higher than leaf and stem extracts. No significant differences were seen between root and seed, or between leaf and stem [25].

4. New approaches
Sea buckthorn can act as promising functional food. Sea buckthorn extract can effectively inhibit prostate cancer growth and proliferation in vitro. Sea buckthorn extract effectively downregulates prostate-specific antigen with other androgen-responsive genes in vitro. Differential extraction using various solvents based on polarity revealed that the end phase aqueous cocktail extracted from leaves of *H. rhamnoides* L. (SKICDDL-3) can effectively target AR and downregulate androgen-responsive genes, PSA, ELL2, EAF2 and CALR significantly in vitro. Colony formation Unit assay and Wound healing assay further show that SKICDDL-3 can effectively inhibit proliferation and migration of castration-resistant C4-2 prostate cancer cells in vitro. Sea buckthorn (*H. rhamnoides* L.) has recently attained worldwide recognition, for its pharmaceutical and nutraceutical potential and is currently cultivated in several parts of the world [26].

The findings of flavonol suggest that mechanisms of growth inhibition by pentamethylquercetin, syringetin and isorhamnetin are different from the apoptosis caused by quercetin, kaempherol and myricetin[27].
Fig. 1. Sea-buckthorn pulp oil batch

Table 2. Ingredients of seabuckthorn fruits (derived from sinensis subspecies)

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Component</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.2</td>
<td>Myristic Acid C14:0</td>
<td>0.39</td>
</tr>
<tr>
<td>2</td>
<td>17.7</td>
<td>Pentadecanoic Acid C15:0</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>20.4</td>
<td>Palmitic Acid C16:0</td>
<td>32.03</td>
</tr>
<tr>
<td>4</td>
<td>21.2</td>
<td>Z-Palmitoleic Acid C16:1 ∞7</td>
<td>28.05</td>
</tr>
<tr>
<td>5</td>
<td>21.5</td>
<td>E-Palmitoleic Acid C16:1</td>
<td>0.11</td>
</tr>
<tr>
<td>6</td>
<td>22.6</td>
<td>Hexadecenedioic Acid C16:2</td>
<td>0.11</td>
</tr>
<tr>
<td>7</td>
<td>22.8</td>
<td>Tricosene</td>
<td>0.09</td>
</tr>
<tr>
<td>8</td>
<td>23.1</td>
<td>Margaric Acid C17: 0</td>
<td>0.10</td>
</tr>
<tr>
<td>9</td>
<td>23.2</td>
<td>Tricosene</td>
<td>0.12</td>
</tr>
<tr>
<td>10</td>
<td>23.9</td>
<td>8-Heptadecenoic Acid</td>
<td>0.20</td>
</tr>
<tr>
<td>11</td>
<td>24.4</td>
<td>Heptadecenoic Acid Isomer</td>
<td>0.03</td>
</tr>
<tr>
<td>12</td>
<td>26.3</td>
<td>Stearic Acid C18: 0</td>
<td>1.20</td>
</tr>
<tr>
<td>13</td>
<td>27.1</td>
<td>Oleic Acid C18: 1∞9</td>
<td>23.83</td>
</tr>
<tr>
<td>14</td>
<td>27.3</td>
<td>Cis-Vaccenic Acid C18: 1∞7</td>
<td>7.90</td>
</tr>
<tr>
<td>15</td>
<td>28.3</td>
<td>Linoleic Acid C18: 2∞6</td>
<td>3.29</td>
</tr>
<tr>
<td>16</td>
<td>31.5</td>
<td>Linoleic Acid C18: 3∞3</td>
<td>1.84</td>
</tr>
<tr>
<td>17</td>
<td>34.8</td>
<td>Arachidic Acid C20:0</td>
<td>0.27</td>
</tr>
<tr>
<td>18</td>
<td>35.9</td>
<td>Gondoic Acid C20:2 ∞9(Gadoleic)</td>
<td>0.23</td>
</tr>
<tr>
<td>19</td>
<td>42.3</td>
<td>Behenic Acid C22: 0</td>
<td>0.04</td>
</tr>
<tr>
<td>20</td>
<td>52.0</td>
<td>Lignoceric Acid C24:0</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>99.98</td>
</tr>
</tbody>
</table>

The antiproliferative effect of *H. rhamnoides* L. leaves extract on acute myeloid leukemia cells was at least partially determined by activation of the S phase checkpoint, which probably led to deceleration of the cell cycle and apoptosis induction [28].

