Biomedicine & Pharmacotherapy 98 (2018) 733–745

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

A comprehensive review of the pharmacological potential of *Crocus sativus* and its bioactive apocarotenoids

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ARTICLE INFO

Keywords:
C. sativus
Saffron
Antioxidant
Anticancer crocin
Crocetin
Safranal

ABSTRACT

Crocus sativus is an herbaceous plant that belongs to family Iridaceae. It is commonly known as saffron and has been used for medicinal purposes since many centuries in India and other parts of the world. Saffron of commercial importance comprises of dried stigmas of the plant and is rich in flavonoids, vitamins, and carotenoids. Carotenoids represent the main components of saffron and their cleavage results in the formation of apocarotenoids such as crocin, picrocrocin, and safranal. Studies conducted during the past two decades have revealed the immense therapeutic potential of saffron. Most of the therapeutic properties are due to the presence of unique apocarotenoids having strong free radical scavenging activity. The mode of action of these apocarotenoids could be: modulatory effects on detoxifying enzymes involved in combating oxidative stress, decreasing telomerase activity, increased the proapoptotic effect, inhibition of DNA, RNA and protein synthesis, and by a strong binding capacity of crocetin with tRNA. The present review focuses on the therapeutic role of saffron and its bio oxidative cleavage products and also highlights the possible molecular mechanism of action. The findings reported in this review describes the wide range of applications of saffron and attributes its free radical scavenging nature the main property which makes this spice a potent chemotherapeutic agent for the treatment of various diseases.

1. Introduction

Crocus belongs to family iridaceae comprising of genera and more than 85 species. *Crocus sativus* is the most important species as its dried stigmas constitute the saffron of commerce. Cultivation of *C. sativus* requires intensive labour and time thereby making it a highly priced spice [1]. Saffron has been in cultivation for many centuries and is presently being cultivated in various parts of the world namely Iran, India, Spain, Greece and Turkey. Growing pharmacological applications have increased the demand of saffron. The increasing demand in the world market is considerd to be the main reason for the expansion of saffron cultivation in other parts of the world.

The main constituents of saffron are carotenoids, glycosides, monoterpenes, aldehydes, anthocyanins, flavonoids, vitamins (especially riboflavin and thiamine), amino acids, proteins, starch, mineral matter, and gums [2]. However, among all these components apocarotenoids such as crocetin, crocin, safranal (the bio-oxidative cleavage products of zeaxanthin) and picrocrocin are considered to be the important bioactive components. Crocin is responsible for intense colour, safranal for odour and picrocrocin for bitter taste. It is mainly because of these components that saffron has gained importance as a therapeutic herb. Crocin (digentiobiosyl8, 8′-diapocarotene-8, 8′-oate; $C_{44}H_{64}O_{24}$), a diester of gentiobiose (disaccharide) and dicarboxylic acid crocetin is considered to be one of the few naturally occurring water soluble carotenoids. The flavouring property of saffron is due to the bitter glycoside, picrocrocin, 4-(β-D-glucopyranosyloxy) −2, 6, 6-trimethylcyclohex- 1- ene- 1- carboxaldehyde. ($C_{16}H_{26}O_7$). Crocin family also includes various glycosyl esters. Glycosylated forms of crocetin i.e. transcrocin-3 and trancrocin-4 are considered to be the abundant forms [3]. Most of the crocin derivatives except crocin-1 are considered to exist in cis- *trans* isomeric forms. Crocetin is the other bioactive constituent of saffron and is insoluble in water and most organic solvents. It is a natural carotenoid dicarboxylic acid. Glucosyl ester of crocetin consists of seven conjugated double bonds and four side chain methyl groups. End groups are esterified with one, two or three glucose units [4]. Earlier reports supported the existence of only six glycosides of crocetin in saffron [5]. However, more crocetin esters and their trans and cis isomers were isolated by using HPLC with UV/vis photodiode array along with spectrometry [4].

Safranal ($C_{10}H_{14}O_{7)}$ is the main component responsible for the

Abbreviations: CSE, *Crocus sativus* extract; ROS, reactive oxygen species; DPPH, DPPH(2,2-diphenyl-1-picrylhydrazyl); GPx, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase; LDL, low density lipoproteins; HPLC, high performance liquid chromatography

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https://doi.org/10.1016/j.biopha.2017.12.090
Received 7 October 2017; Received in revised form 2 December 2017; Accepted 18 December 2017

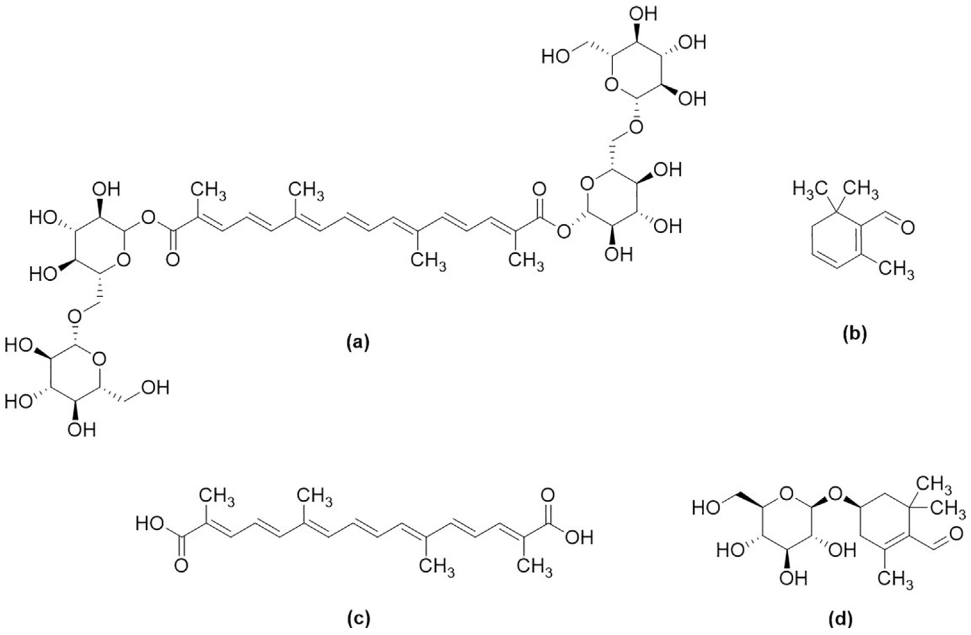

Fig. 1. Structures of (a) Crocin (b) Crocetin (c) Safranal and (d) Picrocrocin.

aroma of saffron and accounts for 60–70% of volatile fraction of saffron [6]. It is a cyclic terpenic aldehyde obtained from picrocrocin and HTCC (4-hydroxy-2, 6, 6-trimethyl-1-cyclohexen-1-carboxaldehyde) during saffron drying process [7] and is considered to be the enzymatic and thermal degradation product during storage process [8]. The structures of saffron components are illustrated in Fig. 1.

