2. Literature review

This chapter represents a review of the current developments in the area of optimization, extraction, isolation and structural characterization of bioactive polyphenolic compounds from plant sources, subsequently its nanoparticles preparation by green chemistry methods and \textit{in vitro} and \textit{in vivo} hepatoprotective effects against drugs induced hepatotoxicity in common carp fish.

2.1. Optimization of the solvent extraction of bioactive polyphenolic compounds from plant sources

Since the 1970s, the World Health Organization has recognized the importance of traditional medicine as an affordable source of health care, including treatment of tropical disease. Thus, the extraction and purification of bioactive compounds from natural sources have become more and more important for the utilization of phytochemicals in the preparation of dietary supplements or nutraceuticals, functional food ingredients, and additives to food, pharmaceutical, and cosmetic products [1]. Different solvent systems have been used for extraction of polyphenols from plant materials. Many parameters such as particle size, extraction solvent, solvent concentration, pH, extraction temperature and time etc. have significantly influenced the extraction yield [2, 3]. The extraction method must enable complete extraction of the compounds of interest and must avoid their chemical modification. Water, aqueous mixtures of ethanol, methanol and acetone are commonly used. However, the conventional approach for the optimization of a multivariable system is usually one variable at a time. This can be very time-consuming and when interactions exist between the variables, it is unlikely to find the true optimum condition. Response surface methodology (RSM) is a very useful tool for this purpose, which was first introduced by Box and Wilson [4]. As a package of statistical and mathematical techniques employed for developing, improving, and optimizing process, RSM can be effectively used to evaluate the effects of multiple factors and their interaction on one or more response variables [5]. One of the advantages of this method is that its ability to take into accounts the interactions among different variables as opposed to traditional
one variable at a time analysis. As an appropriate experimental design, RSM can reduce the number of experiments and provide a mathematical model [6-9].

The tradition assay to study the optimization condition was the one-factor-at-a-time approach, in which only one factor is variable at a time while others are fixed at constant values. But this method is time-consuming and cannot evaluate the interaction effects among the various factors. RSM is an effective technique to overcome these problems. It can explore the relationships between the response values and the independent variables and optimize the processes or products where multiple variables may influence the outputs [10]. RSM has been successfully used to model and optimize biochemical and biotechnological processes related to food systems [11-13]. Gong et al. (2012) reported the optimal extraction parameters of marigold extracts for the highest antioxidant activity by ABTS method were ethanol concentration of 79.7%, extraction temperature of 74.2 °C and time of 8.1 h, and by DPPH assay with 89.3% of ethanol concentration at 81.5°C for 11.1 h, antioxidant activity values were 2.42 and 1.86 mmol TE/g, respectively by successively using response surface methodology [14]. The detail works carried out on this direction (RSM with CCD) have been summarized in Table 2.1

| Table 2.1. Optimization of the solvent extraction of bioactive compounds from various sources by using Response surface methodology with CCD |
| Source | Independent variables | Dependent variables | Findings |
| Black currant leaves and buds | Solvent, pH, buffer, various status of plant materials | TPC, Antioxidant activities | The extraction solvent affected yield: aqueous acetone was better than methanol and acetate or glycine buffer. In aqueous buffer, maximum yields of total phenolics and antioxidant activities |
| | | | Reference [15] |
were obtained at pH 3. Extraction from lyophilized materials yielded extracts with higher phenolic contents and antioxidant activities.

