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Review Article

A comprehensive review on the pharmacological properties of *Diplazium esculentum*, an edible fern

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Abstract

Diplazium esculentum (Family: Athyriaceae), one of the most popular wild edible fern, is considered as a pharmacologically diverse ethnomedicinal plant. The coiled fronds and young leaves of this plant have a wide range ethnomedicinal uses in folk medicine around the world directed for a number of ailments. The present article is the first comprehensive review on the pharmacological activities of this particular edible fern. The structural formulas of all the chemical constituents identified and isolated so far from *D. esculentum* are also provided in the present review. The most thoroughly studied pharmacological activities *viz.*, antioxidant, antimicrobial, immunomodulatory, neuromodulatory, anti-fertility, cytotoxic, etc.) of the *D. esculentum* extracts have been discussed. This comprehensive review will be of help for the future researchers investigating for more potent compounds and their pharmacological activities.

Keywords: bioactive compounds; pharmacological activity; immunomodulation; neuromodulation; antioxidant

1. Introduction

Ferns are one of the most widely used wild edible groups of plants throughout the world. Their origin have been dated back to the Paleozoic era and they constitute the primitive vascular plant group which is found scattered all over the world. Due to their primitive origin, ferns have been well adapted with various changes of the environment than other primitive vascular plants of the world (Wallace et al. 1991). Therefore, ferns are expected to have many useful phytochemicals than other plants. The presence of these diverse groups of phytochemicals largely influence the pharmacological properties of ferns when they are consumed, which ultimately determines its beneficial and / or detrimental effects on human and animal health. It is interesting to note that only a few of the fern groups are used as food throughout the world. The fern stems, rhizomes, leaves, young fronds and shoots, and sometimes the whole plants are used for food (Liu et al., 2012). *Diplazium esculentum* (Koenig ex Retz.) Sw. (Family – Athyriaceae) is one such fern which is considered as one of the most commonly consumed edible ferns throughout the world.

Though there are some literatures on the ethnobotanical and ethnomedicinal studies of *D. esculentum*, the epidemiological studies of this fern have not yet been attempted. The studies done so far on this fern were mainly concerned with its beneficial effects either *in vitro* or *in vivo* in small laboratory animals. Very few researchers have focused on its health impacts. Most of the studies on this fern have been conducted to assess the antioxidant, antimicrobial, antitumor or other beneficial activities (Nanasombat & Teckchuen, 2009; Tongco et al., 2014; Kaushik et al., 2012; Seal, 2012) but very few studies have been performed so far to determine the possible pharmacological and toxicological impacts of this fern on human and animal health. In a nut shell, no comprehensive review of this plant has been reported to date which demonstrates all the bioactivities of this plant. Therefore, the overall aim of the present review is to elucidate the potential therapeutic prospects as well as health deteriorating properties of *D. esculentum* taking consideration on the latest and updated information on this plant, and thereby to advance the existing knowledge of this fern as food and medicine in relation to human health.

2. Brief morphological description

D. esculentum is an edible fern, pantropical in distribution and occurs widely and commonly throughout India, China, Cambodia, Laos, Thailand, Vietnam and Malaysia. It grows in gregarious colonies in open marshy areas, stream banks and canals from sea level to 2,300 m. The rhizome is erect, often forming a slender leaning black trunk to 1 m tall, scaly at the apex. Scales are 1 cm long, dark brown, margins finely toothed, apex long-acuminate. Fronds are 1–2 m long, 0.5–1 m wide, erect to arcuate. Stipe is black and scaly at the base, paler above. Lamina is 2–3-pinnate, 0.5–1.5 m long, 0.5–1 m wide, dark green. Secondary pinnae variable in size, commonly 5–8 cm long, 1.5–2.5 cm wide, margins very shallowly lobed, lobes are toothed, basal lobes longer than the rest, glabrous beneath, veins are simple or forked, lowest 3–5 pairs of adjacent vein groups anastomosing. Sori spreading along most veins; indusium thin, dark brown, margins becoming uneven with age (Roy, 2017).

3. Taxonomic classification

Kingdom: Plantae
Division: Pteridophyta
Class: Polypodiopsida
Order: Polypodiales
Family: Athyriaceae
Genus: *Diplazium*
Species: *D. esculentum*

