Review Article

Pharmacological and therapeutic importance of *Hibiscus sabdariffa*- A review

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ABSTRACT
The phytochemical analysis of *Hibiscus sabdariffa* showed that the plant contained alkaloids, anthocyanins, flavonoids, phenols, saponins, tannins, polyuronides, cardiac glycosides, reducing sugar, carbohydrate, protein, gums, mineral, essential and volatile oils. The recent pharmacological studies showed that *Hibiscus sabdariffa* possessed antibacterial, antifungal, antiviral, anticancer, apoptotic, immunological, antioxidant, hypolipidemic, antidiabetic, smooth muscle relaxant, gastrointestinal antiinflammatory, analgesic, antipyretic, protective effects, wound healing, and wide range of cardiovascular and CNS effects. The current review discussed the chemical constituent, pharmacological and therapeutic effects of *Hibiscus sabdariffa*.

Keywords: *Hibiscus sabdariffa*, pharmacology, chemical constituents, therapeutic, pharmacognosy.

INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. Hibiscus (*Hibiscus sabdariffa*) has a long history of use in Africa and neighboring tropical countries for many conditions, including hypertension, liver diseases, cancer, constipation, and fever. Fresh or dried calyces of *Hibiscus sabdariffa* were used in the preparation of herbal drinks, hot and cold beverages, fermented drinks, wine, jam, jellied confectionaries, ice cream, chocolates, flavouring agents, puddings and cakes. The recent studies revealed that *Hibiscus sabdariffa* contains wide range of bioactive constituents and possessed wide range of pharmacological effects. The current review will highlight the chemical constituent, pharmacological and therapeutic effects of *Hibiscus sabdariffa*.

Plant profile

Synonyms

Abelmoschus cruentus (Bertol.) Walp., *Furcaria sabdariffa* Ulbr., *Hibiscus acetosus* Noronha, *Hibiscus cruentus* Bertol., *Hibiscus fraternus* L., *Hibiscus gossypifolius* Mill., *Hibiscus palmatilobus* Baill., *Hibiscus sanguineus* Griff., *Hibiscus sabdariffa* Rottb. and *Sabdariffa rubra* Kostel(1).

Taxonomic classification

Kingdom: Plantae, **Subkingdom**: Viridiplantae, **Infrakingdom**: Streptophyta, **Superdivision**: Embryophyta, **Division**: Tracheophyta, **Subdivision**: Spermatophytina, **Class**: Magnoliopsida, **Superorder**: Rosanae, **Order** : Malvales, **Family** : Mavaceae, **Genus** : Hibiscus, **Species**: *Hibiscus sabdariffa*(2).

Common names

Arabic
Chi Kujarat, Karkadah, Karkadeeb, Shi Sudani, Shi El-Sudan; **English**: Indian-sorrel, Jamaica-sorrel, Red-sorrel, Roselle, Sorrel; **French**: Oseille de Guinée; **German**: Malventee, Rosella; **Portuguese**: Carurú-de-Guiné, Quiabo-azedo, Quiabo-de-Angola, Quiabo-róseo, Quiabo-roxo, Rosela, Vinagreira; **Spanish**: Rosa de Jamaica, Serení; **Swedish**: Rosellhibiskus(3).

Distribution

It was native to old world tropics, probably in the East Indies or Africa, now cultivated throughout the tropics(4-5). However, roselle may domesticated in Western Sudan before 4000 BC, it was first recorded in Europe in 1576 AD. It carried from Africa to the New World by slaves for use as a food plant. Roselle was called Jamaican sorrel in 1707 in Jamaica, where the regular use of the calyces as food seemed to have been first practiced. The use of the plant was known in Java as early as 1658AD. In the New World, roselle was cultivated in Mexico, Central America, the West Indies, Southern Florida, Texas and California in the late 19th century(6-8). Now, it was said that *Hibiscus sabdariffa* is grown in all parts of the world(9).

Description

The plant is an annual erect shrub, it can also be regarded as a perennial. Culinary varieties are many-branched, bushy, and generally 1–2 m tall. Stems may be green or red, depending on the seed source. It has a strong taproot. The young plants have leaves that are unlobed, but as the plant grows the later-developing leaves are shallowly to deeply palmate, 3- or 5-parted (up to 7-parted). The large flowers have pale yellow petals (may suffused with pink or red) and a dark red eye. The flowers are

usually borne singly in the leaf axils. The sepals at the base of the large flowers and fruit vary from dark purple to bright red (may white) at maturity. The calyx increases from 1 to 2 cm in length before the flower is fertilized, then it reaches 5.5 cm (occasionally longer) at maturity. Flowers are red to yellow, with a dark center containing short peduncles, and have both male and female organs. The seed pods begin ripening near the bottom and proceed to the top(10-13).

Traditional uses

Hibiscus has a long history of use in Africa and neighboring tropical countries for many conditions, including hypertension, liver diseases, cancer, constipation, and fever. Fresh or dried calyces of *Hibiscus sabdariffa* were used in the preparation of herbal drinks, hot and cold beverages, fermented drinks, wine, jam, jellied confectionaries, ice cream, chocolates, flavouring agents, puddings and cakes(14-17)Roselle was used as herbal tea to sooth colds, clear a blocked nose, clear mucous, as an astringent, promoting kidney function, aiding digestion, as a general tonic, diuretic, and antipyretic(10).*Hibiscus sabdariffa* was also used as folk remedy for abscesses, cancer, cough, debility, dyspepsia, dysuria, fever, hangover, heart ailments, neurosis, scurvy and strangury(18).In Mexico, India and Africa infusions of the leaves or calyces were traditionally used as diuretic, cholerectic, febrifugal and hypotensive, to decrease the viscosity of the blood and to stimulate intestinal peristalsis. In India, a decoction from the seeds was used to relieve pain in urination and indigestion. In Chinese folk medicine, it is used to treat liver disorders and high blood pressure(10, 19) In North Africa, calyces preparations were used to treat sore throats, coughs, and emollient leaf pulp was used for the treatment of external wounds and abscesses(20).In Nigeria, a decoction of the seeds was used traditionally to enhance or induce lactation in cases of poor milk production and poor letdown(9). In Iraq, a decoction was used as digestive, diuretic, sedative and refresher(21). In Iran, sour hibiscus tea was reportedly a traditional treatment for hypertension(22). In Uganda, *Hibscus sabdariffa* was used in anemic and sick individuals to improve their health and as an immune booster(23).

Parts used

Calyces, seeds, leaves, shoots and oil(14).

Physicochemical characteristics

The yield of the extracted oil from red and white *Hibiscus sabdariffa* seeds was found to be 21.1%. The oil had a refractive index (1.467, 1.466), saponification value (189.7, 189.1), iodine value (119, 119), peroxide value (4.6, 4.7), acid value (3.57, 3.55), viscosity (22.5, 22.5) and specific gravity (0.90, 0.90) for oil of red and white *Hibiscus sabdariffa* seeds, respectively (24-25).

Chemical constituents

The preliminary phytochemical analysis of *Hibiscus sabdariffa* showed that the different parts of the plant contained alkaloids, anthocyanins, flavonoids, phenols, saponins, tannins, polyuronides, cardiac glycosides, reducing sugar, carbohydrate, protein, gums, mineral, essential and volatile oils(9,26-30). Quantitative determination of the phytochemical constituents showed that calyces contained saponnins 0.009-0.96%, flavonoids 0.43-20.08%, tannins 0.158-17.00%, phenols 0.26-1.10%, glycosides 0.132% and alkaloids 2.14%(31-33).Analysis of *Hibiscus sabdariffa* petals showed the presence of anthocyanins 16.53 mg/g, phenols 7.40 mg/g and flavonoids 3.50 mg/g (12.76 %)(26).The anthocyanins determined in calyx and callus of *Hibiscus sabdariffa* were (mg/g): cyanidin-3-O-glucoside 02.40 ± 0.02 and 08.01 ± 0.04, delphinidin-3-O-glucoside 02.20 ± 0.01 and 27.04 ± 0.07, Cyanidin-3-O-sambubioside 17.11 ± 0.10 and 53.93 ± 0.20, delphinidin-3-O-sambubioside 21.28 ± 0.05 and 07.07 ± 0.05, malvidin-3-O-glucoside (not detected) and 18.19 ± 0.10, petunidin-3-O-glucoside (not detected) and 16.89 ± 0.02 respectively. The sum of the amounts of anthocyanins was 42.99 ± 0.70 and 131.13 ± 1.50 respectively(34-35).The phytochemical study of the calyces of *Hibiscus sabdariffa* revealed identification of 10 compounds phenolic acids (protocatechuic acid and chlorogenic acid); flavonoids (eugenol, gossypetin, kaempferol, quercetin, myricetin, luteolin, rutin and astragalin)(36). Eighteen phenolic compounds were identified *Hibiscus sabdariffa* petals included chlorogenic acid , protocatechuic acid, gossypetrin, sabdaretin, gossypetin, luteolin, gossytrin, hibiscetin, rutin, hibiscetrin, myricetin, eugenol, nicotiflorine, quercitrin, quercetin, kaempferol, astragalin and cyranoside(26).*Hibiscus sabdariffa* extracts contained organic acids, including citric acid, hydroxycitric acid, hibiscus acid, malic acid tartaric acid, oxalic acid and ascorbic acid(14,37). Organic acids amounts of *Hibiscus sabdariffa* calyces were: malic: 560 ± 13, citric acid: 70 ± 2.5, tartaric acid: 46 ± 2.6, oxalic acid: 148 ± 7.2 and acetic acid: 115 ± 5.5 mg/100g calyces(33). Nutritional analysis showed that the fresh calyces of *Hibiscus sabdariffa* contained protein 1.9 g/100 g, fat 0.1 g/100 g, carbohydrates 12.3 g/100 g, fiber 2.3 g/100 g, vitamin C 14 mg/100 g, □-carotene 300 µg/100 g, calcium 1.72 mg/ 100 g, iron 57 mg/100 g, Mg 322.2±2.4 mg/g, K 1505±7.2 mg/g, Al 46±1.0 mg/g, Na 12.5±1.3 mg/g, Mn 7.6±10 mg/g and Cl 24.5±30 mg/g. The leaves contained protein 3.3 g/100 g, fat 0.3 g/100 g, carbohydrate 9.2 g/100 g, phosphorus 214 mg/100 g, iron 4.8 mg/100 g thiamine 0.45 mg/100 g, □ -carotene 4135 µg/100 g, riboflavin 0.45 mg/100 g and ascorbic acid 54