<p>| <strong>Fructus sophorae</strong> | Ethanol concentration, solid–liquid ratio, temperature, and extraction time | Flavonoids (quercetin, kaempferol, and isorhamnetin) | Three aglycon forms of the flavonoids, namely, quercetin, kaempferol and isorhamnetin were quantified by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) to estimate extraction yield. The combined effects of independent variables were studied and the optimal extraction conditions were obtained as ethanol concentration, 74.47%; solid–liquid ratio, 17.99 ml/g; temperature, 89.13 °C; and extraction time, 2.10 h. | [16] |
| <strong>pomegranate</strong> | Solvent type, | Total phenolic | TPC varied from 5506.42 | [17] |</p>
<table>
<thead>
<tr>
<th>Material</th>
<th>Extraction method</th>
<th>Antioxidant parameters studied</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>(Punica granatum L.) peel</td>
<td>solvent to solid ratio, particle size, ethanol concentration (% v:v), temperature (°C) and time (min)</td>
<td>content (TPC), ferric reducing antioxidant power (FRAP), scavenging activity of DPPH radical and yield</td>
<td>The total phenolic content (TPC) ranged from 29.78% to 45.38%. FRAP and DPPH values varied from 24.30 to 63.37 mmol Fe²⁺/100 g dry weight and 60.12–83.52% inhibition, respectively. Extraction yields ranged from 8923.24 mg gallic acid equivalent/100 g of dry weight.</td>
</tr>
<tr>
<td>Olive Seeds (Olive europaea L.)</td>
<td>Extraction time, temperature, extraction cycle</td>
<td>TPC, antioxidant activities</td>
<td>The highest total phenolic compounds content and antioxidant activity were obtained at the optimized extraction time of 12 h, an extraction temperature of 70 °C and an extraction cycle using three stages.</td>
</tr>
</tbody>
</table>
| Apple flesh              | Acetone aqueous solution, solvent to solid ratio, temperature, and time | Ferric-reducing power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays, and by the determination of total phenol content | The optimal condition found was of 60%, 5 mL/g, 30 °C, and 61 min for acetone aqueous solution, solvent to solid ratio, temperature, and time, respectively, resulting in optimal antioxidant capacities of 2,152.96 μmol TE/100 g.
<p>| <strong>Boletus edulis mycelia</strong> | Ratio of dried mycelia to water (X1: 1:40–1:60), extraction time (X2: 6–10 min), and ultrasonic temperature (X3: 50–70 °C) | Polysaccharides | The optimized conditions were 56 °C, 1:55 of ratio of dried mycelia to water, and a time of contact of 8.4 min. Under these conditions, the experimental yield of polysaccharides was 15.48%, which was well matched with the predictive yield of 15.53%. | [20] |
| <strong>Garcinia mangostana Linn.</strong> | extraction time, solid to solvent ratio, and methanol concentration | Total phenolic concentration | Response surface analysis showed that the optimal extraction parameters which gave a maximum TPC yield of 140.66 mg gallic acid equivalent (GAE)/g powder were from a 2 h extraction with 0.05 solid to solvent ratio and | [21] |</p>
<table>
<thead>
<tr>
<th>Flying squid muscle protein (<em>Ommastrephes bartrami</em>)</th>
<th>Enzyme to substrate (E/S) ratio, reaction temperature, and hydrolysis time</th>
<th>DPPH radical scavenging power</th>
<th>The optimum conditions obtained were as follows: E/S ratio of 1.74%, temperature of 51 °C and time of 46 min, under which, DPPH radical scavenging activity of 74.25% was obtained. Moreover, it was found that the optimum hydrolysate of 8 mg/mL displayed relatively stronger inhibitory effect on lipid peroxidation compared with <em>e</em>-Tocopherol of 0.1 mg/mL.</th>
</tr>
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<tr>
<td><em>Agaricus bisporus</em></td>
<td>Ethanol concentration pH and precipitation time</td>
<td>Exopolysaccharides</td>
<td>The optimal levels for ethanol concentration (85%, v/v), pH (8) and precipitation time (22 h) were determined, and EPS production was estimated at 2.71 g/L. The</td>
</tr>
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actual yield of EPS under these conditions was 2.69 g/L.