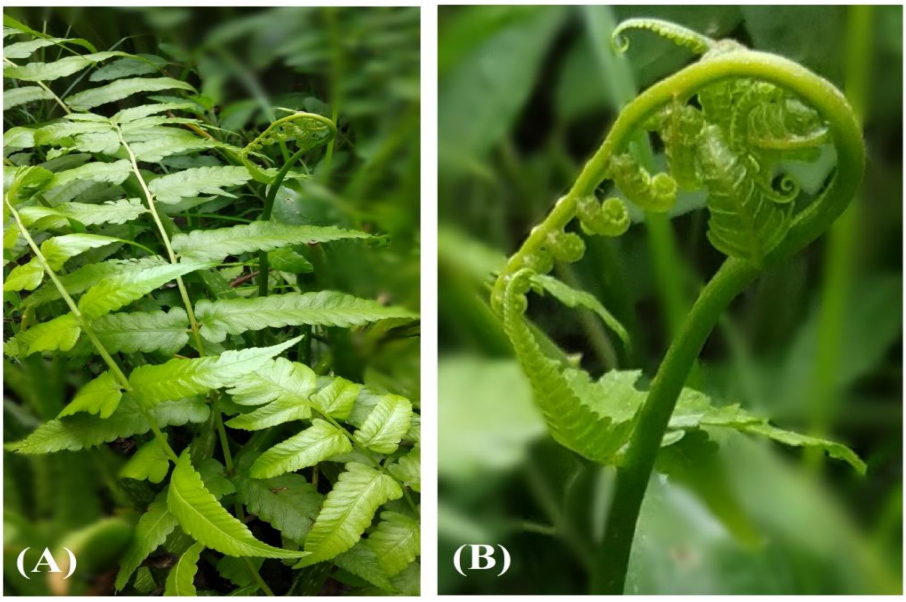

Figure - 1: (A) *Diplazium esculentum* in its natural habitat, (B) Coiled fronds of *D. esculentum*

4. Uses of *D. esculentum* as food

Diplazium esculentum is one of the most common varieties and the most commonly consumed fern throughout Asia and Oceania. In India, young fronds of *D. esculentum* are popularly known as lingra in Northern India, rukja and lochanch in North Eastern India and dheki sak in West Bengal, India. The newly emerging coiled fronds are consumed after cooking as a seasonal vegetable during monsoon season which continues for almost five months. The frond of this fern is generally cooked in oil or butter; using them in a vegetable curry is less preferred (Roy et al., 2013a). In the northeastern India, especially in Sikkim, and in the central and north-western Himalayan states of India (Himachal Pradesh and Uttarakhand), the local folk relish both vegetables and pickles from *D. esculentum*. Natives consider these recipes effective both to counteract constipation and as an appetizer, especially as a pickle (FAO, 2010). Study conducted in the villages of the Parvati valley, Himachal, India revealed that out of the 50 consumed wild edibles, *D. esculentum* is used as a vegetable/pickle by an average of 66% of the inhabitants (Kala, 2005). In Malaysia, this plant is eaten as 'ulam' or green edible leaves, usually consumed with hot sauce. This practice of eating 'ulam' with sauce is also known as 'krawoo' (Rahmat et al., 2003).

5. Ethnomedicinal uses

The Apatani tribe of Arunachal Pradesh, India uses the frond of *Diplazium esculentum* plant as medicine for constipation (Kala, 2005). It has been shown that the local inhabitants of Similipal Biosphere Reserve, Orissa, India used to take honey with decoction of boiled water extract of *D. esculentum* in empty stomach twice a day for 15 days to cure spermatorrhea (Rout et al., 2009). The natives of Adi tribe of Dehang-Debang Biosphere Reserve of Arunachal Pradesh, India use the boiled young fronds of *D. esculentum* as vegetables with boiled rice for laxative purpose (Kagyung et al., 2010). In the Kolli hills of Eastern Ghats, Tamil Nadu, India, the natives use handful of *D. esculentum* leaves to make juice and taken orally twice a day to get relief from cold and cough (Karthik et al., 2011). Moreover, the local people of this region use the frond parts of this plant as laxative and often used to treat colitis and constipation (Perumal, 2010). The local people of Manokwari, West Papua Province use this plant for wound healing and as an ailment of headache (Lense, 2011). In a Sudanese community in Indonesia, *D. esculentum* is used for the treatment of fever, dermatitis and measles (Roosita et al., 2008).

6. Pharmacological properties of *D. esculentum*

6.1. Phytochemical analysis of *D. esculentum*

D. esculentum is reported to possess a diverse group of phytochemicals, *viz.*, alkaloids, anthraquinones, anthranol glycosides, cardiac glycosides, cyanidins, flavonoids, glucosides, leucoanthocyanins, phenolic compounds, phytosterols, saponins, steroids, tannins and terpenoids. (Das et al., 2013, Tongco et al., 2014; Akter et al., 2014). The bioactive compounds present in this fern are esculentic acid, 5-*O*-Methyleriodictyol 7-*O*-(4-*O*-D-xylosyl)-β-D-galactoside, pterosin B, ptaquiloside, lutein, phosphoric acid, phytol, 2,6,10-trimethyl,14-ethylene-14-pentadecene, hexadecanoic acid methyl ester, pentadecanoic acid, Stigmasta-5,22-dien-3-ol, acetate, (3beta), beta-ocimene, 1,2- Benzenedicarboxylic acid, BIS(2-Methylpropyl)ester, 1-Heneicosanol, 5,8,11,14-eicosatetraenoic acid, methyl ester (all Z), ergost-5-en-3-ol, (3beta) and stigmast-5-en-3-ol, (3beta). (Tandon et al., 1980; Srivastava et al., 1981; Gangwar, 2004; Somvanshi, 2006; Wali et al., 2016; Naik et al., 2020). The chemical structures of these bioactive principles are depicted in Figure 2.