2. Therapeutic properties of saffron and its constituents

Saffron has been used in folk medicine as anti spasmodic, eupeptic, to treat menstrual cramps, lumbar pain, cough, broncial spasms, asthma, heart disease, small pox, scarlet fever and colds [9]. During the past two decades the degradative products of carotenoids have gained importance in the modern pharmacological studies and have shown several properties including anti-inflammatory, anti-tumour [10,11] anti-oxidant [11], anxiolytic [12], neuron protection and anti-neuro-degenerative disease [13–16]. Fig. 2 summarises the pharmacological application of saffron and its components.

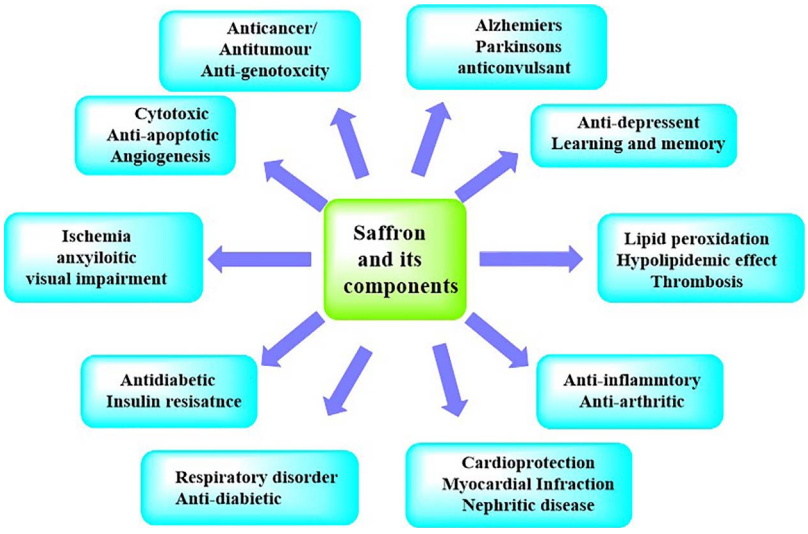

Fig. 2. Pharmacological applications of saffron and its components.

2.1. Free radical scavenging activity

In normal cells, generation of reactive oxygen species (ROS) is a normal process however, uncontrolled generation and concomitant increase of ROS level in the body results in “oxidative stress” which is considered to be the main cause of various diseases [14]. Carotenoids are known to be very efficient physical and chemical quenchers of singlet oxygen, as well as potent scavengers of ROS [3–5].

2.1.1. Saffron extract

Researches have shown that spices containing phenolic and flavonoid compounds have antioxidant activities and are frequently used as antioxidant food supplements [17,18]. The antioxidant property of *C. sativus* could be attributed mainly to its active ingredients such as safranal, crocin, crocetin and carotene, all of which have been reported to have antioxidant properties [16]. Various *in vivo* and *in vitro* studies showed the pharmacological and biological effects of saffron especially of its alcoholic extracts are predominantly because of its antioxidant potential which is considered to be the result of synergistic antioxidant potential of all the bioactive components of saffron. Martinez-Tome et al. [17] reported that saffron extract has significantly scavenged

hydrogen peroxide and has inhibitory effect on lipid peroxidation. Ghardoost et al. [18] demonstrated the protective effects of saffron aqueous extract and crocin against chronic stress induced impairment of learning and memory. In a comparative study saffron extract has shown significant radical scavenging property compared to that of carrot and tomatoes [19]. Radical scavenging activity of plant extracts is considered to be good for its anti-inflammatory activity. Hosseinzadeh and Younesi [20] in an *in vivo* study tested the anti-inflammatory effect of ethanolic and aqueous extracts of saffron stigma and flowers. The extract of stigma was found to inhibit the acetic acid induced writhing reflex and also an important role in curative effect in other complications e.g. in case of lower limb skeletal muscle injury [21] and re-epithelialisation of burnt wounds [22].

2.1.2. Saffron components

Potential of scavenging ROS is a measurement of antioxidant activity; saffron components have been found to decrease ROS production as effectively as the phenolic antioxidants. In a DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging study of crocin and safranal, Assimopoulou et al. [23] attributed the antioxidant activity of *C. sativus* stigma to crocin and safranal and suggested this to their ability to donate a hydrogen atom to the DPPH radical [23]. Nature of the solvent used had significant effect on both the contents. Crocin and safranal showed high radical scavenging activity, 50% and 34% for 500 ppm solution in methanol, respectively. In a comparative study of antioxidant potential of Trolox, BHT (Butylated hydroxytoluene) and saffron components, safranal showed lower radical scavenging potential compared to crocetin whose potential was equivalent to that of Trolox and BHT [24]. Similarly, crocin showed stronger antioxidant potential than α- tocopherol (a strong antioxidant agent), as it reversed the adverse effects of cell membrane damage and increased superoxide dismutase (SOD) level in oxidative stressed neurons [25].

The isolated components of saffron have been found to play important role in the treatment of diseases via different mechanisms of which free radical scavenging activity being the most prominent. These saffron components decreased lipid peroxidation and occurrence of gastric lesions [26], prevented diazinon (DZN) induced rise of oxidative stress marker, several specific inflammatory markers and neuronal damage biomarkers via free radical scavenging activity when evaluated individually. These activities were demonstrated by saffron too [27]. Protective effect against the mitochondrial toxin 3-Nitropropionic acid (3-NPA) along with decrease in the lipid peroxidation in striatal synaptosomes isolated from the brain of rat has also been reported [28]. Saffron and crocin administration also alters and ameliorates the effects of venom induced oxidative stress [29]. Similar observations have been made by Premkumar et al. [30]. Several studies demonstrated that crocin and safranal or saffron itself mediate their effect by modulating redox status of the cells which are under oxidative stress due to hyper generation of ROS [26,34,35].

Zheng et al. [31] established that administration of crocetin increased the resistance of low density lipoproteins (LDL) to *in vitro* oxidation and in *in vivo* conditions, decreased plasma levels of oxidized LDL, signifying antioxidant role of crocetin. Crocetin, because of its strong antioxidant potential has been reported to inhibit lipid peroxidation, increase the activity of Glutathione *S*-transferases (GST), Glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), decrease marker enzymes such as aryl hydrocarbon hydroxylase (AHH), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT) and adenosine deaminase (ADA)

in lung tissues and also inhibit proliferation of lung cancer cells [37,38]. Crocetin reduces glutathione levels induced by indomethacin in non-diabetic and diabetic rats [33], reduces ROS induced lipid peroxidation in primary hepatocytes of rat [34], ROS generated by B*a*P in mice [35] and Ang-II-induced ROS [36]. Antioxidant potential of crocetin has been found to be higher to that of dimethyl crocetin. The possible reason for the superiority of crocetin seems to be because of the presence of hydroxyl moiety of the carboxylic group in crocetin that makes it potent H donor and more reactive to free radicals [32]. In another comparative study of crocetin, safranal and dimethyl crocetin, crocetin was found to be more effective than dimethyl crocetin and safranal [37]. However, the synergistic effect of all the bioactive constituents have been reported to contribute significantly towards the antioxidant potential of saffron [38].