mg/100 g. The seeds contained crude protein 27.78%, crude fatty oil 21.85%, carbohydrate 21.25%, crude fiber 16.44%, potassium 1329 ± 1.47 mg/100 g, sodium 659 ± 1.58 mg/100 g, calcium 647 ± 1.21 mg/100 g, phosphorus 510 ± 1.58mg/100 g, magnesium 442.8 ± 1.80 mg/100 g(38-40). The amino acid composition of *Hibiscus sabdariffa* calyx (mg/g dry matter) were: arginine 3.60- 4.48, cysteine 0.87-1.30, histidine 1.19-1.50, isoleucine 2.70-3.00, leucine 4.21-5.00, lysine 2.77-3.90, methionine 0.65-1.00, phenylalanine 2.32-3.20, threonine 2.36-3.00, tryptophane - 0.45, tyrosine 1.44-2.20, valine 3.33-3.80, aspartame 10.50-16.30, glutamine 7.20- 8.85, alanine 3.46-3.70, glycine 2.47-3.80, proline 5.60-5.82 and serine 2.65-3.50(41,19).The components of the essential oils of air-dried flowers of *Hibiscus sabdariffa* from Lagos- Nigeria, were analysed by means of gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GCMS). The major compounds identified in the essential oil were hexadecanoic acid (64.3%) and linoleic acid (22.7%).The chemical classes of compounds present in the oil were sesquiterpene hydrocarbon (0.2%), oxygenated sesquiterpenes (1.2%), diterpenes (1.6%), aliphatic compounds (0.6%), phenyl propanoids (0.1%) and fatty acids (96.1%). Seventeen compounds were identified in the oil included (%): *n*-nonanoic acid: 0.6, eugenol 0.1, □-caryophyllene: 0.1, 10-*epi*-□-eudesmol: 0.3, ↑-cadinol: 0.5, □-selina-6-en-4-ol: 0.2, bisabolol oxide: 0.2, cadalene: 0.1, tetradecanoic acid: 2.1, hexadecanoic acid methyl ester:2.3, isophytol: 1.6, hexadecanoic acid: 64.3, heptadecanoic acid: 1.2, linoleic acid methyl ester: 2.1, oleic acid: 0.9, stearic acid methyl ester: 0.5 and linoleic acid: 22.7(42).Oils of *Hibiscus sabdariffa* flowers from Iraq contained palmitic (0.27%) and Linoleic (0.7%) acids. Where H2 (saponified of chloroform extract and methylation) s contained palmitic (0.04%), linoleic (0.004%), stearic (0.008%), heptanoic (0.003%) and octanoic (0.011%) acids(43).The main unsaturated fatty acids in the oil of the seed of red and white *Hibiscus sabdariffa* from the North Kordofan, Sudan were oleic (47.0555%, 47.8868%), linoleic (30.5836%, 30.7931%) and elaideic acid (14.359%, 15.1603%) and the saturated acids are palmitic acid (3.9494%, 3.9198%) and myristic acid (1.9609%, 1.9845%)(24). The analysis of seed oil of *Hibiscus sabdariffa* petroleum ether extract using gas chromatography (GC) revealed the presence of linolelaidic acid (26.02%), arachidic acid (20.59%) and palmitic acid (16.05%)(44).Chemical classes isolated from the oil of tea of roselle (*Hibiscus sabdariffa*) from Havana - Cuba, were included terpenoids which comprised the largest class of volatiles 48.2 %, fatty acids 12.4 %, alcohols 8.6 %, phenols 6.6 %, esters 3.9 %, furanoids 3.8 % and others 16.3 %. Eighty-one volatile constituents were identified from the oil of *Hibiscus sabdariffa*, the major constituents were linalool (0.58 mg/kg) and □-terpineol (0.55 mg/kg). However the componuds identified and their percentage were: 2,3-dimethylbutane < 0.01, isobutanol 0.15, 2-pentanone < 0.01, 2-methylbutanal 0.06, 3-methyl-1-butanol 0.14, 2-methyl-1-butanol 0.06, isobutanoic acid 0.01, 2-ethylfuran 0.03, hexanal 0.12, 2-furfural 0.13, 2-methylbutanoic acid 0.02, (E)-2-hexenal 0.02, 2-furfuryl alcohol 0.02, (Z)-3-hexen-1-ol 0.02, a-angelica lactone 0.04, p-xylene < 0.01, heptanal 0.02, 2-acetylfuran 0.02, (E)-2-heptenal 0.03, benzaldehyde 0.03, 5-methyl-2-furfural < 0.01, 2,2,6-trimethyl-6-vinyl tetrahydro furan 0.13, pentyl propanoate 0.02, methyl-2-furoate 0.03, 1-octen-3-ol 0.05, 6-methyl-5-hepten-2-one 0.02, octanal 0.03, a-terpinene 0.01, p-cymene 0.02, limonene 0.07, (Z)-b-ocimene 0.02, 1-propylbenzene 0.05, (E)-b-ocimene 0.06, acetophenone < 0.01, octanol 0.03, cis -linalool oxide (furanoid form) 0.36, m-cymenene < 0.01, trans -linalool oxide (furanoid form) 0.29, linalool 0.58, nonanal 0.16, 2-phenethyl alcohol 0.01, myrcenol 0.03, cis -b-terpineol 0.03, (E)-2-nonenal 0.05, ethyl benzoate 0.01, terpinen-4-ol 0.08, p-cymen-8-ol 0.06, a-terpineol 0.55, methyl salicylate 0.05, decanal 0.05, p-menthen-9-al 0.08, thymoquinone 0.14, geraniol 0.10, decanol 0.03, (E)-anethole 0.06, 1-methylnaphthalene 0.02, indole 0.04, undecanal 0.02, 4-vinylguaiacol 0.04, (E.E)-2,4-decadienal 0.03, methyl decanoate 0.02, methyl anthranilate 0.02, 1,2-dihydro-2,5,8-trimethylnaphthalene 0.02, eugenol 0.23, methyl eugenol 0.07, 2,6-dimethyl naphthalene 0.04, b-caryophyllene 0.04, a-humulene 0.02, geranyl acetone 0.02, b-santalene 0.02, a-calacorene 0.07, (E)-nerolidol 0.15, dodecanoic acid 0.15, g-eudesmol 0.12, tetradecanoic acid 0.13, pentadecanoic acid 0.04, methyl hexadecanoate 0.04, (Z)-9-hexadecenoic acid 0.19, (E)-phytol 0.04, hexadecanoic acid 0.21 and (E)-phytol acetate 0.09(45).