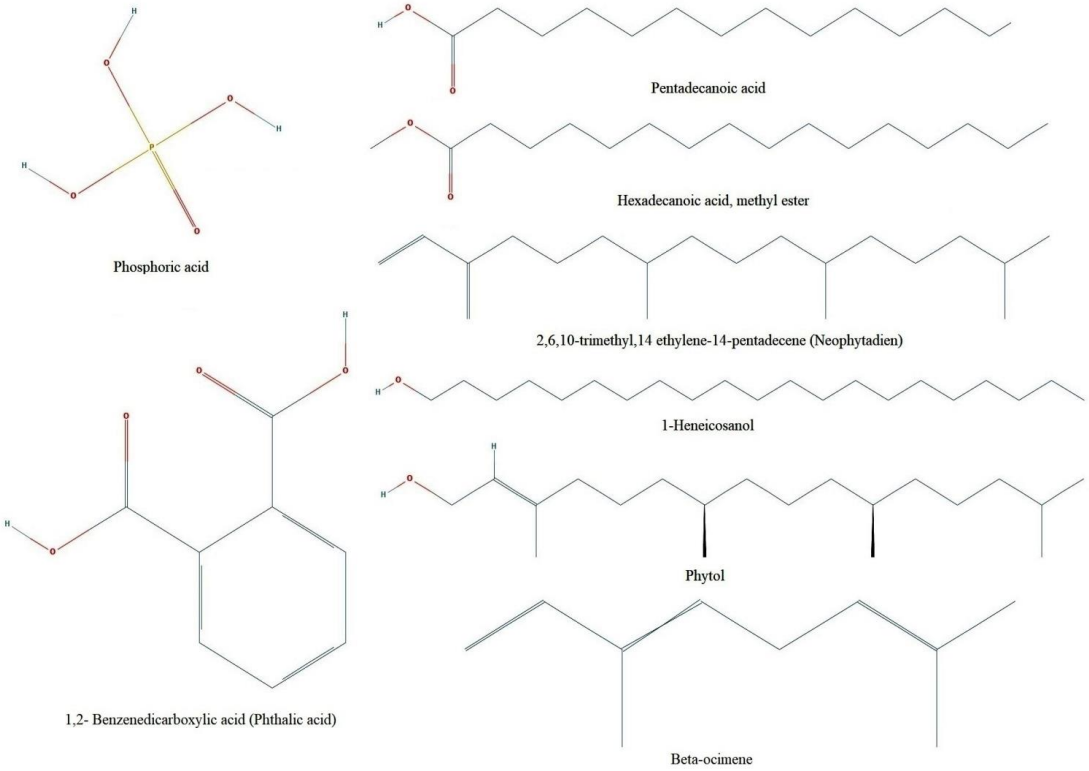

Figure - 2: Bioactive principles in *D. esculentum*

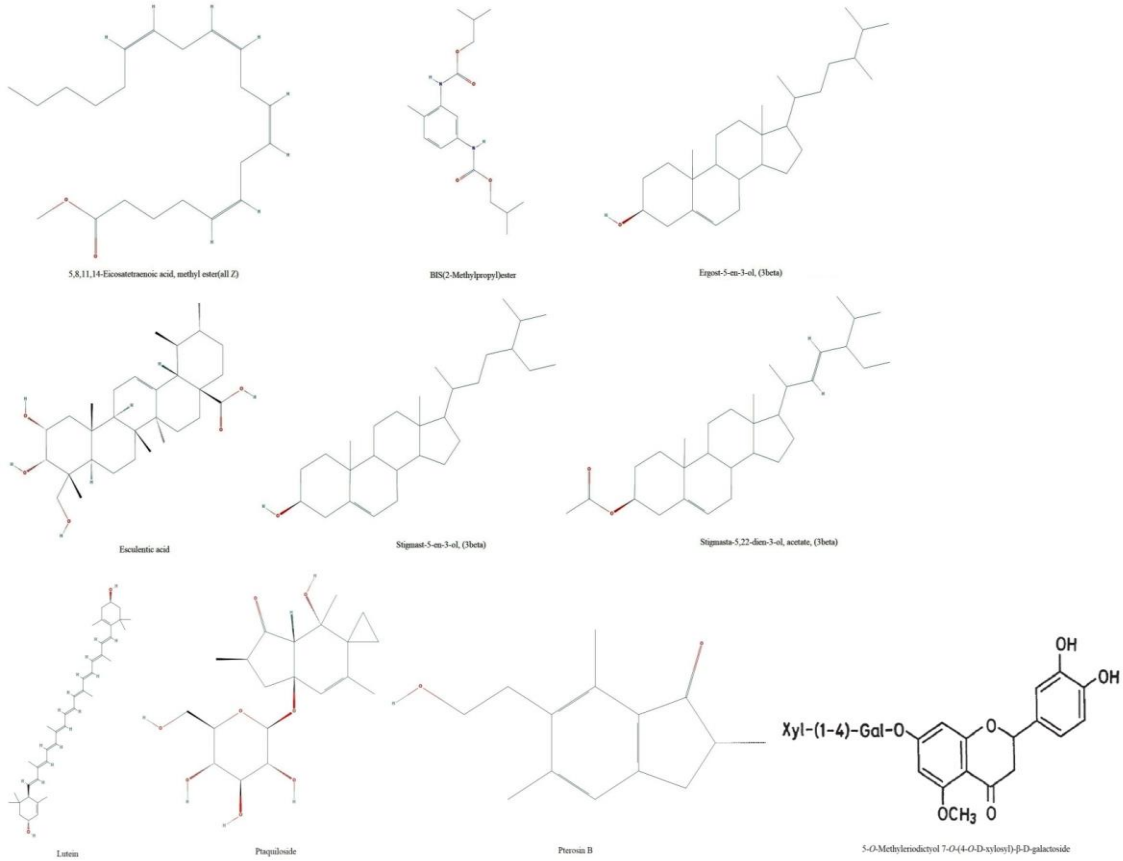

Figure - 2 (Continued): Bioactive principles in *D. esculentum*

6.2. Antioxidant and free radical scavenging activities of *D. esculentum*

The *in vitro* total antioxidant activity evaluated by ABTS, FTC, TBA, FRAP and phosphomolybdenum methods exhibit high percentages of the total antioxidant activity in *D. esculentum* which contains high amount of flavonoid and phenolic compounds that confers moderately high DPPH radical scavenging activity as evident by its IC_{50} value (Roy et al., 2013a; Das et al., 2013, Tongco et al., 2014; Wali et al., 2016). Oxidative stress of the liver was estimated by assessing the antioxidant enzymes catalase and reduced glutathione in Wistar rats. It was observed that the treatment of the rats with hydroalcoholic extract of *D. esculentum* significantly reduced the level of lipid peroxidation. The level of the antioxidant enzymes, i.e., catalase and reduced glutathione was also decreased significantly in a dose-dependent manner, indicating potent antioxidant potential of *D. esculentum* (Junejo et al., 2018). The methanolic extract of *D. esculentum* also possesses scavenging activities against different reactive oxygen species (ROS) and reactive nitrogen species (RNS), including hydroxyl, superoxide, hydrogen peroxide, singlet oxygen, hypochlorous acid, nitric oxide and peroxynitrite. Moreover, the *D. esculentum* extract acted as an iron chelator and also possessed reducing power and lipid peroxidation inhibition property. Moreover, *D. esculentum* is reported to contain significant amount of flavonoids and Phenolic compounds. Both these classes of compounds have good antioxidant potential and their effects on human nutrition and health are considerable (Roy et al., 2013a).

6.3. Antibacterial activity of *D. esculentum*

The aqueous extracts of rhizome and root of *D. esculentum* inhibite the bacterial growth, whereas, the leaf extract did not show any inhibition. The aqueous and alcoholic extracts of rhizome were found to be more effective than the antibiotic. The synergistic effect was observed when the root extract was applied with the antibiotic against *Salmonella arizonae* and *Staphylococcus aureus*. But, the leaf and root extracts did not show any inhibition against *E.coli*. (Amit et al., 2011). The chloroform extract of *D. esculentum* showed strong antimicrobial activity against *Sarcina lutea*, *Salmonella typhimurium*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Shigella boydii*, *Escherichia coli*, *Staphylococcus aureus* and *Vibrio cholera*. The highest zone of inhibition for chloroform extract was observed against *Sarcina lutea* followed by *Salmonella typhimurium*. On the other hand, in case of the aqueous extract, the highest zone of inhibition was found in *Samonella typhimuriun* followed by *Klebsiella pneumonia* (Akter et al., 2014).