2.2. Anti-cancer /antitumor effect

Cancer represents the main cause of mortality in the world and use of synthetic or natural agents (alone or in combination) is considered to be promising strategy for prevention of cancer. Use of medicinal plants and herbs has proven to be good alternative to synthetic drugs and as a potential strategy for cancer prevention.

2.2.1. Saffron extract

In India and other parts of the world, several studies on the therapeutic applications of saffron started more than two decades back. These studies established that the extracts of saffron have cytotoxic, anti-cancer, antitumor (*in vivo* and *in vitro)*, and antimutagenic potential. There are reports of protective effect of saffron when used along with amino acid cysteine and the antioxidant vitamin E against the toxicity of cisplatin [39,40]. Thus, administration of saffron along with other anticancer agents could prove to be more effective in the inhibition of colony formation and nucleic acid synthesis compared to the anticancer agent alone [40].

Topical administration of aqueous extract of saffron (100 mg/kg body wt) significantly reduced dimethylbenzene [*a*] anthracene (DMBA) induced and croton oil promoted skin carcinogenesis, restricted 20-methylchloanthrene (MCA) induced soft tissue sarcomas in mice [41] and dimethyl nitrosamine (DEN) induced hepatic cancer *via* inhibition of apoptosis induction, cell proliferation and also via modulation of oxidative damage and suppressing inflammatory response [42]. Premkumar et al. [43] showed antioxidant and antimutagenic potential of aqueous extract of saffron. Aqueous extract of saffron has been found to prevent certain drug (cisplatin, urethane, cyclophosphamide and mitomycin C) induced genotoxicity and oxidative stress in mice besides increasing hepatic enzymes such as the glutathione--transferase, SOD, catalase and non-enzymatic antioxidants in animals pre-treated with saffron. The study suggests saffron to mediate its role of a promising chemo preventive agent because of its antioxidant activity and modulatory property during lipid peroxidation and detoxification [44].

2.2.2. Saffron components

Bakshi et al. [45] demonstrated inhibition of both *in vitro* and *in vivo* xenograft growth and antitumor activity of crocin in Dalton's lymphoma (DLA) in mice. Long term treatment of crocin on rats bearing colorectal tumours induced by rat adenocarcinoma DHD/K12-PROb cells decreased tumour growth besides significantly increasing their survival time [46]. Anti-genotoxicity (genotoxicity in most of the cases leads to cancer) effect of safranal has been reported by Hosseinzadeh and Sadeghnia and revealed that safranal significantly protected Methyl methane sulfonate (MMS) induced DNA damage [47]. Crocetin treatment markedly suppressed the hepatotoxic lesions caused by the aflatoxin B1 (AFB1) toxicity and the suppression was validated by decreased level of enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatise and gamma-glutamyl transpeptidase) in the rats treated with crocetin [48]. Moreover, in crocetin treated rat hepatocytes decreased level of malondialdehyde (MDA) has also been reported [34]. Umigai et al. [49] evaluated the effect of crocetin on vascular endothelial growth factor (VEGF) induced angiogenesis. Angiogenesis, the formation of new capillaries from pre-existing vasculature is a much-regulated process; loss of regulation would result in excessive neovascularisation that promotes tumour growth

Table 1
Anticarcinogenic effects of saffron extract and its components on different cancer cell lines and proposed mechanism of action.

Extract/ Component	Mechanism of Action	Cell Lines	References
CSE	Induction of Apoptosis	HeLa and HepG2	[51]
		A549,	[52]
		MCF-7	[53]
		HCT-116	[54]
	Induction of cytotoxicity and cell proliferation inhibition	HepG2	[42]
		HCT-116, SW-480 and HT-29	[55]
		A549	[56]
		MCF-7	
	Inhibition of DNA and RNA synthesis	A549, WI38 & VA- 13	[57]
		HeLa	[58]
Crocin	Induction of Apoptosis	HeLa	[59,60]
		MCF-7	[59]
	Induction of cytotoxicity and cell proliferation inhibition	L1210, Hl-60	[61],62]
		HCT-116, SW-480 and HT-29	[55]
		HepG2	[63]
		BxPC-3	[64]
	Inhibition of DNA and RNA synthesis	L1210, P388	[65]
	Apoptotic effect	PC-12	[66]
		KB Cells	[67]
Crocetin	Induction of Apoptosis	HeLa,	[68]
		MCF-7	[53]
		Mia- PaCa-2, Bx-PC3	
		Capan-1 and ASPC-1	[69]
	Induction of cytotoxicity and cell proliferation inhibition	K562, HL-60	[61,62]
		MCF-7, MDA-MB- 231	[70]
	Inhibition of DNA and RNA synthesis	L1210, P388	[65]
		A549	[71]
	inhibiting intracellular nucleic acid synthesis and colony formation	A549, VA13	[71]
	inhibition and inactivation of cell cycle regulatory protein (Cdc-2, Cdc-25c, cyclin B1)	MIA-PaCa-2 cells	[69]
Safranal	Via apoptotic cell death	Neuroblastomacells	[72]
	Via apoptotic cell death	KB Cell Line	[67]

[50]. Crocetin was found to suppress VEGF induced tube formation during angiogenesis besides inhibiting phosphorylation of p38 and protected vascular endothelial (VE)-cadherin expression which plays a major role in the control of vascular permeability [49]. There are reports of anti-proliferative effect *(in vitro)* of saffron, and its components on various cell lines which are summarised in Table 1.

2.2.3. Mechanism of action

Both *in vivo* and *in vitro* inhibition of growth of malignant cells by saffron and its components is reported and inhibition of intracellular nucleic acid synthesis, free radical chain reactions and apoptotic regulation are considered to be the possible molecular mechanisms involved in antitumor effect/anticancer effects of saffron. Inhibition of intracellular nucleic acid synthesis could be possible via different ways e.g. inhibition of DNA adduct formation [71,73], exerting inhibitory effect on cellular DNA and RNA synthesis, but not on protein synthesis, disruption of DNA-protein interactions, e.g. topoisomerases II [74], Crocetin showed anti- genotoxic effects by inhibiting RNA synthesis via suppression of DNA dependent RNA polymerase II enzyme [75], inhibition of DNA, RNA and protein synthesis [51] and via strong binding affinity of crocetin with tRNA [76] in case of cancer cell lines. Bathaie et al. reported the conformational changes induced by the interaction of saffron and its components with DNA, the order of potential of saffron constituents interaction with DNA was crocetin > DMC > > crocin [77]. Components of saffron i.e. crocin, crocetin, dimethyl crocin, and safranal interact with nucleic acids and protect them from harmful damages [76,78] e.g. in case of MMS induced DNA lesions, crocin significantly decreased MMS induced DNA damage in a dose-dependent manner [79] and in other report *Crocus sativus* extract (CSE) and crocin enhanced base excision repair system (BER) [80].