Sea buckthorn may represent a "golden mean" for the treatment of cancers: It has anti-proliferation properties and can induce apoptosis and stimulate the immune system, and sea buckthorn oil counteracts many side effects of chemotherapy by restoring kidney and liver function, increasing appetite, and keeping patients in generally good health. Although the anticancer activity of sea buckthorn has been confirmed by many in vitro and animal in vivo studies, the treatment
and prophylactic doses for humans are unknown[1].

Seabuckthorn (H. rhamnoides L.) constitutes thorny nitrogen-fixing deciduous shrub. Sea buckthorn (SBT) is primarily valued for its very rich vitamins A, B1, B12, C, E, K, and P; flavonoids, lycopene, carotenoids, and phytosterols. and therapeutically important since it is rich in potent antioxidants. Hippophae sp has high-nutritional and medicinal values due to its very rich antioxidant property. It is a widely used plant in traditional medicine for various clinical conditions. Scientifically evaluated pharmacological effects of it are like antiulcerogenic effect, in vitro and in vivo antioxidant effects, cardiac disease, anti-atherogenic effect, radioprotective effects, beneficial effects on experimental injury and clinical diseases of the liver, inhibition of platelet aggregation. A lot of research work is still needed to find cellular and molecular mechanisms of these activities[29].

In vitro, Cytotoxic and anti-proliferative effects of hydro-alcoholic extract of H. rhamnoides Linn (HEHR) seeds were investigated on human leukemia (HL-60) and normal (BHK21) cells while in vivo anti-proliferative effect of HEHR was evaluated on Ehrlich ascite carcinoma (EAC) induced Swiss albino mice. anti-proliferative effect of HEHR due to its interference with the cell kinetics which indicated the reduction in the GSH levels and colony growth. The cytotoxic effect of HEHR is produced by the apoptosis mechanism which involved DNA fragmentation [30].

Accumulation of cholesterol in the aorta was studied using the Sudan-IV staining technique. SBT seed oil feeding to normal rabbits for 18 days caused a significant decline in plasma cholesterol, LDL-C, atherogenic index (AI) and LDL/HDL ratio. The HDL-C levels, HDL-C/TC ratio (HTR) and vasorelaxant activity of the aorta were significantly increased. In cholesterol-fed animals, the TC, TG, LDL-C and AI were significantly increased and showed a decline following seed oil administration. The increase in HDL-C was more marked in seed oil-treated hypercholesterolemic animals. The acetylcholine-induced vasorelaxant activity was significantly decreased in cholesterol-fed animals and could be restored to that of normal values by seed oil administration. These observations suggest that supercritical CO(2) extracted SBT seed oil has significant anti-atherogenic and cardioprotective activity[31].

Sea buckthorn powder (SBP) was administered at varying concentrations (0.6, 0.9, 1.2, 1.5 and 1.8 µg mL$^{-1}$) to cell cultures (BE(2)-M17) with 20 mm Aβ for 72 h. MTS test indicated that SB significantly increased cell viability in Aβ-induced cells up to 95%. Results of Western blot showed maximum of 38% inhibition of Aβ compared to the control (Aβ only). ELISA demonstrated a significantly lower amyloid-β level (6672 pg mL$^{-1}$) than the control (10189 pg mL$^{-1}$). These findings suggest that this plant warrants further investigation as a potential therapeutic agent in the treatment of AD [32].

Naturally occurring vitamin B12 is only found in animal products such as meat, milk, dairy products, fish, oysters and clams, but it is well-known for its absence in plant-based foods. significant amounts of vitamin B12 were detected only in H. rhamnoides (37 µg/100 g dry weight). These initial findings provide the basis for the detection of vitamin B12 also in other plants and can be a good measure of prevention for vitamin B12 deficiency in vegetarians [33].