6.4. Antidiabetic activity of *D. esculentum*

The antidiabetic activity of *D. esculentum* was reported by Junejo et al. (2018). They showed that *D. esculentum* significant lowered the blood glucose level in streptozotocin (STZ) induced diabetic rats. A 45.6% decrease in the fasting blood glucose level was observed in the diabetic rats treated with the hydroalcoholic extract of *D. esculentum* at the dose of 500 mg/kg body weight. Significant glucose level stabilizing activity of *D. esculentum* was evident by the oral glucose tolerance test. The extract at the doses of 250 mg/kg and 500 mg/kg body weight significantly increased the glucose tolerance which indicated that *D. esculentum* could induce glycogenesis to utilize the excess glucose uptake by cells. *D. esculentum* significantly decreased the triglyceride and LDL level and increased the level of HDL which is strongly correlated with the up regulation of the insulin level in the STZ induced diabetic rats. The extract at the dose of 500 mg/kg body weight showed almost equivalent reduction in the cholesterol and LDL level and greater reduction of triglyceride level was observed as compared to the standard metformin hydrochloride treated diabetic rats. Moreover, the extract significantly increased the level of HDL in a dose-dependent manner. *D. esculentum* significantly restored the histological architecture such as regeneration of β-cell and decrease in tissue necrosis in the liver of treated animals compared to the vehicle control group. The extract reduced the vacuolization and dislocation of the pancreatic islets. The extract at the dose of 500 mg/kg body weight did not show any infiltration of the inflammatory cells and normal acinis were observed (Junejo et al. (2018)).

6.5. Antihelminthic activity of *D. esculentum*

The antihelminthic activity of aqueous, ethanolic- and petroleum ether extracts of *D. esculentum* were evaluated on adult Indian earthworm, against the standard piperazine citrate. The ethanolic extract showed dose-dependent increasing paralyzing effect ranging from loss of motility to loss of response to external stimuli, which gradually led to death. The aqueous extract also showed significant activity, but the petroleum ether extract showed the least activity among all the extracts, revealing the fact that the anthelmintic activity of *D. esculentum* increased gradually with increasing polarity (Amit & Singh, 2012).