Another significant way of regulating growth of cancer cells is via apoptosis. Apoptosis (programmed cell death) is a gene-regulated phenomenon induced by many chemotherapeutic agents in cancer treatment [81]. The induction of apoptosis in tumour cells is considered to be very useful in the management and therapy as well as in the prevention of cancer. Several studies have shown cells to be sensitive to saffron and its components which have shown inhibitory effect on the progression of cancer, both *in vivo* and *in vitro,* via apoptosis induction and cell cycle arrest at phases like G0/G1phase [60,61,82] and G2/M phase (in MIA-PaCa-2 cells). Both pro-apoptotic effect of saffron on cancerous cells and anti-apoptotic effect on noncancerous cell lines has been reported [83]. Proapoptotic effects of ethanolic extract of saffron at higher concentrations in lung cancer-derived cell line i.e. cultured carcinomic human alveolar basal epithelial cells (A549) has been reported. However, no such effect was found on non-malignant (L929) cells [84]. Similarly, crocin has been found to exert its pro-apoptotic effect and arrest cell proliferation of human acute promyelocytic leukaemia cell line (HL-60), human hepatocellular carcinoma (KIM-1), a cholangiocarcinoma (KMC-1), B-cell hybridomas, U937 amonocytic cell-line, HeLa cells, human lymphoid leukemia (MOLT-4B) cells and K562 [85] and anti-apoptotic action on in PC-12 cells treated with daunorubicin [86]. Besides the above effects imparted by crocin, down regulatory effect on the expression of the catalytic subunit of the enzyme human Telomerase reverse transcriptase (hTERT) has been found to inhibit telomerase activity and is considered to be one of the possible ways to suppress the proliferation of cancer cells [63]. Apart from the mechanisms cited above for anti-tumour activity of crocin, inhibitory effect on the proliferation of cancer cell lines by the epigenetic changes incurred by crocin is the new addition to the list and is considered as necessary phenomena. These epigenetic alternations result in the inhibition of proliferation of cancer cells' or/and induction of apoptosis, suppression of the telomerase activity and active STAT3, and targeting of microtubules [87].

Administration of crocetin to the experimental animals has been found to reduce activation of hepatic apoptotic pathways [88] and in some conditions observed to arrest cell cycle as well. Cell cycle arrest by crocetin is anticipated to be either via p53-dependent or p53-independent P21 mediated mechanisms [54]. The other mechanisms involved are via regulating cell cycle regulatory proteins (Cdc-2, Cdc-25c, cyclin B1) and apoptosis signalling pathway proteins e.g. safranal, has shown anti-apoptotic potential by up regulating B cell lymphoma -2 (Bcl-2) expression, down regulating Bax and caspase 3 expression with decreased TUNEL positivity [89], inhibition of nuclear factor-kappa B activation, increased cleavage of caspase-3, as well as DNA damage and cell cycle arrest (HepG2 cells) [42]. In benzo[*a*] pyrene-induced genotoxicity, crocetin showed inhibitory effect on DNA adduct formation in C3H1OT1/2 cell lines and aflatoxin B1 activated cytotoxicity and DNA-adduct formation. These effects were mediated by elevating activities of enzymes like GST and glutathione peroxidase [90]. All these mechanisms contribute towards strong cancer preventive activity of crocetin.

Although, saffron and its constituents show apoptotic effect in cancerous cells anti-apoptotic effect in normal cell line has also been reported [91,92]. The differences in sensitivity to the effect of saffron and its bioactive components in normal and the malignant cells attributed to the existence of distinct cell surface receptors, intracellular retention transport and differences in the uptake of certain drugs or in the methods used for the extraction and assessment of toxicity [52,93–95]. The above discussed findings are of great significance indicating saffron as a chemo-preventive agent, however, understanding the detailed molecular mechanism of anticancer/antitumor activity of

saffron and its components needs further understanding. Moreover, elaborate clinical trials are required in order to warrant the use of saffron and/or its main ingredients as natural agents for prevention and treatment of different human cancers.

2.3. Effect on nervous system

Depletion of cellular ATP is the hall mark event of ischemia and other nervous system diseases which results in biochemical and physiological changes that have deleterious effect on various tissues [96]. Brain has high oxygen consumption, high lipid contents especially (PUFA) and high concentration of transition metals. Decrease in antioxidant activity and increase in ROS generation makes brain more susceptible to oxidative stress leading to acute central nervous system (CNS) injury [97]. After ischemia, the production of ROS may increase, leading to tissue damage via several different cellular molecular and biochemical pathways. Radicals can cause damage to cellular components such as lipids, proteins, and nucleic acids (e.g. DNA), leading to subsequent cell death by apoptosis [97].

2.3.1. Ischemia

Oxidative stress in central nervous system CNS results in ischemic reperfusion, Alzheimer's disease, Parkinson's disease, seizures and other neurological and neurodegenerative disorders [97]. During the last few decades, an overwhelming attention has been paid to the use of plant products as alternative medicines to negate the effect of ROS and cellular ischemia reperfusion injuries mediated by ROS.

Saffron and its components have been found to act as good curative agents in ischemia and neuronal disorders because of which use of saffron has been suggested in focal ischemia [98] autoimmune encephalomyelitis in C57BL/6 mice, cerebral ischemia [99], hippocampal ischemia [80,81] and renal ischemia reperfusion [100]. Saffron has antioxidant activity that is possibly the main cause of attenuation in cerebral ischemia induced oxidative damage in rat hippocampus [101]. To investigate the effects of saffron in brain disorders, Ghazavi et al. [102] used ethanolic extracts of saffron in mice and found that antioxidant potential was increased which was reflected by increased level of glutathione and its dependent enzyme, by suppressing the increased content of MDA, glutamate and aspartate.

In case of cerebral ischemia pre-treatment of crocin markedly inhibited oxidizing reactions in mice micro vessels besides modulating the ultrastructure of cortical microvascular endothelial cells (CMEC) [103]. In ischemic stroke rat model, crocin reduced MDA level, increased the activity of SOD and GPx [28], reduced lipid peroxidation and increases antioxidant capacity to counter the decrease in the activity of enzymes such as SOD, Na^+K^+-ATPase, catalase etc.

2.3.2. Memory impairment

Saffron extract has been reported to attenuate morphine-induced memory impairment [104], ethanol induced memory impairment in mice [105,106], scopolamine induced memory impairment in Morris water maze model in rats with no effect on intact memory [107]. Saffron intake significantly reversed the Al (aluminium) induced changes in monoamine oxidase (MAO) activity and the levels of MDA and GSH, besides, significantly decreasing AChE activity in cerebral tissues of Al^+ saffron group in Al induced memory impaired rats [108]. Hosseinzadeh and Nassiri-Asl [109] reported that saffron extract and crocin improved spatial cognitive abilities following chronic cerebral hypo perfusion and is involved in memory modulation and information retrieval [110]. Subsequent studies revealed that crocin played preventive role in learning impairment, combating oxidative stress damage and in enhancing memory in aged mice [88]. All these positive effects of saffron and its components can be attributed to its antioxidant property that is responsible for scavenging free radicals involved in the memory impairment.