2.2. Isolation and characterization of bioactive flavonoids from aquatic fern *Azolla microphylla*

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain [24]. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed [25]. Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bioprospecting of new plant-derived drugs [26]. The ongoing growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine [27]. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies [28]. The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them [29, 30]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [31]. Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host. So the identification of bioactive compound in plants, their isolation, purification and
characterization of active ingredients in crude extracts by various analytical methods is important. The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemicals in them [29, 30]. The instant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges [32], therefore they are more prone to extinction [33]. Conversely, because information on the use of plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing socioeconomic changes [34]. Furthermore, the indigenous knowledge on the use of lesser-known medicinal plants is also rapidly declining. Continuous erosion in the traditional knowledge of many valuable plants for medicine in the past and the renewal interest currently, the need existed to review the valuable knowledge with the expectation of developing the medicinal plants sector [35].

In the present review focused on isolation of flavonoids and various aspects of aquatic fern *Azolla microphylla*. Azolla species are unique among floating macrophytes, because they can grow in waters devoid of combined nitrogen, as they live in a symbiotic relationship with N₂ fixing cyanobacteria such as *Anabaena azollae* living in the dorsal lobe cavities of *Azolla* leaves. The doubling rate of *Azolla* spp. can be less than 2 days and large amounts of carbon are fixed via the *Azolla–Anabaena* association [36]. The *Azolla–Anabaena azollae* symbiosis is an important N₂-fixing association between eukaryotic fern and prokaryotic cyanobacterium. This host–symbiont combination is exploited as biofertilizer for many agricultural crops [37, 38]. The agronomic potential of *Azolla* for growing rice has been recognized nearly globally [39]. *Azolla* has been used alone or in combination with other inorganic nitrogen fertilizers [40, 41]. The continuous application of chemical fertilizers has led to ecological imbalances, including diversified soil problems and nitrate pollution *etc.* [42]. Nitrate pollution has
emerged as a large scale problem due to the over-utilization of fertilizers over a long period of time to increase crop yields. These problems of overuse of nitrogen fertilizers affect the biofertilizing efficacy of the biological nitrogen-fixers.

Floating ferns of the genus *Azolla* are exposed to a variety of consumers in their freshwater habitats. Nonetheless, they are able to achieve dense surface coverage, presumably due to some endogenous protective factor(s). A variety of animals have been reported to feed on *Azolla* including several species of fish and snails [43, 36]. In general, palatability, nutritive value and digestibility determine the desirability of a food source. Species of *Azolla* have been found to differ in their desirability to fish. The Nile Tilapia (*Oreochromis niloticus*), for example exhibits a strong preference for *Azolla filiculoides* in comparison to *Azolla pinnata* [44]. Abraham and Vidhu, (2012) reported on the phytochemical profile of the *Azolla microphylla* shows that tannins, phenols, sugar, anthroquinone glycosides and steroids [45]. *Azolla microphylla* is also rich in protein, vitamin and minerals and is used as food supplements for dairy cattle, pigs, ducks and chickens resulting in increased milk production, enhancement of weight of cattle, pigs, ducks, broiler chickens and production of eggs with layered yolk, as compared to conventional ones.

More than 4,000 flavonoids have been recognised, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks. The flavonoids have provoked considerable interest recently because of their potential valuable effects on human health. They have been several biological properties including anti-inflammatory, hepatoprotective anti-thrombotic and antiviral activities many of which may be associated, partially at least, to their antioxidant and free-radical-scavenging ability. The antiradical property of flavonoids is directed mostly toward HO and O₂ as well as peroxyl and alkoxyl radicals [46]. Furthermore, as these compounds present a strong affinity for iron ions their antiperoxidative activity could also be ascribed to a concomitant capability of chelating iron [47, 48].

Das and Pereira, (1990) have shown that a carbonyl group at C-4 and a double bond between C-2 and C-3 are also important features for high antioxidant activity in
flavonoids [49]. Butein and other 3, 4-dihydroxychalcones are more active than analogous flavones because of their ability to achieve greater electron delocalization. Likewise, isoflavones are frequently more active than flavones because of the stabilising effects of the 4-carbonyl and 5-hydroxyl in the former [50]. In the antioxidant action of o-dihydroxyfavonoids metal chelation is an important factor [51].