Pharmacological effects

Antibacterial and antifungal effects

Extracts and fractions of *Hibiscus sabdariffa* were tested against some pathogenic bacteria of human [Gram positive (*Corynebacterium diphtheria, staphylococcus aureus, staphylococcus capitis*), and Gram negative (*Pseudomonas aurogenosa* and *protus merabeles*)], from the all extracts, the fraction of (Chloroform-Ethanol) gave the highest effect, it gave inhibition range: (26-34 mm)(43). The antimicrobial activity of the roselle water and ethanol extracts was tested against *Bacillus subtilis* (ATCC6633), *Staphylococcus aureus* (ATCC6538) and *Escherichia coli* (ATCC 8739). The inhibition of the roselle ethanol extract against *B. subtilis* and *S.*

aureus was slightly higher than that of water extract but this difference was not significant. However, *E. coli* was strongly inhibited by the Roselle water extract at concentrations of 25 and 50 mg/ml(46). The antibacterial effects of *Hibiscus sabdariffa* calyces extracts were evaluated against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 15380), *Haemophilus influenzae* (ATCC 10211), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 12344) and *Streptococcus pneumoniae* (ATCC 6305). The zone of growth inhibition exerted by the ethanolic extract of calyces against *Pseudomonas aeruginosa* (ATCC 27853): 15mm , *Klebsiella pneumoniae* (ATCC 15380): 27mm , *Haemophilus influenzae* (ATCC 10211):20mm , *Staphylococcus aureus* (ATCC 25923): 29mm, *Streptococcus pneumoniae* (ATCC 6305): 29mm. The zone of growth inhibition exerted by the ethyl acetate of calyces: *Escherichia coli* (ATCC 25922): 11mm, *Streptococcus pyogenes* (ATCC 12344): 11mm. The zone of growth inhibition exerted by the methanolic extract of calyces against *Escherichia coli* (ATCC 25922): 16mm, *Pseudomonas aeruginosa* (ATCC 27853):29, *Klebsiella pneumoniae* (ATCC 15380): 28, *Haemophilus influenzae* (ATCC 10211): 27, *Staphylococcus aureus* (ATCC 25923): 25, *Streptococcus pyogenes* (ATCC 12344): 34mm. The zone of growth inhibition exerted by the aqueous extract of calyces against *Escherichia coli* (ATCC 25922): 30mm, *Pseudomonas aeruginosa* (ATCC 27853): 31mm, *Klebsiella pneumoniae* (ATCC 15380): 22mm, *Haemophilus influenzae* (ATCC 10211): 25mm, *Staphylococcus aureus* (ATCC 25923): 23mm, *Streptococcus pyogenes* (ATCC 12344): 20mm and *Streptococcus pneumoniae* (ATCC 6305): 19mm(47).The antimicrobial potential of leaves and seeds methanolic extracts of *Hibiscus sabdariffa* was studied against Gram-positive, Gram-negative bacteria and fungal strains. Leaves methanolic extracts of *Hibiscus sabdariffa* possessed antibacterial effects against Gram-positive bacteria (*Bacillus subitilis* NCTC: 8236 and *Staphylococcus aureus* ATCC: 25923), as well as Gram-negative bacteria (*Escherichia coli* ATCC: 25922, *Pseudomonas aeruginosa* ATCC: 27853, *Klebsiella pneumoniae* ATCC: 53657 and *Proteus vulgaris* ATCC: 6380). The leaves methanolic extracts of *Hibiscus sabdariffa* also showed an intermediate antifungal activity against two reference fungal strains (*Candida albicans* ATCC: 7596 and *Aspergillus niger* ATCC: 9765). The seeds methanolic extracts of *Hibiscus sabdariffa* did not show any activity against the tested bacterial and fungal strains(48).The antibacterial activity of methanol extract of *Hibiscus sabdariffa* calyces was studied against five hospital isolates of multidrug resistant *Acinetobacter baumannii* (MDR *A. baumannii*). The methanol extract exhibited significant antibacterial properties against the non-MDR *A. baumannii* as well as the MDR *A. baumannii* strains with a zone of inhibition ranging from (11.3 ± 0.3) to (13.6 ± 0.3) mm. Values of minimum inhibitory concentration and minimum bactericidal concentration ranged from 25 to 50 and 50 to 100 mg/ml, respectively. The percentage inhibition of *Hibiscus sabdariffa* extract (10 mg/disc) with respect to gentamicin (10 mg/disc) revealed that *Hibiscus sabdariffa* was much more effective than gentamicin(49).The antimicrobial potency of *Hibiscus sabdariffa* leaf extracts were evaluated against *Klebsiella pneumonia*, *Salmonella typhi* and *Shigella dysenteriae*. Mean zones of inhibition of the aqueous leaf extracts for the 20 and 40 mg/ml for *K. pneumonia* was (15.33±0.58 and 18.67±0.76), *S. typhi* (15.50±0.50 and 16.33±0.58) and *S. dysenteriae* (17.83±0.76 and 19.17±1.04). Hexane extracts showed no activity against the test organisms. The minimum inhibitory concentration and the minimum bactericidal concentration for the aqueous leaf extract were: *K. pneumonia* (10.0 and 15.5 mg/ml), *S. typhi* (10.0 and 12.5 mg/ml) and *S. dysenteriae* (7.5 and 12.5 mg/ml) respectively(50). The antimicrobial activity of concentrations of 10%, 5%, and 2.5% methanol extract of *Hibiscus sabdariffa* was studied against *Escherichia coli* O157:H7 isolates from food, veterinary, and clinical samples. The results revealed that the most potent concentration was 10%, then 5%, and finally 2.5%. The overall mean zone of inhibition for the *Hibiscus sabdariffa* extract was 12.66 mm for 10%, 10.75 mm for 5%, and 8.9 mm for 2.5%. The highest inhibition zones (11.16 mm) were observed against veterinary samples, and the lowest (10.57 mm) against the food samples(51). Aqueous extracts from the dried calyces of *Hibiscus sabdariffa* was tested for antimicrobial activity against the foodborne pathogens *Escherichia coli* O157:H7 strains ATCC 43894 and *Staphylococcus aureus* strains SA113 and ATCC 27708. Against *E. coli*, the results of 20 mg/ml filtered extract were not different from those of the control, whereas autoclaved extracts reduced viable cells ca. 3 to 4 log CFU/ml. At 60 mg/ml, both extracts inactivated cells after 24 h. There were reduced populations of both strains of *S. aureus* (ca. 2.7 and 3 log CFU/ml, respectively) after 24 h of incubation in 40 mg/ml filtered extracts(52).The methanol extract of the dried calyces of *Hibiscus sabdariffa* were investigated for antibacterial activity against Gram positive and Gram negative bacteria. The highest antibacterial activity of *Hibiscus sabdariffa* calyces was recorded against *S. aureus* (18.5 ± 0.5 mm), followed by *S. epidermidis* (17.5 ± 1.5 mm), *S. enteric* (17.5 ± 1.5 mm), *K. pneumonia* (17.5 ± 0.5 mm), *P. aeruginosa* (15.5 ± 0.5 mm), *E. coli*(14.5±0.5 mm), *P. vulgaris* (14.5±0.5 mm),

and *B. cereus* (13.5 ± 1.5 mm)(53).The antimicrobial combinatory effect of the aqueous extract of *Hibiscus sabdariffa* (AEHS) with antibiotics (clarithromycin, amoxicillin, metronidazole) were evaluated against *Helicobacter pylori* strains. AEHS exerted remarkable bacteriostatic effect against all *Helicobacter pylori* tested strains with MICs values ranging from 9.18 to 16.68 µg/ml. Synergy effect of aqueous extract of *Hibiscus sabdariffa* with clarithromycin or metronidazole was obtained against four of seven *Helicobacter pylori* strains tested with ∑FIC ranging from 0.21 to 0.39. The additive effect of aqueous extract of *Hibiscus sabdariffa* with amoxicillin was obtained against five of seven *Helicobacter pylori* strains tested with ∑FIC ranging from 0.61 to 0.91(54).

The antifungal effect of *Hibiscus sabdariffa* extract was evaluated against *Candida albicans*, the biofilm forming capacity of *Candida albicans* strains in the present of the *Hibiscus sabdariffa* extract was also studied. The minimum inhibitory concentration values of *Hibiscus sabdariffa* extract were ranged from 0.5 to 2.0 mg/ml. Time-kill experiment demonstrated that the effect was fungistatic. Furthermore, *Hibiscus sabdariffa* extract inhibited biofilm production of all the isolates(55).The antifungal effect of *Hibiscus sabdariffa* extract, in combination with voriconazole or fluconazole was evaluated against *C. albicans* isolates. Six strains of fluconazole-resistant *C. albicans* isolates were obtained from patients with recurrent candiduria. When the extract was used in combination with voriconazole, a high degree of synergism was observed(56).

Antiviral activity

The antiviral effects of aqueous extracts of *Hibiscus sabdariffa* (HE) was studied against human norovirus surrogates (feline calicivirus (FCV-F9) and murine norovirus (MNV-1)) and hepatitis A virus (HAV) at 37 °C over 24 h. FCV-F9 titers were reduced to undetectable levels after 15 min with both 40 and 100 mg/ml HE. MNV-1 was reduced by 1.77 ± 0.10 and 1.88 ± 0.12 log PFU/ml after 6 h with 40 and 100 mg/ml HE, respectively. HAV was reduced to undetectable levels by both HE concentrations after 24 h(57). The aqueous extract of *Hibiscus sabdariffa* (AEHS) and its bioactive constituent protocatechuic acid (PCA), were evaluated *in vitro* for their antiviral activity against HSV-2 clinical isolates and anti-enzymatic activity against urease. PCA showed potent anti-HSV-2 activity compared with that of acyclovir, with EC50values of 0.92 and 1.43 µg/ml, respectively, and selectivity indices > 217 and > 140, respectively. AEHS exerted anti-urease activity, with an IC50 value of 82.4 µg/ml(58). The antiviral effects of aqueous *Hibiscus sabdariffa* extracts was evaluated against Aichi virus (AiV) (a foodborne pathogen that causes gastroenteritis). AiV did not show any significant reduction with 1:1 (100 mg/ml) or 1:5 (40 mg/ml) diluted aqueous hibiscus extracts after 0.5, 1, or 2 h at 37 °C. However, AiV titers were reduced to non-detectable levels after 24 h with all the three tested concentrations. AiV was reduced by 0.5 and 0.9 log PFU/ml with undiluted extracts (200 mg/ml) after 2 and 6 h, respectively(59). The leaves extracts of *Hibiscus sabdariffa* (red and green leaved) were studied for antiviral activities against Measles Virus (MV) as well as the effects of the extracts on Hep-2 cells were studied. Ethanol extract of the leaves showed no toxicity to the Hep-2 cells at all concentrations used (5, 10 and 15 mg/ml). The pre-inoculative treatment of Hep-2 cells with plant extracts showed that *Hibiscus sabdariffa* had antiviral activities only at 10 and 15 mg/ml on MV. The post-inoculative treatment of Hep-2 cells with plant extracts showed that *Hibiscus sabdariffa* at 5, 10 and 15 mg/ml concentrations, had antiviral activities on MV(60).