2.3.3. Neurodegenerative diseases

Neurodegenerative diseases such as Alzheimer's Disease (AD) and Parkinson's are associated with the formation of toxic amyloid structures because of their ability to bind to the hydrophobic patches of intermediate structure of proteins [111]. Ebrahim-Habib et al. reported that saffron has the capacity to inhibit the aggregation and deposition of amyloid β in the human brain and prevent short-term memory problems besides being both safe and effective in mild to moderate depression in Alzheimer's disease [112]. High antioxidant properties saffron significantly contributed to inhibition of Aβ fibrillogenesis in a concentration and time-dependent manner. The amphiphilic character of crocin was found to make it more effective in preventing accumulation of toxic amyloid structures [113]. However, trans-crocin 4 at lower concentrations than dimethyl crocetin showed its effect by inhibition on A-beta fibrillogenesis via oxidation of the amyloid beta--peptide fibrils in Alzheimer's disease [19]. Intracerebroventricular (ICV) injection of streptozotocin (STZ) to rodents has been frequently used as an animal model for sporadic AD [114] treatment *with* saffron (30 mg/kg) for 3 weeks was found to improve cognition deficits induced by ICV injection of STZ in rats [115]. However, the subsequent evaluation suggested crocin to be the main component of CSE to antagonizes the cognitive deficits caused by STZ-ICV in rats and has potential of being used in the treatment of neurodegenerative diseases [115]. Another therapeutic approach to AD is by inhibiting acetylcholinesterase (AChE) which is involved in acetylcholine breakdown, saffron had a moderate (up to 30%) inhibitory effect on AChE activity and is suggested to play an important therapeutic approach for the treatment of AD [18].

In case of Parkinson's disease (mainly characterized by the degeneration of neurons in the substantia nigra,) enhancement of antioxidant system along with depletion of Thiobarbituric acid (TBARS) by crocetin results in prevention of deleterious effects of compounds like 6-hydroxy dopamine (involved in inducing Parkinson's disease) and also reduction in dopamine utilization by tissues was suggested as a possible mechanism [116]. In another case, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced Parkinson's disease, the protective effect of saffron pre-treatment on dopaminergic cells in the substantia nigra pars compacta (SNc) and retina in a mouse model was reported [117]. Yoshino et al. [118] investigated the antioxidant effects of crocetin on reactive oxygen species such as hydroxyl radical in stroke-prone spontaneously hypertensive rats, a high-oxidative stress model, and observed significant inhibition of hydroxyl radical generation compared with the control. Based on the literature, beneficial effects of the saffron and its components on neurodegenerative disorders such as Alzheimer and Parkinson's disease are mainly due to their interactions with cholinergic, dopaminergic and glutamatergic systems.

2.3.4. Anti-convulsant role

Hosseinzadeh and Khosravan [119] indicated that the aqueous and ethanolic extracts of *C. sativus* have anticonvulsant activity in pentylene tetrazole (PTZ) and maximal electroshock seizure (MES) induced seizures. Similar effects were shown by safranal by decreasing the incidence of both minimal colonic seizures and generalized tonic colonic seizures, reduced seizure period and mortality percentage and delayed the onset of convulsions contrary to that of crocin which did not show any effect [120]. The effect of safranal is believed to be mediated partly through GABA (A)-benzodiazepine receptor complex [102–105]. It is assumed that saffron's anticonvulsant and analgesic properties and its effects on morphine withdrawal and rewarding properties of morphine might be due to an interaction between saffron, GABA and opioid system [121]. Although, the results shown by saffron and its components are promising and can be used for the development of new therapeutics for the treatment of these diseases but more *in vivo* studies and clinical trials on humans are required.

2.3.5. Mechanism of action

From the studies conducted so far, it can be concluded that use of saffron and its components delay, prevent and alleviate ischemia, neuronal disorders and neurodegenerative diseases by modulating molecular and biochemical pathways of the cells. Antioxidant properties of saffron and its constituents are responsible for inhibiting aggregation and fibrillar formation in neuroprotective disease [19] and also in other complications, where it is involved in preventing lipid peroxidation by quenching the hyper generated ROS like, superoxide radical, nitric oxide (NO) and TBARS.

In other detoxification mechanisms, glutathione (GSH) and its related enzymes have a central role to play [122,106–107]. Glutathione is an antioxidant that detoxifies ROS especially in neurons and loss of glutathione is being considered an early possible signal in apoptotic cell death and increase in ROS generation [123]. On the other hand, MDA occurs naturally and is assayed as a biomarker of oxidative stress. ROS degrades polyunsaturated lipids forming MDA that is an electrophilic species and is considered as a potential mutagen. Thus, the components that increase the GSH pool and induce related enzymes that can decrease MDA could play important role in the inhibition of the genotoxicity, carcinogenicity, ischemia and other complications. There are various reports of saffron and its constituents showing reduction in lipid peroxidation, improving total antioxidant capacity, increasing GSH pool, decreasing MDA level as well as antioxidant enzyme activities such as superoxide dismutase, catalase, and GSH-related enzymes [101]. The effect of saffron and its components on the biochemical and physiological parameters are summarised in Fig. 3.

2.3.6. Role in depression and anxiety

Depression is one of the most commonly diagnosed psychological disorder [124]. The risk of this disease involves trauma, stress, and viral infections [125]. Although therapeutic approaches for the combating depression exist, however, pharmacotherapy is currently the most commonly used outpatient treatment for depression [126]. Many antidepressant medications have side effects which leads to the increase in herbal psychopharmacology research. Herbal derivatives are emerging as popular alternatives to prescribed medications for the treatment of depression [127].

2.3.6.1. Saffron extract. Based on the recent findings and from clinical trials, saffron has emerged as a promising medicinal herb for the treatment of depression. Halataie et al. reported the positive effect of aqueous and ethanolic extract of saffron, safranal and crocin, in stress-induced reduction in food intake, weight gain and anorexic time in mice. Aqueous extract of saffron and crocin were also found to reduce the side effects of electroshock stress in mice [128]. There are additional reports that support the anti-depressant activity of aqueous and alcoholic extracts of saffron [109]. In a separate comparative studies, saffron was found to be as effective as imipramine [129] and fluoxetine in the treatment of mild to moderate depression [130].

2.3.6.2. Saffron components. Metabolic syndrome (MetS) is a condition which is associated with increased depressive symptoms, Jam et al. assessed the effect of preparation of crocin i.e. 30 mg of crocin (2 tablets of 15 mg) on volunteers and reported that the symptoms of depression in subjects with MetS were reduced [131]. Georgiadou et al. [132] revealed the positive effects of crocins in Obsessive-Compulsive Disorder (OCD) and suggested the possible role of crocins in the treatment of compulsive behaviour. They also pointed towards the possibility of a functional interaction between crocin and the serotonergic system. Safranal has also been reported to have significant antidepressant activity [133] however; major contribution to this effect has mainly attributed to crocin 1 and crocin 2 forms of crocin [134]. These experiments suggest saffron to be safe and effective antidepressant agent with crocin being the main responsible component. Although, the trials showed positive and promising effects but comprehensive and extensive study is required to analyse the other parameters (toxicities and adverse effects) of saffron, while