2.3. Green synthesis of nanoparticles

Materials scientists are conducting research to develop novel materials with better properties, more functionality and lower cost than the existing ones. Several chemical, physical and biological synthesis methods have been developed to enhance the performance of nanomaterials displaying improved properties with the aim to have a better control over the particle size, distribution and morphology. Synthesis of nanomaterials to have a better control over particle size, distribution, morphology, purity, quantity and quality by employing environmental friendly economical processes has always been a challenge for the researchers. Metal nanoparticles are being extensively used in various biomedical applications due to their small size to volume ratio and extensive thermal stability. Gold nanoparticles (GNPs) are an obvious choice due to their amenability of synthesis and functionalization, less toxicity and ease of detection. The present review focuses on various methods of functionalization of GNPs and their applications in biomedical research. Functionalization facilitates targeted delivery of these nanoparticles to various cell types, bioimaging, gene delivery, drug delivery and other therapeutic and diagnostic applications. This review is an amalgamation of recent advances in the field of functionalization of gold nanoparticles and their potential applications in the field of medicine and biology. Synthesis methods and properties of nanomaterials studied in this research work are reviewed below.

Since Faraday’s pioneering work in 1857 on the synthesis of colloidal gold by reducing NaAuCl4 with a solution of phosphorus in carbon disulphide [52, 53], several physical and chemical methods have been developed to produce gold NPs. Synthetic techniques based on the reduction of metal ions with sodium citrate or sodium borohydride, followed by surface modification of the produced particles with suitable capping
ligands and organic solvents [54, 55], raised environmental concerns, because of the toxic compounds used in the process. Also, it is difficult to obtain NPs of defined size and shapes (e.g., spheres, rod, cubes, hexagons, etc.) in high yield. Current synthetic methods result in mixed shape NPs that require expensive and low-yield purification procedures, such as differential centrifugation [56]. These limitations invite new eco-friendly (“green chemistry”) methodology for production of nanocrystals with desired shape. The detail of recent research works carried out on the preparation of noble gold nanoparticles on this direction has been summarized in below.

Khalil et al. 2010 studied the biological synthesis of gold nanoparticles (AuNPs) of various shapes (triangle, hexagonal, and spherical) using hot water olive leaf extracts as reducing agent. The high phenolic content of the hot water extract of olive leaves having strong anti-oxidant properties helped in the reduction of gold cations to AuNPs. The characterization of AuNPs revealed that the morphology of the AuNPs depends on the extract concentration and pH of the used medium. At higher concentration of the extract and basic pH, the pseudo-spherical particles are capped by phytochemicals [57].

Babu et al. 2011 synthesized GNPs using ethanolic fraction of *Fagopyrum esculentum* leaf extract. The synthesis process offers a ‘green’ opportunity for the production of nanoparticles with an optimum time of 16 s. The crystallinity of GNPs was confirmed by XRD analysis. FT-IR, NMR and EDX analyses demonstrated the presence of biomolecules on the surface of GNPs arising from the strong reducing molecules such as phenolic compounds, flavonoids and antioxidants which are involved in the reduction of gold salts to GNPs [58].

Andeani et al. (2011) biosynthesized gold nanoparticles using dried flowers extract of *Achillea wilhelmsii* plant [59].

Sougata Ghosh et al. (2012) studied the *Gnidia glauca* flower extract mediated synthesis of gold nanoparticles and evaluation of its chemocatalytic potential. The authors suggested that GGFE can provide an environmentally benign rapid route for synthesis of AuNPs that can be applied for various purposes. Biogenic AuNPs synthesized using GGFE exhibited excellent chemocatalytic potential [60].
Nagaraj Basavegowda et al. (2012) studied the plant mediated synthesis of gold nanoparticles using fruit extracts of *Ananas comosus* (L.) (Pineapple) and evaluation of biological activities. The authors reported the synthesized gold nanoparticles were generally found to be effective as antimicrobial agents against some important human pathogens like *E.coli* and *Streptobacillus* sp. which are affecting and cause diseases like food poisoning and rat-bite fever to human beings respectively [61]. Das et al. (2012) synthesized gold nanoparticles using *Amaranthus spinosus* leaf extract and also studied their optical properties of gold nanoparticles [62].