Anticancer and apoptotic effects

The anticancer effect of roselle juice were evaluated using different cell lines like ovarian (Caov-3), breast (MCF-7, MDA-MB-231) and cervical (HeLa) cancer cell lines. It possessed the strongest anti-proliferative potency towards the MCF-7 cancer cells(61).The anticancer effect of *Hibiscus sabdariffa* extract (HSE) was evaluated on a panel of human tumor cell lines, multiple myeloma (MM) cells (RPMI 8226) and oral squamous cell carcinoma (OSCC) cells (SCC-25). In both RPMI 8226 and SCC-25 cells, HSE impaired cell growth, exerted a reversible cytostatic effect, and reduced cell motility and invasiveness(62). Human gastric carcinoma (AGS) cells were susceptible to Hibiscus polyphenol-rich extract (0.95 mg/ml HPE inhibited its growth by 50%). AGS cells underwent DNA fragmentation, and had an increase in the distribution of hypodiploid phase (apoptotic peak, 52.36%) after a 24-h treatment with HPE (2.0 mg/ml). The effect of HPE on AGS cells might be mediated via p53 signaling and p38 MAPK/FasL cascade pathway, as demonstrated by an increase in the phosphorylation of p53 and the usage of a specific p38 inhibitor(63). The cytotoxic effects of *Hibiscus sabdariffa* aqueous extract (HSE) against human breast adenocarcinoma cell line (MCF-7) and fetal foreskin fibroblast (HFFF) were investigated. Different concentrations of water extract of calyces were added and the percentage of cell survival was determined after 24, 48, and 72 hours using an MTT assay. Apoptosis induction was assessed by DNA fragmentation. At the concentration of 0.5 mg/ml of the extract and after 72 hours incubation, the number of viable MCF-7 cells was less than 50%. The extract was not cytotoxic against normal HFFF cells in all tested concentrations. HSE induced apoptosis only in MCF-

7 cells(64). The methanol extract from calyces of *Hibiscus sabdariffa* showed significant selective activity against a leukemia line (K-562), with IC50 values of 0.12-1.16 mg/ml, with concentration-dependent, cytotoxic and cytocidal effects(65).The cytotoxic activities of *Hibiscus sabdariffa* leave extracts from different extraction methods were tested against human prostate cancer cell line (PC-3) using sulforhodamine B (SRB) assay. The results showed that the 95% ethanolic extract of *Hibiscus sabdariffa* dried leaves possessed potent cytotoxicity against prostate cancer cell line with an IC50 of 8.58±0.68 µg/ml(66). The possible anticancer effects of *Hibiscus sabdariffa* extract was studied against human leukemic THP-1 cells. It exhibited a concentration dependent antiproliferative effect against THP-1 leukemic cells with IC50 values of 15.47 mg/ml(67).Roselle-anthocyanins (HA) showed apoptosis of human cancer cells (HL-60) in a dose- and time-dependent manner. It also increased phosphorylation in p38, c-Jun and cytochrome c release, and expression of tBid, Fas, and FasL genes(68). Protocatechuic acid inhibited the survival of human promyelocytic leukemia (HL-60) in a concentration and time dependent manner. It induced apoptosis via reduction of retinoblastoma phosphorylation and down regulation of Bcl-2 protein expression. The cells underwent intranucleosomal DNA fragmentation and morphological changes characteristics of apoptosis, while the action against gastric carcinoma cells by inducing apoptosis was through JNK/MAPK signaling pathways(69-70). *Hibiscus sabdariffa* leaf extract (HLE) dose-dependently inhibited the migration and invasion of human prostate cancer LNCaP (lymph node carcinoma of the prostate) cells under non-cytotoxic concentrations. HLE also exerted an inhibitory effect on the activity and expressions of matrix metalloproteinase-9 (MMP-9). The HLE-inhibited MMP-9 expression appeared to be a consequence of nuclear factor-kappaB (NF-□B) inactivation because its DNA-binding activity was suppressed by HLE. Molecular data showed that all these influences of HLE might be mediated via inhibition of protein kinase B (PKB, also known as Akt) /NF-□B/MMP-9 cascade pathway, as demonstrated by the transfection of *Akt1* overexpression vector. The inhibitory effect of HLE was proven by its inhibition on the growth of LNCaP cells and the expressions of metastasis-related molecular proteins *in vivo*(71). The anticancer activity of Hibiscus leaf polyphenolic (HLP) extract was studied against melanoma cells. HLP was rich in epicatechin gallate (ECG) and other polyphenols. The results revealed that both HLP and ECG induced the caspases cleavages, Bcl-2 family proteins regulation, and Fas/FasL activation in A375 cells. In addition, The cells presented AVO-positive after HLP treatments. The results indicated that the anticancer effect of HLP, partly contributed by ECG(72). The protective effect of anthocyanin extract of roselle (HAs) was studied in N-nitrosomethylurea (NMU)-induced leukemia of rats. Leukemia was induced by intravenous injection of 35 mg/kg bw of NMU dissolved in physiologic saline solution. HAs groups received different doses of HAs (0.1 and 0.2%) daily, orally, after NMU injection. After 220 days, when compared with the NMU-only group, HAs significantly prevented loss of organ weight and ameliorated the impairment of morphology, hematology, and histopathology. Treatment with HAs caused reduction in the levels of AST, ALT, uric acid, and MPO. Oral administration of HAs (0.2%) also remarkably inhibited progression of NMU-induced leukemia by approximately 33.3% in rats(73). An *in vivo* micronucleus assay using albino mice was used to examine the anticlastogenic effects of a crude aqueous extract of *Hibiscus sabdariffa* fruits in bone marrow cells of mice. Various doses of freshly prepared crude extract of *Hibiscus sabdariffa* (50, 100 and 150 mg/kg bw) were given for 7 days as a dietary supplement followed by a single dose of sodium arsenite (2.5 mg/kg bw). The results showed that sodium arsenite effectively induced micronuclei in polychromatic erythrocytes (PCEs). Administration of a crude extract of *Hibiscus sabdariffa* significantly reduced micronuclei in PCEs(74). The 80% ethanol extract of *Hibiscus sabdariffa*, showed antimutagenic and chemopreventive activity in chemical induce colon carcinogenesis(74).The *Hibiscus sabdariffa* extract inhibited mutagenicity of 1-nitropyrene (1-NP) in a dose-response manner. The inhibition rate on HeLa cells of *Hibiscus sabdariffa* extract was also dose-dependent. The HAE did not induce DNA fragmentation(75). The effect of *Hibiscus sabdariffa* aqueous extract was investigated against cyclophosphamide (CPA, 25 mg/Kg) induced damage to DNA in male wistar rats by micronucleus test. The aqueous extract was prepared by infusion and each animal received a daily dose of 400 mg/Kg by gavage for 15 consecutive days of treatment. The group treated with the aqueous extract of *Hibiscus sabdariffa* revealed a 91% reduction in micronucleus frequency when compared with the positive control group(76). Delphinidin 3-sambubioside (Dp3-Sam), (the anthocyanin, isolated from the dried calices of *Hibiscus sabdariffa*) induced a dose-dependent apoptosis in human leukemia cells (HL-60) as characterized by cell morphology, DNA fragmentation, activation of caspase-3, -8, and -9, and inactivation of poly(ADP)ribose polymerase (PARP). Dp3-Sam induced Bid truncation, mitochondrial membrane potential (□Ψm) loss, and

cytochrome c release from mitochondria to cytosol. Dp3-Sam also caused a time- and dose-dependent elevation of intracellular reactive oxygen species (ROS) level in HL-60 cells, and antioxidants such as *N*-acetyl-l-cysteine (NAC) and catalase could effectively block Dp3-Sam-induced ROS generation, caspase-3 activity, and DNA fragmentation(77).The chemopreventive properties of *Hibiscus sabdariffa* on human gastric carcinoma cells were investigated. With the using of a set of apoptotic detection assays, it appeared that HSE induced cytotoxicity and apoptosis of AGS cells in a concentration-dependent manner but was ineffective in Chang liver cells. The result also revealed increased phosphorylation in p38, JNK and c-Jun, cytochrome c release, and expression of Fas, FasL, Bax, and t-Bid in the HSE-treated AGS cells. The results indicated that HSE mediated AGS apoptosis *via* the JNK/p38 signaling cascade(78). Hibiscus anthocyanins (HAs) inhibited the serum-stimulated proliferation of smooth muscle cell (SMC) and resulted in cell apoptosis. The HAs inducing cell apoptosis was dose dependent. HAs induced apoptosis via activating p38 MAP kinase that subsequently phosphorylates target protein c-Jun and transduces the signal to further activate the apoptotic protein cascades that contain Fas-mediated signaling (Fas/caspase-8 signaling module) and activating p53 and inducing bax expression(79).