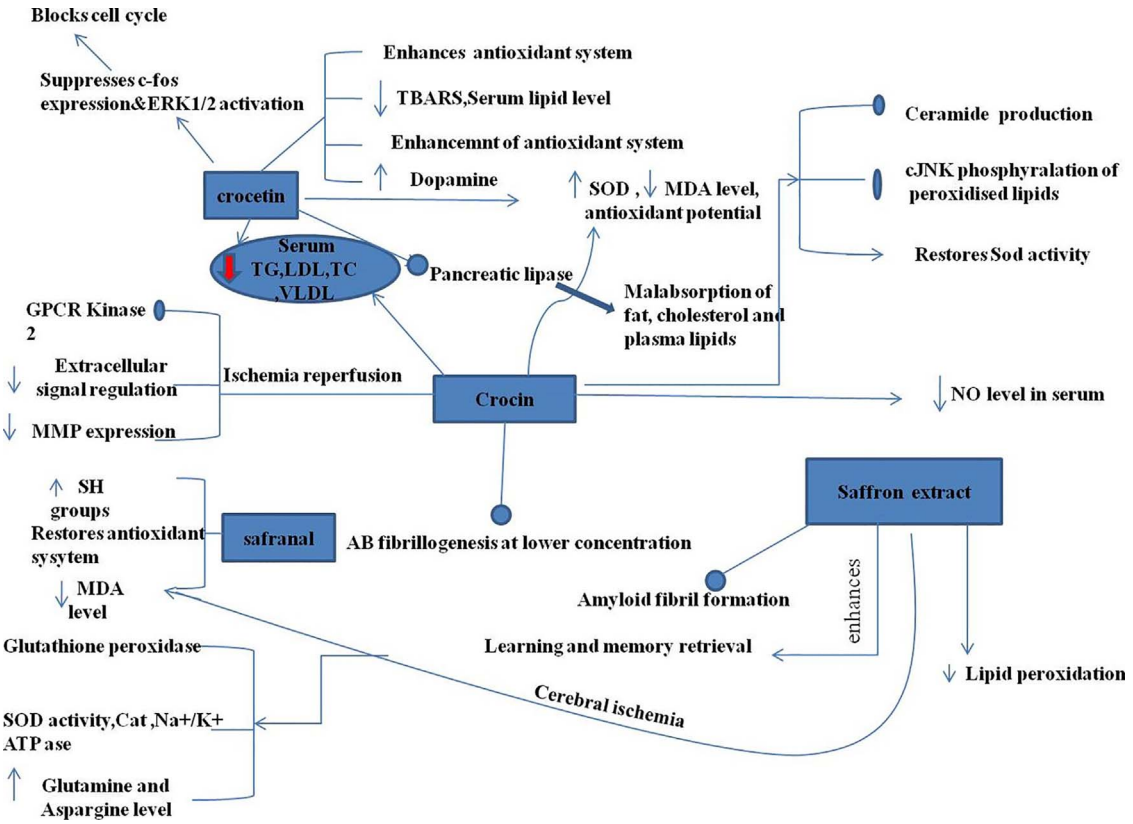

Fig. 3. Effect of saffron and its components on various biochemical and physiological parameters.

being used in the treatment of depression.

2.4. Visual impairment

Antioxidant property of saffron also plays a crucial role as a curative agent in visual impairment [135]. Continuous exposure of albino rats to light was found to cause photoreceptor and retinal stress. Saffron given as a dietary supplement counteracted the effects of continuous light exposure, besides maintaining both morphology and function by acting apoptotic regulator [136]. Components of saffron have shown their effect via acting on signalling pathways e.g. crocin plays a role in the activation of PI3K/AKT signalling pathway in ganglion cell layer after retinal injury [137] and was used in ischemic retinopathy where was found to increase retina and choroid blood supply besides facilitating the recovery of retina functioning [138]. Oral administration of crocetin prevents *N*-methyl-D-aspartate (NMDA) induced retinal damage in murine retina by acting and inhibiting the caspase pathway [139] and trans- crocetin, an isoform of crocetin decreases NMDA induced membrane depolarisation but does not inhibit non NMDA components indicating that trans-crocetin is involved in the antagonistic effect of CSE on NMDA receptors [140]. In another study, protective effects of crocetin against retinal damage induced by oxidative and endoplasmic reticulum stress, besides inhibiting the increase in caspase −3 and −9 activities after retinal damage have been reported [141].

2.5. Anti-arthritic activity by acting as anti-inflammatory agent

Arthritis/Rheumatoid arthritis is associated with inflammation. Saffron and its components show anti-inflammatory activity that in turn has positive effect in the treatment of arthritis. Saffron extract treatment showed anti-inflammatory activity in xylene induced ear edema in mice and chronic inflammation in formaldehyde induced arthritis [20]. *In vitro* analysis revealed anti-inflammatory activity of crocin by acting on cyclooxygenase pathway and inhibiting both cyclooxygenase 1 (COX1), cyclooxygenase 2 (COX2) enzymes and by inhibiting production of prostaglandin E (2) (PGE -2) in a dose dependent manner [142]. Revitalizing effects by crocin treatment were reported in arthritis induced cartilage and bone deterioration and inflammation. Crocin treatment was found to re-establish the antioxidant status of the system (GSH, SOD, CAT and GST) which was altered due to arthritis moreover, crocin played its role as potent inhibitor of the elevated levels of bone joint exo-glycosidases, cathepsin-D and tartarate resistant acid phosphatases. Matrix metalloproteinases (MMPS) produced by interleukin 1β are the contributors of osteoarthritis [143] studied inhibitory effect of crocin on the expression of MMP-1 and MMP-3, hence, alleviating inflammation in osteoarthritic condition. Due to these properties crocin is regarded as an effective anti-arthritic agent that can equally nullify the arthritis associated secondary complication [143]. Saffron and crocin have been found to show mitigating effects on arthritis by modulating cartilage deteriorating enzymes, inflammatory mediators, MMP'S and antioxidant stress.

2.6. Hypolipidemic effect

Balanced catabolic and anabolic processes are required to keep normal lipid levels in the body and saffron and its components have been found to stabilise the disturbed lipid metabolism [144]. Hyperlipidemia is one of the main risk factors in the initiation and progression of atherosclerotic lesions and cardiovascular complications and is associated with the increased levels of serum cholesterol, LDL, very low density lipoproteins (VLDL), fatty acids, TGs and disturbance of other biochemical parameters [145]. Hence, lowering the elevated levels of these parameters could be an important strategy in prevention of coronary heart disease and related complications. Premkumar et al. [59] reported that saffron and its constituents show decreased elevated levels of triglycerides (TGs), total cholesterol (TC), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine aminotransferase (ALT), MDA, GSHPx, GSH, and Glutathione disulphide (GSSG) in serum and increasing effect on SOD, CAT, fluorescence recovery after photobleaching (FRAP), and SH values in liver tissue. Saffron was found to be more effective as compared to its individual constituents possibly because of the synergistic role played by the constituents to quench free radicals and ameliorate the damages of hyper-lipidemia [38].