6.6. Cytotoxic activity of *D. esculentum*

The cytotoxic potential of *D. esculentum* was evaluated by Akter et al. (2014) using brine shrimp lethality bioassay which can screen a large array of bioactive principles in crude samples. This method is very useful and has been used in predicting cytotoxic and antitumor activity of extracts (Mongelli et al., 2002). It was observed that both chloroform- and methanol extracts produced concentration dependent increase in percent mortality of brine Shrimp nauplii indicating the possible cytotoxic potential in these extracts. On the other hand, it was found that *D.esculentum* shoots ethanolic extracts did not inhibit the proliferation of MDA-MB-231, MCF-7, Caco-2 and HepG2 cell line (Rahmat et al., 2003).

6.7. Hepatoprotective activitiy of *D. esculentum*

D. esculentum significantly restored the hepatic injury in rats treated with carbon tetrachloride (CCl_4), as indicated by significant dose-dependent decrease in the serum markers *viz.*, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP). It was observed that CCl_4 treatment did not significantly increase the ALP and total bilirubin level in the group pre-treated with 200 mg/kg and 400 mg/kg body weight of the *D. esculentum* extract, respectively (Junejo et al., 2018).

6.8. Immunomodulatory activities of *D. esculentum*

Significant losses of the body weight as well as the relative spleen weight were observed in boiled *D. esculentum* fed Swiss albino mice. The dose- and time dependent decrease in the number of the peritoneal macrophages, plaque forming cells as well as the progressive decrease in the degree of the hemagglutination titre in the *D. esculentum* treated mice indicated its immunosuppressive potential. *In vitro* studies showed that *D. esculentum* inhibit lymphocyte proliferation as well as it caused hemolysis (Roy et al., 2013b). Moreover, a dose-dependent reduction in the level of Th1 (IL-2 and IFN-γ) and Th2 (IL-4 and IL-10) cytokine production by T cells in *D. esculentum* treated mice has been documented, indicating that *D. esculentum* when fed in chronic doses to Swiss albino mice, induced the Th1/Th2 imbalance, resulting in severe immunosuppression (Roy & Chaudhuri, 2015). Moreover, the chloroform and acetone extracts of *Diplazium esculentum* at a dose of 100mg/kg. p.o. showed significant reduction in the carrageenan induced paw edema in rat which was comparable to that of the standard drug ibuprofen, suggesting the anti-inflammatory activity of this plant (Kaushik et al., 2011). The aqueous and ethanolic extracts of *Diplazium esculentum* was also investigated for anti-anaphylactic and mast cell stabilizing activity. Both the extracts showed anti-anaphylactic activity by reducing mast cell degranulation induced by horse serum and triple antigen (DPT) in Wister rats. It was observed that pretreatment of rats with *D. esculentum* extracts stabilized the mast cell membrane and thereby inhibited the production of

nitric oxide synthase resulting in a decreased level of nitric oxide in both serum and peritoneal fluid (Das et al., 2012).

6.9. Mosquito larvicidal property of *D. esculentum*

Potent larvacidal activity of methanol extract of *D. esculentum* was first observed against *Culex* larvae. The third or fourth instar of *Culex* larvaes was used to determine the larvicidal activity. An LC_{50} value of 149.279 ppm indicated that the methanolic extract of *D. esculentum* is a potent larvicidal agent Halimatussakdiah et al., 2018). The crude ethanolic extract of the aerial parts of *D. esculentum* was evaluated against the third instar larvae of *Anopheles gambiae* and *Culex quinquefasciatus*, and found to have potential larvicidal effect. Increased mortality was observed in both mosquito species as the concentration of the extract gradually increased. The extract was found to be more potent against *A. Gambiae* larvae. This difference in susceptibility could be attributed to the difference in the physiological characteristics between the two mosquito species. (Umohata et al., 2020).

6.10. Neuromodulatory activity of *D. esculentum*

The effect of *D. esculentum* on central nervous system (CNS) of mouse was evaluated using actophotometer. It was observed that the aqueous leaf extract of *D. esculentum* significantly increased the locomotor activity in a dose-dependent manner. The aqueous extract (dose: 100 mg/kg body weight) significantly stimulated the CNS when compared to the control groups (Kaushik et al., 2012). Moreover, the aqueous extract of *D. esculentum* was found to be a potent analgesic. It was observed that apart from its effect on CNS, *D. esculentum* has marked beneficial effects against, peripherally and inflammatory pain models (Chawla et al., 2015).

In vivo acetylcholinesterase activity in the mice treated with *D. esculentum* indicated the dose-dependent decrease in the rate of the conversion of the substrate acetylthiocholine iodide in to acetyl- and choline group by the enzyme acetylcholinesterase (Roy, 2017). On the other hand, significant dose-dependent increases in the acetylcholinesterase– and NADH oxidase inhibitory activities, as well as low IC_{50} values for acetylcholinesterase- and NADH oxidase inhibition of the *D. esculentum* extract were observed, indicating its effectiveness as a good anticholinesterase and NADH oxidase inhibitor. It was proposed that *D. esculentum*, being an edible fern, may also be a good dietary source of acetylcholinesterase- and NADH oxidase inhibitor and thereby, can be used for the management of oxidative stress-related neurodegenerative disorders (Roy et al., 2015).