2.4. Hepatoprotection

Till date there is no effective medicine for hepatic diseases which is primarily caused by excess consumption of alcohol, high doses of acetaminophen, chemotherapeutic agents, hepatic viral infection, dantrolene sodium, valporic acid, peroxidised oil and isonicotinic acid hydrazide, etc. Consequently, control of liver diseases has become a major goal of modern medicine. The drugs offered by modern medicine for the treatment of liver diseases are corticosteroids and immunosuppressants which provide only symptomatic relief mostly without influencing the disease process and their use is associated with the risk of relapse and danger of side effects [63]. In traditional systems of medicine, like Ayurveda, medicinal plants and their formulations are used to cure liver diseases. Some of these plants and herbal preparations have been evaluated for their protective actions against hepatotoxins. Some of the polyherbal preparations were proved to be antihepatotoxic in action as evidenced by clinical trials.

2.5. Hepatoprotective effects of medicinal plants

Reen et al. (2001) studied the effect of different solvent extracts of various *Swertia* species in primary monolayer cultures of rat hepatocytes against carbon tetrachloride- and paracetamol-induced toxicity. The primary monolayer cultures of rat hepatocytes damaged due to carbon tetrachloride and paracetamol administration resulted in at the indicated concentrations reduced GSH by almost 50 and 80%, respectively, while the lactate dehydrogenase enzyme leakage was almost 15% above the untreated control.
The hexane and methanol extracts of *Swertia purpurascens*, *Swertia chirata*, *Swertia paniculata* and *Swertia cordata* exhibited better activity compared with other species investigated [64].

Bhanwra et al. (2000) studied the effect of aqueous leaf extract of *Azadirachta indica* in paracetamol-induced hepatotoxicity in rats. The liver damage due to paracetamol administration resulted in elevation in the activities of serum transaminases and gamma glutamyl transpeptidase (GGT). The extract of *A. indica* (500 mg/kg) significantly reduced the elevated activities of these enzymes in serum. *A. indica* was also found to be effective in reducing paracetamol-induced liver necrosis as evidenced by histopathological studies [65].

The ethanolic extract of *Trianthema portulacastrum* L. (Aizoaceae) exhibited a significant dose dependent (100 mg, 200 mg/kg p.o. 10×) protective effect against paracetamol and thioacetamide induced hepatotoxicity in albino rats. The degree of protection was measured by using biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin (BRN), and total protein (TP). The plant extract completely prevented the toxic effects of paracetamol (acetaminophen) and thioacetamide on the above serum parameters. The authors suggested that the protective action of *Trianthema portulacastrum* L. extract contains an alkaloid trianthemine, ecdysterone (a potent chemosterilant), saponin and punarnavine of these compounds have potential hepatoprotective activity against paracetamol and thioacetamide treatment [66].

Aqueous-ethanolic extract (50%, v/v) of leaves of *Cassia occidentalis* L. (Caesalpiniaceae), commonly known as ‘Kasondi’ exhibited hepatoprotective effect against paracetamol and ethyl alcohol intoxication in rats. The authors suggested that the few anthraquinones present in *C. occidentalis* may be responsible for hepatoprotective activity [67].

Marzouk et al. (2011) studied the hepatoprotective and antioxidant effects of hydroalcoholic extract of *Cichorium endivia* L. leaves (HCE) against acetaminophen-induced oxidative stress and hepatotoxicity in male rats. Oral administration of
acetaminophen produced liver damage in rats as manifested by the significant increase in liver MDA and serum total lipids, total cholesterol, creatinine, total bilirubin and enzyme activities (AST, ALT and ALP). While a significant decrease in the levels of liver GSH, GST, SOD, CAT, serum total protein and albumin was recorded. Pre-treatment of rats with *C. endivia* leaves extract or silymarin for 21 days succeeded to modulate these observed abnormalities resulting from acetaminophen as indicated by the pronounced improvement of the investigated biochemical and antioxidant parameters. These results substantiate the potential hepatoprotective and antioxidant activity of *C. endivia* leaves extract [68].