Cardiovascular effects

Hibiscus sabdariffa crude extract induced endothelium-dependent relaxant effects on isolated thoracic aorta of male Wistar rats. The endothelium-dependent relaxations were resulted from NOS activation(80). Roselle calyx infusion was found to lower significantly ($P<0.05$) both systolic and diastolic pressure in spontaneously hypertensive and normotensive Wistar-Kyoto rats at doses of 500 and 1000 mg/ kg bw. The reduction in blood pressure in both groups was positively correlated with weight(81). The aqueous extract of *Hibiscus sabdariffa* calyx attenuated the development of salt-induced hypertension in Sprague-Dawley rats treated for 12 weeks(82-83) The aqueous extract of petals of *Hibiscus sabdariffa* (HS) also attenuated the established stages of 2-Kidney, 1-Clip renovascular hypertension in Sprague-Dawley rats(84). *Hibiscus sabdariffa* ingestion in rat (10%, 15% and 20% of the water extract in drinking water for 10 consecutive weeks) significantly reduced Systolic (SBP), diastolic (DBP) and left ventricles (LV) mass in a dose-dependent fashion but did not affect the heart rate. It significantly increased surface area and length density of myocardial capillaries by 59%, 65% and 86%, and length density by 57%, 77% and 57%, respectively(85).The effect of roselle extract (*HSE*) (100 mg/kg/bw *HSE, orally* for 21 consecutive days) on blood pressure, serum lipid profile, oxidative stress marker levels and histological changes to the heart induced by nicotine (0.6 mg/kg/*bw)* were studied in rats. *HSE* significantly ($P>0.05$) reduced the heart rate but without effect on blood pressure. *HSE* increased the high-density lipoprotein concentration significantly ($P<0.05$) in nicotine- treated rats, without any significant changes in total cholesterol, triglyceride and low-density ipoprotein concentration. *HSE* treatment was also found to reverse malondialdehyde level, superoxide dimustase enzyme activity and protein concentration significantly ($P<0.05$) in nicotine-treated rats(86). The anti-hypertensive activity of aqueous calyx extract of *Hibiscus sabdariffa* was investigated on salt induce hypertensive albino rats for 28 days. The extract treated groups showed a significant ($P<0.01$) reduction in diastolic and systolic blood pressure when compared to the normotensive and hypertensive rats. There was no significant difference ($P>0.05$) between the drug treated and the extract treated groups during this treatment(87). Intravenous injection of aqueous extract of *Hibiscus sabdariffa* calyces to anaesthetized cats lowered the blood pressure in a dose-response manner. The inhibitory effects were resistant to a number of standard receptor blockers but the hypotensive influence was partially blocked by atropine(88).In a clinical study on hypertensive subjects, it was found that roselle reduced the mean arterial pressure, comparable to the effect of captopril(89).The daily consumption of extract of hibiscus sepals significantly decreased systolic and diastolic blood pressure in adults with pre to moderate essential hypertension and type 2 diabetes. The results revealed that the effectiveness of extract was equivalent to captropril, but less effective than lisinopril(90). A controlled and randomized clinical trial was carried out to compare the antihypertensive effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* with captopril. Patients with diagnosed hypertension and without antihypertensive treatment for at least 1 month were included, they were recieved either an infusion prepared with 10 g of dry calyx from *Hibiscus sabdariffa* (9.6 mg anthocyanins content), daily before breakfast, or captopril 25 mg twice a day, for 4 weeks. The results showed that *Hibiscus sabdariffa* was able to decrease the systolic blood pressure (BP) from 139.05 to 123.73mm Hg ($P<0.03$) and the diastolic BP from 90.81 to 79.52mm Hg ($P<0.06$). At the end of the study, there were no significant differences between the BP detected in both treatment groups ($P>0.25$). The rates of therapeutic effectiveness were 0.7895 and 0.8438 with *Hibiscus sabdariffa* and captopril, respectively ($P>0.560$), whilst the tolerability was 100% for both treatments(89). A randomized, double-blind, placebo-controlled clinical trial was conducted to determine the antihypertensive effects of *Hibiscus sabdariffa* tea consumption on 65 pre- and mildly hypertensive adults with no blood

pressure-lowering medications. They used either three 240-ml servings/day of brewed hibiscus tea or placebo beverage for 6 wk. At 6 wk, hibiscus tea lowered systolic BP (SBP) compared with placebo. Diastolic BP was also lower, although this change did not differ from placebo. The change in mean arterial pressure was of borderline significance compared with placebo. Participants with higher SBP at baseline showed a greater response to hibiscus treatment(91). Polyphenols from *Hibiscus sabdariffa* calices were administered to patients with metabolic syndrome (125 mg/kg/day for 4 wk) and spontaneously hypertensive rats (125 or 60 mg/kg in a single dose or daily for 1 wk). *Hibiscus sabdariffa* extract improved metabolism, displayed potent anti-inflammatory and antioxidant activities, and significantly reduced blood pressure in both humans and rats(92). Clinical trials confirmed the antihypertensive effect of the watery infusions. The results showed that the treatment decreased blood pressure (BP) from 146.48/97.77 to 129.89/85.96 mmHg, reaching an absolute reduction of 17.14/11.97 mmHg (11.58/12.21%, P<0.05). The treatment showed therapeutic effectiveness of 65.12 % as well as tolerability and safety of 100 %. BP reductions and therapeutic effectiveness were lower than those obtained with lisinopril (P<0.05)(93). The antihypertensive activity of roselle could be mediated by many mechanisms included inhibition of angiotensin-converting enzyme activity and subsequent renin-angiotensin-aldosterone system, (especially anthocyanins delphinidin-3-O-sambubioside, cyanidin-3-O-sambubioside and related flavonoid glycosides), enhancement of vascular activity by Na+ -K+ -ATPase and Ca2+-Mg2+-ATPase, enhancement of NO production, as endotheliumderived relaxing factor (EDRF), attenuation of the discharge of the sympathetic nervous system and diuretic effects(94-102).

Effects on lipid profile and body weight

The hypolipidemic effect of ethanolic extract of the leaves of *Hibiscus sabdariffa* (HSEE) (100, 200, and 300 mg/kg) was investigated in hyperlipidemic rats. Administration of HSEE (200 mg/kg and 300 mg/kg) together with continuous cholesterol feeding for four weeks caused significant reduction in serum cholesterol level by 18.5% and 22%, respectively (P<0.05); serum triglyceride level by 15.6% and 20.6%, respectively (P<0.05); serum LDL level by 24% and 30%, respectively (P<0.05), and serum VLDL level by 15.5% and 20.5%, respectively (P<0.05), as compared to cholesterol group. However, no significant change in HDL level was observed(103). The effect of *Hibiscus sabdariffa* dried calyx ethanolic extract on the serum lipid profile was studied in Sprague-Dawley rats. The rats were fed during 4 weeks with either a basal diet, containing high cholesterol (1%), cholic acid (0.25%), lard oil (10%), or a supplemental diet with *Hibiscus sabdariffa* extract at 5%, 10%, and 15% levels (SD5, SD10, SD15). Weight gain and faeces dry weight were both very significantly less (P< or = 0.01) in SD10 and SD15 groups as compared to the control group. Triacylglycerols and LDL levels were both significantly less (P< or = 0.05) in all groups (SD5, SD10, and SD15) as compared to the control. For total lipids, SD10 and SD15 showed significantly lower levels (P< or = 0.05), whereas very significant differences (P< or = 0.01) were observed in SD5 group. All groups had lower cholesterol levels compared to controls; however, only the SD5 group was statistically significant (P< or = 0.05)(104). The effects of *Hibiscus sabdariffa* calyx aqueous extract on the serum cholesterol, body weight and liver marker enzymes activities were studied in normal albino rats. The aqueous extract was orally administered (100 – 800 mg/kg bw for 28 days) to normal male albino rats. *Hibiscus sabdariffa* administration significantly reduced serum cholesterol and body weight in a dose and duration dependent pattern(105). The hypolipidemic and antiatherosclerotic effects of *Hibiscus sabdariffa* extract (HSE) were investigated in rabbits with experimental atherosclerosis. Rabbits were fed with a normal diet, high cholesterol (1.3%), lard oil (3%) diet (HCD) with or without 0.5 or 1% HSE for 10 weeks. The levels of triglyceride, cholesterol, and low-density lipoprotein cholesterol (LDL-C) were decreased in the serum of rabbits fed HCD plus HSE than in the serum of rabbits fed HCD. Feeding HSE (0.5 and 1% in the diet) to rabbits significantly reduced severe atherosclerosis in the aorta. Histopathological examination showed that HSE reduced foam cell formation and inhibited smooth muscle cell migration and calcification in the blood vessel of rabbits (106).The antioxidant and antihyperlipidemic activities of the extracts of leaves and calyces of *Hibiscus sabdariffa* were investigated by studying *in vitro* inhibitory activity on lipid peroxidation and *in vivo* effects on cholesterol induced hyperlipidemia. Highest antioxidant activity was exhibited by ethanolic extract of calyces followed by ethanolic extract of leaves followed by aqueous extract of leaves of *Hibiscus sabdariffa*. In cholesterol induced hyperlipidemic model, the groups of rats treated with extracts of calyces and leaves of *Hibiscus sabdariffa* showed a significant decrease in the serum TC, LDL-C, VLDL-C, TAG values along with an increase in serum HDL-C levels. The treated groups also showed significant decrease in the atherogenic index, LDL-C: HDL-C risk ratios, and in the levels of SGOT, SGPT and ALP activities compared to cholesterol induced hyperlipidemic control group(107). The effects of aqueous extract of *Hibiscus sabdariffa* (Hs) on body weight gain and its protective effects on the liver by improving lipid metabolism were studied in high fat diet-induced obese C57BL/6NHsd mice. Oral