Crocin has been reported to show hypolipidemic effect in diet-induced hyper-lipidemic rats, by reducing serum TG, total cholesterol and LDL and VLDL levels [146]. Hypo-glyceridemic and hypo-cholesterolemic effect of crocin is also reported in quails kept on hyper-lipidemic diet [147]. Selective inhibition of pancreatic lipase by crocin via competitive inhibition plays an important role in lipid lowering property [146]. Reduction of serum total cholesterol (TC), LDL and TG in quails was also prominent on treatment with crocetin [148]. Cousin and Miller [149] reported that intraperitoneal injection of crocetin was more effective in showing hypolipidemic effect compared to that of the subcutaneous injection.

Crocetin along with crocin showed inhibitory effect on the increased serum TG, cholesterol and LDL level in hypolipidemic rats [147,148], while, crocin and safranal showed dose dependent hypotensive effect. Between these two components, safranal was found to be more potent than crocin for lowering blood pressure [150].

2.7. Role in myocardial infarction and atherosclerosis

Myocardial infarction (MI) is an acute condition of myocardial necrosis that is because of imbalance between coronary blood supply and myocardial demand. MI results in the alterations of several biochemical parameters such as lipid peroxidation, hyper-lipidemia, free radical damage and hyperglycaemia [151]. Saffron has been found to play an important role in electrophysiological remodelling of the atrioventricular (AV) node during atrial fibrillation by increasing the AV nodal refractoriness and zone of concealment [152]. Boskabady et al. [153] documented a potent inhibitory effect of aqueous extract of saffron on the calcium channel of guinea-pig isolated heart. Dose dependent preventive effect of saffron in isoproterenol (ISO) induced myocardial infarction was elucidated by histopathological and ultrastructural examinations by Goyal et al. [154] in male Wistar rats. However, dose dependent effect of crocin in modulation of oxidative stress by maintaining the redox status of cell was reported and it was found that crocin exhibits regression and inhibition of atherosclerosis via apoptosis in bovine aortic endothelial cells by increasing Bcl2/Bax ratio expression [155]. Intravenous administration of crocin was found to reduce myocardial injury and LDH and creatine kinase level however, the same effect was not observed when administered orally possibly because of the inefficient absorption of crocin through gastrointestinal tract [155,114]. There are other reports related to cardio protective role of saffron and its constituents [156].

Main causative factor for atherosclerosis is the disturbance in lipid metabolism. Anti-atherosclerotic effects of saffron were observed mainly because of crocetin that decreased the level of cardiac markers e.g. LDH, creatine kinase (CK) and MDA besides increasing mitochondrion potential in noradrenaline treated cardiac myocytes [148]. The role of crocetin in atherosclerosis has been reported by He et al. [148] in quail and suggested it to be a potential anti atherosclerotic agent. Crocetin administration significantly decreased cholesterol deposits in aorta, atheroma, foam cells, and atherosclerotic lesions in the crocetin-fed animals [148]. Crocetin has been found to reduce cholesterol level indirectly in the blood, hence severity of atherosclerosis [148]. Possible reason for decreasing cholesterol level is because of the suppression of NF-κB crocetin which in turn decreases the vascular cell adhesion molecule-1 (VCAM-1) expression [31]. his Anti-atherosclerotic effect of crocetin has been attributed to its antioxidant activity that decrease ROS induced MDA levels (index of lipid peroxidation) [157]. The protective effect of crocetin against norepinephrine (NE) induced

cardiac hypertrophy, was evaluated. Crocetin was found to improve the myocardial pathological and histological changes induced by NE via the modulation of endogenous antioxidant enzymatic activities which reduced the content of lipid peroxidation (LPO), increased the GSH-Px and SOD activity in cardiac hypertrophy [158].

Changes in the biochemical parameters like increased level of MDA, decreased activity of antioxidant enzymes, accumulation of free radicals near myocardial cell membranes, effect on ion Ca^{2+}channels and many others are also associated with myocardial damage [159]. Dose dependent inhibition of ADP and collagen induced platelet aggregation by crocetin via inhibition of Ca^{2+} elevation in stimulated platelets has also been reported [160]. Thus, the possible mechanism involved in cardio protective effect of saffron is mainly because of antioxidant activity, recovery and up-regulation of antioxidant enzymes [161] e.g. GPx and by inhibition of cardiac calcium channels that result in decreased heart rate and contractility, and finally reduction in heart workload and hence prevention of heart from injury.

2.8. Role in lung pathology and respiratory tract

Extracts of saffron and safranal show preventive effect in lung pathology during lung inflammation of sensitized guinea pigs [162]. Safranal (0.2, 0.5 and 0.75 ml/kg) administered to guinea pigs 30 min before the initiation of 10 min exposure to citric acid aerosol (an irritant agent) was found to reduce the cough count significantly as compared to saline treated group [163]. The effect of safranal on histamine (H1) receptors was evaluated in guinea pig tracheal tissue in organ bath; results obtained inferred that safranal possibly acts as a competitive antagonist of histamine H1 receptors [164]. Bukhari et al. [165] investigated the anti-oxidizing potential of *Crocus sativus* and its main constituents in bronchial epithelial cells, where stress was induced in these cells by a combination of different cytokines that resulted in an increase in nitric oxide production (NO), induced nitric oxide synthase (iNOS) levels, peroxynitrite ion generation, and cytochrome c release. Treatment with saffron and its constituents, safranal and crocin resulted in a decrease of NO, iNOS levels, peroxynitrite ion generation, and prevented cytochrome c release. However, safranal was found to significantly reduce oxidative stress in bronchial epithelial cells via iNOS reduction besides preventing apoptosis in these cells. Moreover, in the murine model of asthma study, anti-inflammatory role of safranal was characterized by increased airway hyper-responsiveness, and also reduced iNOS production, bronchial epithelial cell apoptosis, and Th2 type cytokine production in the lungs.

2.9. Antidiabetic role

Diabetes mellitus is a metabolic disorder associated with chronic disorder of fat, carbohydrate, protein metabolism and oxidative defense system. Increased oxidative stress and impaired antioxidant defense systems results in the initiation and progression of diabetes-associated complications and diabetes mellitus is encephalopathy is one of the severe complications in patients with diabetes mellitus [166]. Saffron extract has antioxidant properties which contributed to protective activities of saffron. Saffron at 40 and 80 mg/kg significantly increased serum tumor necrosis factor (TNF-α) (a marker for inflammation, and oxidative stress parameters) and decreased blood glucose levels, glycosylated serum proteins, and serum advanced glycation end products (AGEs) levels. AGEs are known to initiate and aggravate the pathological damage in diabetic encephalopathy. Furthermore, significant increase in HDL and decreased cholesterol, triglyceride, and LDL has been reported. These findings demonstrated a protective effect of saffron extract on hyperglycemia and hyperlipidaemia conditions and also a remarkable decrease in the oxidative stress in diabetic encephalopathy rats [167].