Shivashiri et al. (2012) studied the hepatoprotective action of celery (*Apium graveolens*) leaves in acetaminophen-fed freshwater fish (*Pangasius sutchi*). The authors suggested that the APAP exposed fish showed elevated levels of both circulating and tissue hepatotoxic markers (AST, ALT and ALP), reduced hepatic glycogen and lipid contents (TG and cholesterol), increased tissue lipid peroxidation markers (TBARS, LHP and PCO), altered tissue levels of enzymatic (SOD, CAT, GPx and GST) and non-enzymatic (GSH) antioxidants and cellular thiol levels (T-SH, P-SH and NP-SH), and reduced hepatic ions (Na, K and Ca²⁺) and abnormal liver histology. The abnormalities associated with APAP exposure were reversed on treatment with CE [69].

Yin et al. (2010) studied the hepatoprotective and antioxidant effects of *Glycyrrhiza glabra* extract against carbon tetrachloride (CCl₄)-induced hepatocyte damage in common carp (*Cyprinus carpio*). The authors suggested that *Glycyrrhiza glabra* extract (2.5, 5 and 10 µg/ml) was added to the carp primary hepatocytes before (pretreatment), after (post-treatment) and both before and after (pre- and post-treatment) the incubation of the hepatocytes with CCl₄. CCl₄ at 8 mM in the culture medium produced significantly elevated levels of lactate dehydrogenase (LDH), glutamate oxalate transaminase (GOT), glutamate pyruvate transaminase (GPT) and malondialdehyde (MDA) and significantly reduced levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Pre-treatment (5 µg/ml) and pre- and post-treatment (5 and 10 µg/ml) of the hepatocytes with *Glycyrrhiza glabra* extract significantly reduced the
elevated levels of LDH, GOT, GPT and MDA and increased the reduced levels of SOD and GSH-Px by CCl4; post-treatment of the hepatocytes with Glycyrrhiza glabra extract at 5μg/ml reduced the GPT and GOT levels and increased the GSH-Px level, but had no effect on the other parameters at all the studied concentrations. The results support the use of Glycyrrhiza glabra extract as a hepatoprotective and antioxidant agent in fish [70]. Jia et al. (2011) studied the in vitro and in vivo hepatoprotective and antioxidant effects of Astragalus polysaccharides against carbon tetrachloride-induced hepatocyte damage in common carp (Cyprinus carpio). The authors suggested that in vitro, APS (200, 400 and 800 μg/ml) was added to the carp primary hepatocytes before (pretreatment), after (post-treatment) and both before and after (pre- and post-treatment) the incubation of the hepatocytes with CCl4 at 8 mM in the culture medium. APS at concentrations of 200, 400 and 800 μg/ml significantly improved cell viability and inhibited the elevation of glutamate pyruvate transaminase (GPT), glutamate oxalate transaminase (GOT), lactate dehydrogenase (LDH) and malondialdehyde (MDA) and significantly increased the reduced level of superoxide dismutase (SOD). In vivo administration of APS at the doses of 1.5 and 3 g/kg in the diet for 60 days prior to CCl4 intoxication significantly reduced the elevated activities of GPT, GOT and LDH and increased the reduced levels of total protein and albumin in the serum; meanwhile, the reduced levels of SOD, glutathione and total antioxidant capacity (T-AOC) were markedly increased and the MDA formation was significantly inhibited in liver tissue. The results support the use of APS as a hepatoprotective and antioxidant agent in fish [71].