administration of the Hs extract reduced fat tissue accumulation, diminished body weight gain and normalized the glycemic index as well as reduced dyslipidemia compared to the obese mice group that did not receive Hs treatment. Hs treatment also attenuated liver steatosis, down-regulated SREBP-1c and PPAR-□, blocked the increase of IL-1, TNF-□ mRNA and lipoperoxidation and increased catalase mRNA(108). The effect of a standardized *Hibiscus sabdariffa* calyces aqueous extract on body weight was evaluated in an obese mice model induced by the administration of monosodium glutamate. *Hibiscus sabdariffa* aqueous extract was orally administered (120 mg/kg/day) for 60 days to healthy and obese mice. *Hibiscus sabdariffa* administration significantly reduced body weight gain in obese mice and increased liquid intake in healthy and obese mice. Triglycerides and cholesterol levels showed non-significant reductions in animals treated with *Hibiscus sabdariffa* (109). *Hibiscus sabdariffa* water extract (HSE) treatment reduced fat accumulation in the livers of hamsters fed with fat diet (HFD) in a concentration-dependent manner. Administration of HSE reduced the levels of liver cholesterol and triglycerides, which were elevated by HFD. Analysis of the effect of HSE on paraoxonase 1, an antioxidant liver enzyme, revealed that HSE potentially regulated lipid peroxides and protected organs from oxidation-associated damage. The markers of liver damage such as serum alanine aminotransferase and aspartate aminotransferase levels that were elevated by HFD were also reduced by HSE treatment. The effects of HSE were as effective as treatment with anthocyanin; which indicated that anthocyanins present in the HSE may play a crucial role in the protection established against HFD-induced obesity(110-111).The effect of *Hibiscus sabdariffa* (Hs) calyx extract on fat absorption-excretion and body weight was studied in rats. Rats were fed with either a basal diet (SDC = Control diet) or the same diet supplemented with Hs extracts at 5%, 10% and 15% (SD5, SD10 and SD15). Only SD5 did not show significant increases in weight, food consumption and efficiency compared to SDC. The opposite occurred in SD15 group which showed a significant decrease for these parameters. The SD10 responses were similar to SD15, with the exception of food consumption. In both SDC and SD5 groups, no body weight loss was observed; however, only in the latter group there was a significantly greater amount of fatty acids found in feces(112).*Hibiscus sabdariffa* polyphenols (HPE) exhibited more potency to decrease plasma cholesterol and LDL cholesterol than the crude extract HSE, and increased HDL cholesterol dose-dependently. It decreased the lipid content of hepatocyte through the activation of AMPK and reduction of SREBP-1, thus inhibiting the expression of fatty acid synthase and HMG-CoA reductase. LDLR and LDL binding of HepG2 cells were enhanced when treated with HPE(113). The effects of *Hibiscus sabdariffa* on adipogenic differentiation of 3T3-L1 cells were studied at the cellular and molecular levels. Hibiscus extract inhibited the adipocyte differentiation of 3T3-L1 preadipocytes induced by insulin, dexamethasone, and isobutylmethylxanthine (IBMX) in a dose-dependent manner. Hibiscus blocked the cytoplasmic lipid accumulation when administered at the onset of differentiation and 4 days after induction of differentiation. The inhibitory effect of hibiscus on adipogenic lipid accumulation of preadipocytes was significant ($P<0.01$) between control cells and cells treated with hibiscus(114). *Hibiscus sabdariffa* extract inhibited the adipocyte differentiation through the modulation of PI3-K/Akt and ERK pathway that play pivotal roles during adipogenesis(115).The cholesterol-lowering potential of *Hibiscus sabdariffa* extract (HSE) was investigated in human subjects, a clinical study was conducted using an oral preparation of HSE capsules. The study consisted of 42 volunteers with a cholesterol level of 175 to 327 mg/dl. They were randomly divided into 3 groups: group I (1 capsule of HSE during each meal), group II (2 capsules), and group III (3 capsules). HSE caused significant decrease in serum cholesterol level in subjects from groups I and II after 4 weeks. HSE after 2 weeks, decreased serum cholesterol levels in all groups ($P<0.05$ for groups I-III) compared with baseline values by 7.8% to 8.2%, while, a reduction in serum cholesterol level by 8.3% to 14.4%, was recorded after 4 weeks. The serum cholesterol level for 71% of group II volunteers was significantly lowered with a mean reduction of 12% ($P<0$ .05)(116). In a sequential randomized controlled clinical trial, 60 patients with diabetes were randomly assigned into two groups: sour tea (*Hibiscus sabdariffa*, ST) and black tea (BT). They were instructed to consume sour tea or black tea two times a day for 1 month to investigate the hypolipidemic effects of sour tea in patients with diabetes and compare them with black tea. In the *Hibiscus sabdariffa* group, the mean of high-density lipoprotein-cholesterol (HDLc) increased significantly ($P = 0.002$) at the end of the study, whereas changes in apolipoprotein-A1, and lipoprotein (a) were not significant. Also, a significant decrease in the mean of total cholesterol, low density lipoprotein-cholesterol, triglycerides, and Apo-B100 were seen in this group. In the BT group, only HDLc showed significant change ($P = 0.002$) at the end of the study, while, the changes in the other measures were not statistically significant(117).A triple blind randomized placebo-controlled clinical trial was carried out to determine the effects of *Hibiscus sabdariffa* (HS) calices on controlling dyslipidemia in 72 obese adolescents.

They received 2 grams of fine powdered calices of *Hibiscus sabdariffa* per day for one month, while controls received placebo powder with the same dietary and physical activity recommendations and duration of exposure. Full lipid profile and fasting blood sugar were measured before and after the trial. In the *Hibiscus sabdariffa* calyces treated group, serum total cholesterol, low density lipoprotein cholesterol and serum triglyceride showed a significant decrease but high density lipoprotein cholesterol level was not changed significantly(118).A clinical trial was carried out to confirm the metabolic-regulating and liver-protecting effect of *Hibiscus sabdariffa* extracts (HSE). Subjects with a BMI ≧ 27 and aged 18–65, were randomly divided into control and HSE-treated groups, for 12 weeks. the results revealed that consumption of HSE reduced body weight, BMI, body fat and the waist-to-hip ratio. Serum free fatty acids were also lowered by HSE. Anatomic changes revealed that HSE improved the illness of liver steatosis. Ingestion of HSE was well tolerated and there was no adverse effect during the trial(119). A total daily dose of 100 mg of *Hibiscus sabdariffa* extract powder (HSEP) was orally administered in capsules for one month to determine its effect on lipid profiles of individuals with dyslipidemia associated with metabolic syndrome (MeSy). The MeSy patients treated with HSEP had significantly reduced glucose and total cholesterol levels, increased HDL-c levels, and an improved TAG/HDL-c ratio, a marker of insulin resistance ($P<0.05$). Furthermore, a triglyceride-lowering effect was observed in MeSy patients treated with HSEP plus diet, and in individuals without MeSy treated with HSEP. Significant differences in total cholesterol, HDL-c, and the TAG/HDL-c ratio were found when the means of absolute differences among treatments were compared ($P<0.02$)(120). In a double blind, placebo controlled, randomized trial, sixty subjects with serum LDL values in the range of 130-190 mg/dl and with no history of coronary heart disease were randomized into experimental and placebo groups. The experimental group received 1 gm of the extract for 90 days, while the placebo received a similar amount of maltodextrin in addition to dietary and physical activity advice for the control of their blood lipids. Body weight, serum LDL cholesterol and triglyceride levels decreased in both groups, there were no significant differences between the experimental and placebo group. At a dose of 1 gm/day, *Hibiscus sabdariffa* leaf extract did not appear to have a blood lipid lowering effect(121).