Crocetin was also found to prevent AGEs-induced bovine endothelial cell (BEC) apoptosis through ROS inhibition and ($[Ca^{2+}]i$) stabilization, showing preventive effects in diabetes-associated vascular complications [168]. The effect of crocetin on insulin resistance and its related abnormalities induced by high-fructose diet were investigated in male Wistar rats [169]. Compared to the control rats fed on normal diet, fructose-fed rats developed a series of pathological changes including insulin resistance, hyperinsulinemia, dyslipidaemia and hypertension. Additional changes include, decreased expression of both protein and mRNA of adiponectin (an insulin-sensitizing adipocytokine), enhanced TNF-α and leptin in fructose-fed rats. Treatment of crocetin to primary cultured rat adipocytes was found to alleviate free fatty acid (FFA)-induced insulin insensitivity and dysregulated mRNA expression of adiponectin, TNF-α and leptin, suggesting the preventive role of crocetin in insulin resistance and related diseases [169]. End products of advanced glycation AGEs cause endothelial cell apoptosis that leads to vascular complications. Crocetin because of its antioxidant capacity and calcium antagonistic activity is considered to be possible remedy for diabetic vascular complications [170].

3. Other miscellaneous roles

3.1. Effect on blood cell count

Saffron in comparison to safranal has shown more positive effect on total and differential count of WBC in blood of sensitized guinea pigs. However, safranal was more effective in the improvement of eosinophil and lymphocyte [162].

3.2. Nephrotoxicity

Saffron extract has been found to show a dose dependent reduction of gentamicin induced nephrotoxicity and significant decrease in the MDA levels in Wistar rats [171]. Similarly, Boroushaki demonstrated that saffron treatment reduces Hexachlorobutadiene (HCBD) (a potent nephrotoxin) induced nephrotoxicity in a dose dependent manner [172].

4. Toxicology

Saffron and its constituents have been used to treat various ailments and its scope has widely increased during the last few decades as it has proved to be important as therapeutic medicine. However, to evaluate the appropriate effect of saffron and its constituents for its further development, assessment of toxicity is important [121]. According to the toxicity classification, substances with a LD50 value within the range of 1–5 g/kg are considered a practically low-toxic and substances that show LD50 higher than 5.0 g/kg are considered to be practically non-toxic [173]. In case of saffron 1.5gm/day of saffron has been considered to be safe, 5 gm/kg as toxic and 20 gm /kg as lethal dose. Saffron and its components are usually water soluble that possibly makes it safe and non-toxic even at higher doses [174]. However, toxic effects are reported in case of pregnancy where continuous dosage of above 10 gm saffron was enough to cause abortion [175]. It is for this reason that use of saffron is not recommended during pregnancy [175]. But, in case of ATRA (All-Trans Retinoic Acid) sensitive cancers crocetin is recommended because of it being lesser toxic than ATRA to pregnant women [176]. In some studies, there are also reports of allergies e.g. anaphylactic reaction mediated by immunoglobin [177] however, these kind of sensitivities were found to occur only at higher doses of saffron and was found to be very rare.

As crocin, showed a variety of interesting pharmacological activities, its acute and sub-acute toxicity has been evaluated in mice and rats where long-term treatment of crocin has not been found to induce metabolic changes in rats which suggested crocin being practically nontoxic at pharmacological doses [178]. The non-mutagenicity of crocin and its other form i.e. di-methyl crocetin has also been reported [179]. Acute and sub-acute toxicity studies of safranal have shown it to

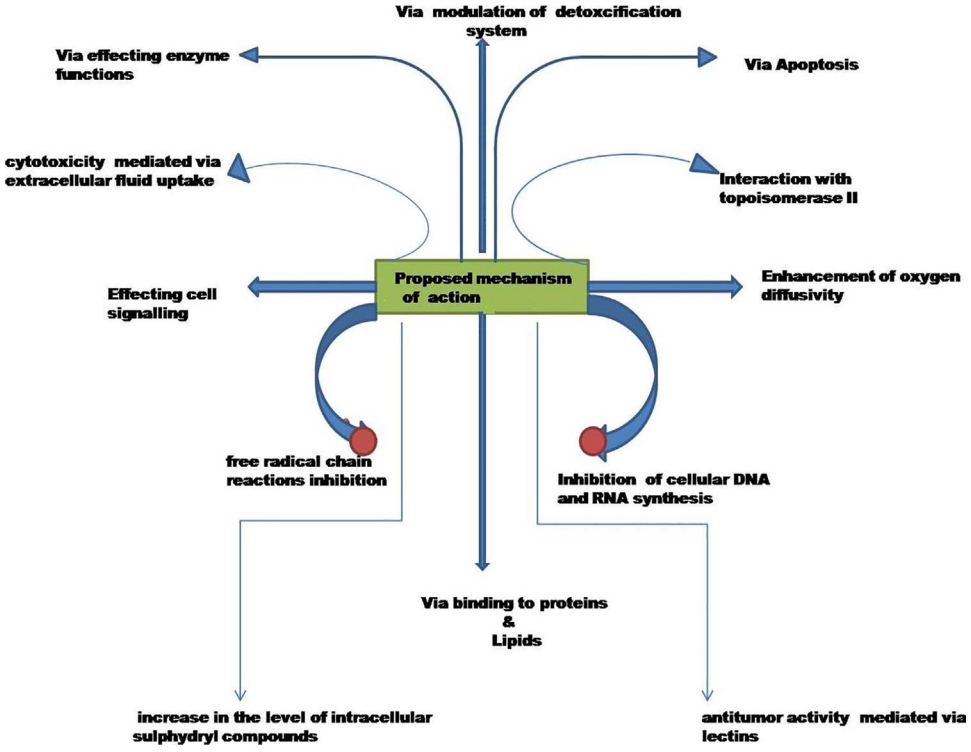

Fig. 4. Possible mechanism of action contributing to therapeutic role of saffron and its components.

be low toxic when administered intra-peritoneally and practically non-toxic via oral administration in both mice and rats [180].

Available literature suggests that saffron and its components at lower concentration or even at pharmacological doses is safe. However, it can be toxic at higher concentration and lethal when taken at very higher concentration.

5. Conclusion

Oxidative stress associated with hyper generation of ROS has been found to be responsible for various clinical conditions including, cancer, atherosclerosis inflammation, ischemia reperfusion and malignant disease etc. Hyper generation ROS causes oxidative damage to DNA or proteins that effects the expression of the proto-oncogenes and tumour suppressor genes, dysregulating metabolic and signalling pathways and other abnormal conditions. Saffron and its components regulate the redox status of the cell by imparting their effect via regulation of metabolic and cell signalling pathways, however, the hallmark for the mechanism of action is radical scavenging activity/antioxidant activity. (Mechanism of action involved is graphically summarised in Fig. 4)

The findings mentioned in this review are certainly encouraging to use saffron and its components for the treatment of various disease including as a potent chemo preventive agent in cancer. However, there is a need to investigate and dissect a clear mechanism of action of saffron and its individual components, to check toxicity effects more extensively. Although clinical trial on humans have been carried out in case of depression and the results are positive however, more trials needs to be performed in other disease to validate in *vitro* and *in vivo* results.

Acknowledgements

The authors thank Department of Biotechnology, GOI and Coordinator, Bioinformatics centre (DBT-BIF), School of Biotechnology for providing necessary facilities.

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