6.11. Effect of *D. esculentum* on the reproductive functions

It was observed that after chronic treatment (135 days and 180 days) at the dose of 320 mg/kg body weight, the percentage inhibition of sperm viability was increased remarkably up to 40.51% and 53.12%, respectively (Roy et al., 2013c). There were significant decreases in body weight of boiled aqueous preparation of *D. esculentum* treated mice (160 and 320 mg/kg bw) after 135 and 180 days of treatment when compared to the control group. The relative weights of epididymis, seminal vesicle and prostate of animals treated with the *D. esculentum* at the dose of 160 and 320 mg/kg bw were decreased significantly after 135 and 180 days of treatment. Study revealed significant decreases in α-glucosidase and fructose level in the testis of *D. esculentum* treated mice. Significant decreases in the glycogen-, protein-, sialic acid-, and citric acid contents have also been observed in *D. esculentum* fed mice. Moreover, it was proposed that significant decrease in the prostatic citric acid level along with the decreased acid phosphatase level in testis might alter the testosterone level in *D. esculentum* treated mice (Roy & Chaudhuri, 2017).

D. esculentum caused various structural abnormalities in testis as indicated by the histological examinations. Chronic dose of *D. esculentum* (320 mg/kg body weight for 180 days) induced alterations in the structure and organization of seminiferous tubules that include reduced epithelial cell height and lack of sperms in the lumen, detachment of spermatogenic cells from the basement membrane, appearance of intraepithelial vacuolations, and clumping of the sperms inside the seminiferous tubules. All these changes could halt the normal spermatogenesis at the primary spermatocytic cycle. Not only that, these alterations were proposed to hamper mitochondrial-, and plasma membrane, and junctional complexes between the adjacent Sertoli cells (Roy & Chaudhuri, 2017).

D. esculentum has been reported to decrease the fertility as well as fecundity in Swiss albino mice in a dose- and time-dependent manner when compared to the respective control groups. The percentages of fertility and fecundity were decreased significantly with increasing dose of boiled aqueous preparation of *D. esculentum*. The sub-chronic and chronic treatments at the dose of 320 mg/kg body of *D. esculentum* have been reported to cause 100% fertility losses. Similarly, 100% losses of fecundity were observed in mice that were treated with 160 and 320 mg/kg bw of BDE for 180 days (Roy & Chaudhuri, 2017).

All the biochemical and histological alterations that was observed in the mice treated with chronic dose of boiled aqueous preparation of *D. esculentum*, did not show any sign of reversal after the withdrawal for 60 days, as evident by the seminiferous tubular morphology where statistically significant difference exist with the control group. It was also hypothesized that the disturbance in the Th1/Th2 cytokine homeostasis due to chronic treatment of *D. esculentum* might cause infertility and recurrent spontaneous abortion (RSA) in female mice (Roy & Chaudhuri, 2017).

6.12. Effect of *D. esculentum* on general physiology, and liver and kidney functions

D. esculentum caused significant loss of the body weight as well as the relative organ weights in mice, indicating decreased metabolic activity, resulted in organ and system failure. Feeding of frozen- and shade dried samples of *D. esculentum* to rats and guinea pigs showed decreased body weight, increased spontaneous and decreased forced motor activity, alterations in the values of blood glucose and total leukocyte count, and increased mortality in guinea pigs (Gangwar, 2004).

Serum biochemistry revealed increased levels of several liver enzymes like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminae (SGPT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), acid phosphatase (ACP) and γ-glutamyl transferase (GGT), and metabolic products like total bilirubin (liver), urea (kidney) and creatinine (kidney) in the blood of *D. esculentum* fed mice, indicating malfunctioning in these organs that might induce metabolic diseases and age-related degenerative disorders which are closely associated with the oxidative processes in the body. Histological examination of liver and kidney of mice from *D. esculentum* treated group revealed alterations in their tissue architecture. Disorganized vasculature and other associated changes were seen in liver and kidney of *D. esculentum* fed mice with distinct disorganization was observed only in those mice that were treated with chronic doses (180 days) of boiled *D. esculentum* (Roy, 2017).

7. Discussion

Phytochemicals are chemical compounds or chemical constituents formed in the plant's normal metabolic processes. The chemicals are often referred to as "secondary metabolites" of which there are several classes including alkaloids, steroids, terpenoids, catecholamines, tannins, saponins, anthraquinones, coumarins, fats, flavonoids, glycosides, gums, iridoids, mucilages etc. These naturally occurring phytochemicals are generally considered to be safe alternatives for synthetic drugs and found to be useful in the prevention of several diseases, and also have

antimicrobial, antifungal, antiparasitic, antiviral, anti-allergic, antispasmodic, antihyperglycemic, anti-inflammatory, and immunomodulatory properties (Rabi & Bishayee, 2009; Wagner & Elmadfa, 2003; Sultana & Ata, 2008; Shah et al., 2009). The present study reviewed several promising pharmacological activities which are intrigued by extensive variety of potential phytoconstituents of *D. esculentum*.

One of the major groups of secondary metabolites present in *D. esculentum* is alkaloids which is reported to act on the nervous system as stimulators, exhibit acetylcholinesterase inhibition activity, enhance memory in animals and is also being investigated for the treatment of Alzheimer's disease (Zhang et al., 2002; Hirasawa et al., 2003, Ma & Gang, 2004). *D. esculentum* is also reported to possess flavonoids and Phenolic compounds. Among flavonoids, some exhibit neuroprotective activity against cytotoxic stress, suggesting their possible use in treatment of neurodegenerative diseases such as stroke and Alzheimer's disease. The mechanism of action of flavonoids is through scavenging or chelation (Cook & Samman, 1996). Phenolic compounds are also very important plant constituents because their hydroxyl groups confer scavenging ability (Diplock, 1997).