There have been numerous bioactive in Hippophae sp, some of which are rare in the plant kingdom e.g., the ratio of palmitoleic or Omega 7 to γ-linolenic acid or Omega 6. Vitamin C is present in very high amounts (up to 900 mg%). In comparison with citric fruits, sea buckthorn berries have about a 14-times higher amount of vitamin C than oranges. The oil used internally has positive effects on the digestive system lowering inflammation. Oral application is adjuvant in the treatment of gastric, duodenal, and intestinal ulcers. It has been shown to reduce inflammation processes in the vagina and cervix. A high amount of vitamin C makes it suitable for immune deficiencies; due to its antioxidant activity, it removes free radicals and strengthens the immune system. Hippophae oil lowers blood
cholesterol, which helps to prevent atherosclerosis. Seabuckthorn was tested and shown to significantly increase the level of beneficial high-density lipoprotein (HDL) cholesterol fraction. It reduces the risk of thrombophlebitis and is enrolled in the control of bleeding. Febrile states respond positively to oil, as well as symptoms of rheumatoid disease. Some of the lipophilic components (α- and γ-linolenic acids) of Seabuckthorn oil positively influence brain functions and the central nervous system by an antidepressant effect. Its advantage as an adjuvant in cancer therapy is that fastens regeneration after the use of chemotherapy [31, 34].

The favoring feature of oil is that it is considered safe, with no potential harmful effects. It can be consumed by pregnant and breastfeeding women. The suggested pharmaceutical form that would be ideal for the application of oil is capsules, because of the problem with rancidity (presence of unsaturated fatty acids). Different fractions of fruits were investigated for antioxidant activity and its relationship to different phytoneutrients. The capacity of the crude extracts, such as the phenolic and ascorbate extracts, to scavenge radicals decreased significantly with increase d maturation. The antioxidant capacity of the lipophilic extract increased significantly and corresponded to the increase in total carotenoids [35].

Seabuckthorn is primarily found in cold-temperate regions of Eurasia and was first located in China. Berries are the most prominent feature of the plant. Phytochemical studies reveal the presence of a wide variety of compounds like flavonoids, carotenoids, polyunsaturated fatty acids, minerals, vitamins, Omega 3, 6, 9 and rarest Omega 7 and about 190 bioactive compounds [3].

The pharmacological evaluation confirmed the ethnomedical claimed biological actions and other beneficial effects on the skin of H. rhamnoides using scientifically accepted protocols and controls, although some of the studies require more elaborative studies. Its full application in dermatology may be attributed to the presence of a variety of flavonoids, vitamins, and unsaturated fatty acids. Great use of the plant in the traditional system for dermatological aspect, demands further comprehensive phytochemical work based on its actual use by the traditional population. Demonstration of the plant in the traditional system, pharmacology, cosmeceuticals not only demands its further therapeutic studies but also warrants focus towards its cultivation and propagation across the globe [3].

Signs of irritation (corneal epithelial inflammation/corrosion, dilatation of blood vessels in the bulbar conjunctiva, conjunctival chemosis, dots on the margin of the cornea, oedema on the margin of the conjunctiva/cornea, eyelid irritation, and other possible signs of irritation) were evaluated and scored from photographs taken of the eyes and eyelids at study visits. In part one, the Hippophae spray was well tolerated. In part two, OSDI decreased significantly (P = 0.022) in the Seaberry spray eye compared to the reference spray, indicating a beneficial effect on symptoms. In part three, OSDI in the SB spray eye decreased significantly compared to the untreated control (P = 0.0007). Symptom sums and frequencies of dryness (sum P = 0.0046, frequency P = 0.0016) and watering (sum P = 0.0003, frequency P = 0.013) in the daily logs were lower in the eye treated with Seaberry spray [36]. Aqueous extract of Seabuckthorn (H. rhamnoides L.) leaves and evaluation of its therapeutic role in oxidative stress-induced cataract in isolated goat lenses using Vitamin E as reference compound. Results showed the potential to delay the onset and/or progression of cataracts, at least during in vitro conditions. Results indicate the possibilities of evaluating this extract for its use as an ant cataract agent during in vivo conditions [37].
5. conclusion

Great use of the plant in the traditional system for dermatological aspects, demands further comprehensive phytochemical work based on its actual use by the traditional population. Anti-inflammation is the most important applicable ingredient of this miracle berry.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors’ Contribution

All authors had equal role in study design, work, statistical analysis and manuscript writing.

Informed Consent

The authors declare not used any patients in this research.

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