Antidiabetic effects

The inhibitory effect of aqueous extracts of two varieties (red and white) of *Hibiscus sabdariffa* calyces on carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) was studied as a possible mechanism for their antidiabetes properties. The extracts caused inhibition of α-amylase and α-glucosidase activities *in vitro*. The IC50 revealed that the red variety (25.2 µg/ml) exhibited higher α-glucosidase inhibitory activity than the white variety (47.4 µg/ml), while the white variety (90.5 µg/ml) exhibited higher α-amylase inhibitory activity than the red variety (187.9 µg/ml). However, the α-glucosidase inhibitory activities of both calyces were higher than that of their α-amylase inhibitory activities (122). The antidiabetic and antioxidant effects of purple roselle extract were studied in streptozotocin(STZ)- induced diabetes in rats. After 21 days treatment, roselle extract lowered blood sugar (both curative and preventive), increased of antioxidant capacity, and improved insulin production (123).The effects of *Hibiscus sabdariffa* UKMR-2 (HSE) variety (100 mg/kg/bw orally for 28 consecutive days) on fertility of streptozotocin-induced diabetic was studied in rats. Administration of HSE significantly lowered the level of fasting blood glucose and increased plasma insulin level. Sperm quality was improved with significantly higher sperm concentrations ($P<0.05$) and sperm motility ($P<0.001$) as well as lower percentage of sperm abnormality ($P<0.05$) as compared to the diabetic group. Plasma follicle-stimulating hormone (FSH) level was significantly elevated ($P<0.05$) in HSE group than in diabetic group while no significant alteration in plasma testosterone and luteinizing hormone (LH) level were seen between groups(124). The effect of oral administration of aqueous extract of *Hibiscus sabdariffa* (HS, at a 12 hr interval, daily for 7, 14 and 21 days, respectively) was evaluated on blood glucose, serum sodium and serum potassium concentrations in albino rats. The results revealed a significant decrease ($P<0.05$) in blood glucose level after 21 days of administration of HS and a significant decrease ($P<0.05$) in serum sodium concentration at 7 and 14 days of administration(125).The mechanism underlying the antidiabetic effect of ethanolic extract of *Hibiscus sabdariffa* calyxes (HS-EE, 0.1 and 1.0 g/kg/day, respectively, for 6 weeks) was investigated in streptozotocin-induced diabetic rats. HS-EE 1.0 g/kg/day significantly decreased the blood glucose level by 38 ± 12% in diabetic rats but not in normal rats. In normal rats, treatment with 1.0 g/kg HS-EE increased the basal insulin level significantly as compared with control normal rats (1.28 ± 0.25 and 0.55 ± 0.05 ng/ml, respectively). Diabetic rats treated with 1.0 g/kg HS-EE also showed a

significant increase in basal insulin level as compared with the control diabetic rats (0.30 ± 0.05 and 0.15 ± 0.01 ng/ml, respectively). Microscopic histological examination showed that HS-EE 1.0 g/kg significantly increased the number of islets of Langerhans in both normal rats (1.2 ± 0.1 and 2.0 ± 0.1 islet number/10 low-power fields (LPF) for control and HS-EE treated group, respectively) and diabetic rats (1.0 ± 0.3 and 3.9 ± 0.6 islet number/10 LPF for control and HS-EE treated group, respectively)(126).The effect of *Hibiscus sabdariffa* polyphenol extract (HPE) was investigated in streptozotocin (STZ) induced diabetic nephropathy. The results revealed that HPE reduced kidney mass induced by STZ significantly, as well as improving hydropic change of renal proximal convoluted tubules in the rats. HPE also significantly reduced serum triglyceride, total cholesterol and LDL in STZ induced rats. Treatment with HPE significantly increased the activity of catalase and glutathione and reduced lipid peroxidation (thiobarbituric acid-reactive substances)(127). The protective effect of *Hibiscus sabdariffa* polyphenolic extract (HPE) was investigatedin in type 2 diabetic rat model. Treatment with HPE reduced hyperglycemia, especially at the dose of 200 mg/kg. HPE decreased serum triacylglycerol, cholesterol, and the ratio of low density lipoprotein/high density lipoprotein (LDL/HDL). Diabetes promoted plasma advanced glycation end product (AGE) formation and lipid peroxidation, while HPE significantly reduced these elevations. Immunohistological observation revealed that HPE inhibited the expression of connective tissue growth factor (CTGF) and receptor of AGE (RAGE), which was increased in type 2 diabetic aortic regions. HPE also recovered the weight loss found in type 2 diabetic rats(128). The possible protective effects of *Hibiscus sabdariffa* calyxes aqueous extract (HSL,100mg/kg/day, orally for 28 consecutive days), as an antidiabetic and antioxidant agent against oxidative liver injury in streptozotocin-induced diabetic were investigated in rats. Supplementation of HSL significantly lowered the level of fasting blood glucose and increased plasma insulin level compared to negative control ($P<0.05$). Alanine aminotransaminases and aspartate aminotransferase level were found to be significantly reduced in the treated group compared with negative control(129). The antidiabetic, hypolipidimic, antioxidant and histopathological effects of hydroalcoholic extract of flower *Hibiscus rosa-sinensis* (HEFHR) were studied in alloxan induced diabetes in rats. HEFHR possessed significant and sustained oral antidiabetic activity, comparable with the hypoglycemic effect of glibenclamide and sulphonylurea. Flower extract of HRS was more efficacious in lipid lowering effect and in antioxidative activity than glibenclamide. After 28 day treatment with flower extract, size of islets was significantly increased and necrosis and atrophy of islets were significantly improved(130). The effects of *Hibiscus sabdariffa* (HSE) on diabetic nephropathy was tested in streptozotocin induced type 1 diabetic rats. HSE was capable of reducing lipid peroxidation, increasing catalase and glutathione activities significantly in diabetic kidney, and decreasing the plasma levels of triglyceride, low-density lipoprotein and increasing high-density lipoprotein value. In histological examination, HSE improved hyperglycemia-caused osmotic diuresis in renal proximal convoluted tubules in diabetic rats. The results also showed that it up-regulated Akt/Bad/14-3-3□ and NF-□B-mediated transcription. Accordingly, HSE ameliorated diabetic nephropathy via improving oxidative status and regulating Akt/Bad/14-3-3□ signaling(131).

Antioxidant effect

In studying the antioxidant effect of aqueous extracts of two varieties (red and white) *Hibiscus sabdariffa* calyces, the red variety possessed higher antioxidant capacity as exemplified by the •OH scavenging abilities, Fe2+ chelating ability, and inhibition of Fe2+-induced pancreatic lipid peroxidation *in vitro*(122). The antioxidant activity of the dried petal extracts of *Hibiscus sabdariffa* was investigated using the DPPH scavenging assay. The IC50 values of the roselle extract was 0.24 mg/ml while that of ascorbic acid was 0.35 mg/ml(26). The antioxidant activity was performed using wistar rats. The treatments were administered via oral route and at single dose for seven days, followed by injection of doxorubicin. The calyx extract of *Hibiscus sabdariffa* had attenuated the side effect of doxorubicin ($P<0.05$) regarding the markers of oxidative stress(36). *Hibiscus sabdariffa* leaves extracts showed good antioxidant activity as all the three extracts (aqueous, ethanol and ethyl acetate) exhibited good DPPH radial scavenging activity with IC50 values ranging from 46.13 ± 0.37 to 94.16 ± 0.56 µg/ml(30,65).The calyx extracts of *Hibiscus sabdariffa* from Ankara- Turkey showed that the total phenol contents of the extracts were between 16.4-49.1 mg gallic acid/g extract. They showed strong antioxidant effects, however, the inhibitory effects of the extracts in DPPH radical scavenging assay, were not dose dependent. On the other hand there was no correlation between the phenol content and radical scavenging activities of the extracts(132).The *in vitro* antioxidant activities of *Hibiscus sabdariffa* leaves extract were measured by ABTS radical cation decolorization assay, they showed strong antioxidant activity(133). The antioxidant activity of ethanolic seed extract of *Hibiscus sabdariffa* (100 and mg/kg HS) was investigated in toxicity induced by chronic administration of sodium nitrate (25mg/kg) in wistar rats. The animals were sacrificed at the end of 60 days and blood samples were taken for analysis of

total protein and some haematological indices. The haematological toxicity induced by chronic administration of sodium nitrate were alleviated by the antioxidant effect *Hibiscus sabdariffa* (134). The polyphenolic content and antioxidant activity of methanol, ethanol, acetone and water extracts of *Hibiscus sabdariffa* calyx were studied using 2,2-diphenyl-1-picryl hydrazine (DPPH) inhibition and lipid peroxidation inhibition. Methanol extract gave the highest inhibition to DPPH (78%) and was only significantly different ($P<0.05$) from acetone and water extracts. Ethanol gave the highest inhibition to lipid peroxidation (26%) but was not significantly different ($P<0.05$) from the other solvent extracts. There was a stronger correlation obtained between total phenolic content and inhibition of DPPH ($r = 0.969$) compared to total flavonoid content and DPPH ($r = 0.742$)(135).*Hibiscus sabdiriffa* showed antioxidant activity, the roselle extracts blended at various proportions with fruit (mango, papaya and guava) juices increased total phenolics (54.6-10.8 mg gallic acid/ 100 g) and antioxidant activity (1.80-1.37 mmol/l0) (136). The effect of an aqueous *Hibiscus sabdariffa* extract (HSE) on the systemic antioxidant potential (AOP; assayed by ferric reducing antioxidant power (FRAP)) was compared with a reference treatment (water) in eight healthy volunteers. HSE caused significantly higher plasma areas under the curve (AUC) of FRAP, an increase in Ae(0-24) of FRAP, ascorbic acid and hippuric acid, whereas malondialdehyde excretion was reduced. The increased urinary hippuric acid excretion after HSE consumption indicated a high biotransformation of the ingested HSE polyphenols, most likely caused by the colonic microbiota. Furthermore, the main hibiscus anthocyanins as well as one glucuronide conjugate were quantified in the volunteers' urine (0.02% of the administered dose)(137).The antioxidant and drug metabolizing potentials of hibiscus anthocyanin extract was investigated in CCl4- induced oxidative damage of rat liver. Hibiscus anthocyanin extract significantly increased the CCl4-mediated decrease in antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase). However, the level of nonenzymic antioxidant molecules (vitamins C and E) were significant preserved by hibiscus anthocyanin extract. There was an induction of phase II drug-detoxifying enzymes: glutathione S-transferase, NAD(H): quinone oxidoreductase, and uridyl diphosphoglucuronosyl transferase by 65, 45, and 57%, respectively. Accordingly, *Hibiscus sabdariffa* anthocyanin extract can act as a prophylactic drug by intervening as a free radical scavenger both *in vitro* and *in vivo* as well as inducing the phase II drug detoxification enzymes(138).