D. esculentum is also investigated for potential bioactive principles among which esculentic acid and lutein are important. Esculentic acid is a triterpene which showed potent anti-inflammatory activity in mice. It is reported to inhibit pro-inflammatory cytokines like TNF-α and IL-6, inflammatory mediators *viz.*, NO & PGE-2 in macrophages, and decrease the COX-2 protein expression in mice (Niu et al., 2014). On the other hand, lutein, an oxygenated carotenoid, acts as an antioxidant, protecting cells against the damaging effects of free radicals. It is hypothesized that they protect against visual disorders and cognition diseases, such as age-related macular degeneration (AMD), age-related cataract (ARC), cognition diseases, ischemic/hypoxia induced retinopathy, light damage of the retina, retinitis pigmentosa, retinal detachment, uveitis and diabetic retinopathy (Jia et al., 2017). Dietary odd-chain saturated fatty acids (OCFAs) like pentadecanoic acid and heptadecanoic acid are associated with lower risks of cardiometabolic diseases, and their higher dietary intake is associated with lower mortality (Venn-Watson et al., 2020). On the other hand, hexadecanoic acid methyl ester shows a variety of pharmacological activities viz., antioxidant, antifungal, hypocholesterolemic, antimicrobial, pesticide, and nematicide activities (Mustapha et al., 2016; Arora et al., 2017). Other beneficial bioactive constituents like 1-heneicosanol and β-ocimene possess antibacterial and antifungal, antitumor, and pest resistance activities, whereas, phytol possesses anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immunomodulating, and antimicrobial effects (Arancibia et al., 2016; Russo & Marcu, 2017; Islam et al., 2018).

Apart from these beneficial phytochemicals, *D. esculentum* possesses some toxic compounds as well, among which saponins and tannins are pharmacologically most important. Study revealed that both the crude and boiled *D. esculentum* possess hemolytic activity which is due to the presence of considerable amount of saponins in plant. Saponins have the capacity to destroy cell membrane, therefore may be related to the hemolytic potential. On the other hand, tannins inhibit protein availability through denaturation. Tannins are heat resistant compounds that can withstand high temperature during boiling. Thus, the toxic effects shown by *D. esculentum* could be attributed to tannins and other heat stable compounds (Roy et al., 2013b). Among the bioactive principles, ptaquilosides and pterosin B are the most prominent in this fern. *D. esculentum* collected from the high-altitude area of Harsil-Gangotri (Northern India) had 19 mg/kg Ptaquiloside (Somvanshi et al., 2006). Shade- and freeze dried samples of *D. esculentum* showed the presence of 10.94 to 16.36 mg/kg of pterosin B (Gangwar, 2004). During metabolism, ptaquiloside undergoes a series of reactions and produces a reactive aglycone dienone intermediate, the inactive pterosin B and DNA adducts. Ptaquiloside is activated at alkaline pH, which is considered as the reason for the location of tumors in the urinary bladder of ruminants and the ileum of rats (Smith et al., 1994).

Among other bioactive phytoconstituents, pthalic acid is the most important. Phthalic acid is already reported to present in considerable amount in *D. esculentum*. Phthalates are the diesters of phthalic acid which was reported to reduce the ovarian weight and increased uterine weight in female mice. In utero or lactational exposure of pthalates alters steroidogenic gene expression in the ovary, decreases estrogen synthesis, reduces ovarian weight, and reduces ovulation. Exposure of phthalates during fetal development altered follicular recruitment and development, eventually causing premature ovarian failure. Low molecular weight phthalates are also suspected of acting as hormones and cause infertility, overweight, and diabetes in men (Lyche, 2017; Barakat & Ko, 2018; Henkel, 2018).

One of the most important properties of *D. esculentum* investigated so far is the acetylcholinesterase inhibitory activity, which can explain the neuroimmune function of this plant. Acetylcholine (ACh) is a ubiquitous neurotransmitter and found even in the roundworm *Caenorhabditis elegans*, one of the simplest organisms with a nervous system (Rakowski et al., 2013; Kosinski & Zaremba, 2007). The wider significance of ACh is in understanding the biological effects of tested toxins and/or medical drugs: as any immunological effects of Acetylcholinesterase (AChE) inhibitors can involve both CNS and PNS, this has to be taken into consideration in interpreting any findings (Pohanka, 2014). The cholinergic system is tightly associated with the cholinergic anti-inflammatory pathway dominantly located in blood and mucosa. This pathway is a regulatory link between nerve terminations in blood and macrophages expressing the α7 nicotinic acetylcholine receptor (α7 nAChR) on their surface (Pohanka, 2012; Wessler & Kirkpatrick, 2008; Rosas-Ballina & Tracey, 2009). Discovery of the cholinergic anti-inflammatory pathway allows us to understand how the CNS is involved in the regulation of innate immunity (Pohanka, 2014). Acetylcholinesterase (AChE) bound on erythrocytes plays an important role in termination of cholinergic anti-inflammatory pathway activation (Pohanka, 2012; Silva-Herdade & Saldanha, 2013). The bioactive constituents like lutein and phytol, present in *D. esculentum* can inhibit AChE, and thus aid in the continued progression of the cholinergic anti-inflammatory pathway. The detailed mechanism of *D. esculentum* mediated regulation of the cholinergic anti-inflammatory pathway is illustrated in the Figure 3.