2.6. Hepatoprotective and antioxidant effects of green synthesized of nanoparticles

Roy et al. (2012) studied the engineered Andrographolide nanoparticles mitigate paracetamol hepatotoxicity in mice. The authors suggested that the functionalized nanoparticles could localize rapidly in the liver and up regulate hepatic GSH store. Hepatic localization of nanoparticles also favorably promotes antioxidant capacity in the liver. Thus, new AG nanoparticles can be useful in several areas including as a protective agent against liver damage in APAP overdose [72].
Chen et al. (2012) studied the effects of nanogold on the alleviation of Carbon tetrachloride-induced hepatic injury in rats. The authors suggested that the male SD rats were subjected to liver injury induction by CCl4, then the rats were fed with zero to high dose (0, 1, 5 or 10 ppm) of nanogold water every day for 4 weeks. Biochemical analyses on liver functions were then performed to evaluate the therapeutic effects of nanogold. The results revealed that gold nanoparticles lowered serum aspartate aminotransaminase (AST) and alanine aminotransferase (ALT) and exerted serum total protein (TP)-recovering effects, which might be partially associated with the elevation of anti-inflammatory cytokine IL-10 level. In addition, serum triglyceride (TG) level fell after continuous ingestion of nanogold. Finally, the experimental animals recovered body weight after 4 weeks of nanogold ingestion. This is the first report indicating inflammation-alleviating effects of nanogold on hepatic injury [73].

Kabir et al. (2014) studied the silymarin coated gold nanoparticles ameliorates CCl4-induced hepatic injury and cirrhosis through down regulation of hepatic stellate cells and attenuation of Kupffer cells. The authors suggested that silymarin coated gold nanoparticles were administered intragastrically once per day for 14 weeks in a dose of 30 mg kg\textsuperscript{-1} of body weight. Hepatoprotective and antifibrotic activities of silymarin coated gold nanoparticles were assessed in terms of reduction in serum enzymes (ALT, AST, ALP), through histopathology and immunohistochemistry techniques. It also reduced the CCl4-induced damaged area as well as fibrotic area to 0% as assessed by histopathology. The Alpha SMA and Kupffer cells were also reduced in number around the portal traid area by the silymarin coated gold nanoparticles. These hepatoprotective and antifibrotic effects were better than the positive control silymarin. The results suggest the therapeutic effect of silymarin coated gold nanoparticles in CCl4-induced liver injury and cirrhosis by promoting extracellular matrix degradation, hepatic stellate cells inactivation with strong enhancement of hepatic regenerative capacity. Silymarin coated gold nanoparticles could be administered for up to 14 weeks without inducing side effects or alterations of the histological structure of kidneys, heart, pancreas and lungs [74].
Yancai et al. (2012) studied the in vivo biodistribution and hepatoprotective effects of two formulated silybin nanosuspensions on targeted liver of mice. The results suggested that silybin nanosuspensions, administrated either intravenously or orally, presented significant ($P \leq 0.05$) hepatoprotective effect by reducing the serum marker enzymes such as AST, ALT, ALP, TBIL and GGT. Histopathological study further confirmed the hepatoprotective activity of the two silybin nanosuspensions formulations when compared with the CCl$_4$ treated control group. These results indicate that the nanosuspensions approaches could be used to improve the drug target delivery and therapeutic efficacy of the silybin [75].

Mishra et al. (2013) studied the nanosuspension of Phyllanthus amarus extract for improving oral bioavailability and prevention of paracetamol induced hepatotoxicity in Sprague–Dawley rats. The authors suggested that an oral dose of PAE at 125 and 250 mg kg$^{-1}$ and PAN at 25 and 50 mg kg$^{-1}$ showed a significant hepatoprotective effect relatively to the same extent ($P < 0.001$) by reducing levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bile salts. These biochemical assessments were supported by rat hepatic biopsy examinations. Moreover, the results also indicated that the hepatoprotective effect of 50 mg kg$^{-1}$ PAN was effectively better than 125 mg kg$^{-1}$ PAE ($P < 0.001$), and an oral dose of PAN that is five times less than PAE could exhibit similar levels of outcomes [76].

Reference


quercetin and diosmetin on iron-loaded rat hepatocyte cultures. Biochem Pharmacol. 45: 13-19