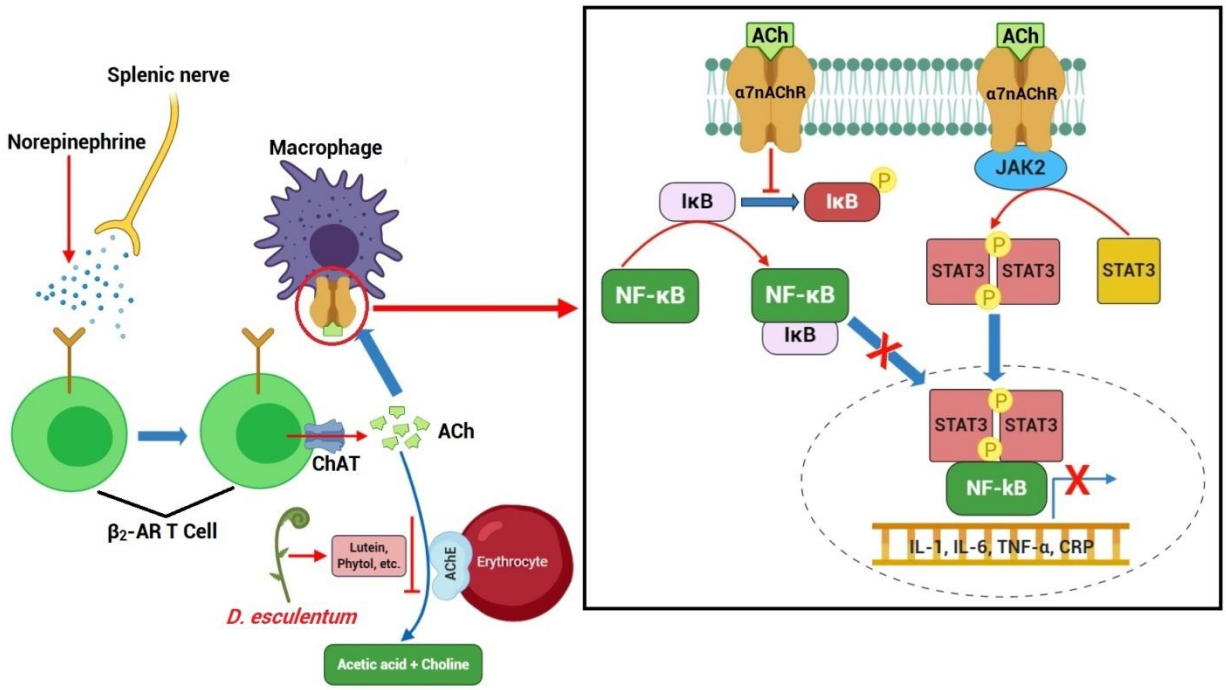

Figure - 3: ***The modulation of the cholinergic anti-inflammatory pathway via protection of ACh from splitting by cholinesterase and thus enhancing the pathway.*** Acetylcholine (ACh) is released by β_2-adrenergic receptor T cells coupled with choline acetyltransferase (β_2-AR T Cells + ChAT) following the stimulation of splenic nerve and subsequent release of norepinephrine, which binds β_2-AR cells stimulating ACh release. At this point, the enzyme, acetylcholinesterase (AChE), which is bound to the erythrocytes, comes in to play and it cleaves the ACh molecule in to acetic acid and choline group, terminating the stimulation of the cholinergic anti-inflammatory pathway (Cuoco et al., 2016). The bioactive principles of *D. esculentum* (lutein, phytol, etc.) inhibit the AChE, and therefore, the circulating ACh molecules bind to macrophages via the α7nAChR activating IκB or JAK/STAT pathway to inactivate the NF-κB-mediated transcription of pro-inflammatory cytokines, such as IL-1, IL-6, TNF-α and CRP.

8. Conclusion and future perspectives

Diplazium esculentum have survived from the Paleozoic times and have adapted with many more various changes of environment than the other primitive vascular plants (Wallace et al., 1991). Therefore, this plant is expected to have many useful phytochemicals than other plants. It is interesting to note that not all the ferns are edible, only a few of them are used as food throughout the world, and *D. esculentum* is one of them. It is the most commonly consumed fern in the world. The fern stems, rhizomes, leaves, young fronds and shoots, and sometimes the whole plants are used for food (Liu et al., 2012). In recent years, more and more researches have reported the food and ethnomedicinal uses of this fern in different parts of the world but very few studies have been conducted so far to assess the pharmacological or toxicological impact of this plant on human health. This plant is enormous source of a wide range of compounds having diverse pharmacological and medicinal properties. The present review is the first attempt to summarize all the available information regarding the chemical profiles and pharmacological properties of this edible plant which may serve as the baseline data to do extensive studies towards the discovery of more potent compounds and further investigations for their biological activities. Therefore, further research should be carried out on *D. esculentum* to uncover the unidentified compounds, their functions including synergistic effects and mechanisms of action. Experimental studies including clinical trials are highly required to establish disease-specific optimal dosing and the optimal method to deliver the therapeutic agent.

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