Effects on smooth muscles

Addition of an aqueous extract of *Hibiscus sabdariffa* calyces (2.5 ml/bath approximately 125 mg of starting crude material) inhibited the tone of various isolated muscle preparations (rabbit aortic strip, rhythmically contracting rat uterus, guinea-pig tracheal chain and rat diaphragm). Other muscles were stimulated (quiescent rat uterus and frog rectus abdominis)(88). The methanol extracts of *Hibiscus sabdariffa* showed a significant ($P<0.01$) dose dependent relaxant effect ($IC50 = 350$ µM) on rat ileal strip comparable to the effect shown by nifedipin and papaverine as reference compounds. The extract when administered intraperitoneally, it also significantly ($P<0.05–0.01$) reduced the intestinal transit (13-35%) in rats ($IC50 = 250$ µM)(28). The aqueous extract of *Hibiscus sabdariffa* calyces extracts induced rat bladder and uterine contractility in a dose-dependent manner via a mechanism unrelated to local or remote autonomic receptors or calcium channels(139). The Vascular effects of crude extract of dried and powdered calyces of *Hibiscus sabdariffa* were evaluated on isolated thoracic aorta of male Wistar rats. The *Hibiscus sabdariffa* crude extract induced endothelium-dependent relaxant effects. The endothelium-dependent relaxations were resulted from NOS activation(80).

Gastrointestinal effects

The gastro-protective potential of *Hibiscus sabdariffa* against indomethacin-induced gastric ulcer was evaluated in the rat. 70% alcoholic extracts of *Hibiscus sabdariffa* (100, 400, 800 mg/kg) were gavaged to rats for 4 consecutive days. Gastric ulcers were induced by the one time gavage of indomethacin (30mg/kg). The animals were killed 6 h after the indomethacin administration. Ulcer index was significantly and dose- dependently decreased in rat treated with *Hibiscus sabdariffa*(140). The anti-ulcerogenic property of ethanolic extract of dried calyces of *Hibiscus sabdariffa* in different ulcer models was studied in Wistar albino rats. The extract at adose of 250 and 500 mg/kg bw, orally showed a significant effect in cold restraint stress, pylorus ligation, necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl) and indomethacin-induced gastric ulcer models. The extract was also significantly decreased the basal gastric acid secretion, significantly increased gastric wall mucus secretion and non-protein sulfhydryl concentrations in gastric tissue and significantly reduced the ethanol-induced elevated levels of malondialdehyde in the rat stomach(141). The antidiarrheal effect of the ethanolic calyx extract of *Hibiscus sabdariffa* was studied using castor oil-induced diarrheal model mice. The extract demonstrated a significant antidiarrheal activity against castor oil-induced diarrheal in mice,

it decreased the frequency of defecation and increased the mean latent period at the doses of 250 and 500 mg/kg bw (P<0.01)(142).The effects of aqueous extracts of the calyces of *Hibiscus sabdariffa* on intestinal transit was studied in experimental rats. The dried calyces of *Hibiscus sabdariffa* was pulverized and 10% extracts of powder were administered orally to rats at varying doses (0.5/100g, 1ml/100g, 2ml/100g bw). After 30 minutes, each animal was then given 1.5 ml of a dye solution orally. One hour after administering the dye, each rat was sacrificed and the intestine carefully dissected out. The length of the intestine and the transit point of the orally administered dye were then measured. The transit point was calculated as a percentage of the total length of the intestine. *Hibiscus sabdariffa* caused significant reduction in the transit points of the dye(143).

Effect on urinary system

The diuretic activity of *Hibiscus sabdariffa* aqueous extract was evaluated on *in vivo* and in situ models. The aqueous extract was administrated in increasing doses and the diuresis produced and disposal of electrolytes were evaluated. The renal filtration rate with plant extract, furosemide and amiloride were evaluated in isolated kidney. The diuretic and natriuretic effect of *Hibiscus sabdariffa* aqueous extract showed a dose-dependent behavior. The pharmacological constants of natriuretic effect was ED50 = 86 mg/kg and Emax = 0.9 mEq/100 g/5 h. In the *in vitro* model, renal filtration was increased 48% with the aqueous extract of *Hibiscus sabdariffa* and an additive effect was recorded when *Hibiscus sabdariffa* aqueous extract was perfused with furosemide (144). The diuretic, natriuretic, and potassium sparing effects of *Hibiscus sabdariffa* were due in part to the modulation of aldosterone activity by the presence of compounds potentially responsible for this modulation, as anthocyanins, flavonoids, and chlorogenic acid(102).Supplementation of aqueous extract of *Hibiscus sabdariffa* at different doses (250, 500 and 750 mg/kg bw) significantly lowered the deposition of stone-forming constituents in the kidneys and serum of urolithiatic rats. These findings were confirmed by the histological investigations(145). The possible beneficial effects of aqueous extracts of *Hibiscus sabdariffa* calyces and anthocyanins were evaluated in an adenine-induced chronic kidney disease (CKD) model. Rats were orally treated, for 28 consecutive days, either adenine alone or together with either aqueous extract of *Hibiscus sabdariffa* calyces (5 and 10%) or anthocyanins (50, 100 and 200 mg/kg of anthocyanin concentrate) and for comparative purposes, two groups of rats were given lisinopril (10 mg/kg). When either *Hibiscus sabdariffa* aqueous extract or the anthocyanins isolated from it was administered along with adenine, the adverse effects of adenine-induced CKD were significantly lessened, in a dose-dependent manner. The effects were similar to those obtained by administration of lisinopril(146).

Antiinflammatory, analgesic and antipyretic effects

The essential oil of *Hibiscus sabdariffa* exhibited excellent anti-inflammatory activity in lipopolysaccharide (LPS)-stimulated macrophage RAW 264.7 cells. The nitric oxide (NO) inhibition rate reached 67.46% when the concentration of the essential oil was 200 μg/ml. Further analysis showed that the anti-inflammatory activity of the essential oil extracted from *Hibiscus sabdariffa* might be exerted through inhibiting the activation of NF-□B and MAPK (JNK and ERK1/2) signaling pathways to decrease NO and pro-inflammatory cytokine (IL-1, IL-6, TNF-□, COX-2, and iNOS) production(147). The antiinflammatory effect of seed of *Hibiscus sabdariffa* was tested in rats. The oral administration of petroleum ether extract of *Hibiscus sabdariffa* seeds reduced the paw edema significantly that was induced by carrageenan in dose dependent manner. After 3 h of the treatment at 4 and 8 ml/kg bw, paw edema was reduced by 27.9% (P<0.05) and 34.2% (P<0.01), respectively. In contrast, the ethanolic extract of *Hibiscus sabdariffa* seeds did not show significant reduction in paw edema (inhibition 0%) even at the maximum dose (400 mg/kg bw). In vascular permeability test, oral administration of diclofenac sodium at 10 mg/kg bw, and petroleum ether extract of *Hibiscus sabdariffa* seeds (at 4 and 8 mg/kg bw) significantly (P<0.01) inhibited the dye leakage induced by acetic acid as compared to control. In cotton pellet induced granuloma test, granuloma formation was inhibited significantly after administration of petroleum ether extract of *Hibiscus sabdariffa* seeds for 6 consecutive days as compared to control group. The test dose (4 and 8 ml/kg bw) showed 30.3% and 27.2% of inhibition (P<0.01) respectively as compared to the control group. The peripheral analgesic activity of petroleum ether extract was measured by acetic acid induced writhing test. *Hibiscus sabdariffa* seed petroleum ether extract exhibited a significant level of inhibition in abdominal writhes produced by acetic acid especially with high dose (8 ml/kg bw, 45.0%, P<0.001) compared to control group(44).The anti-inflammatory activity of methanolic leaves extract of *Hibiscus sabdariffa* (250 and 500 mg/kg bw) was investigated in adult Wistar rat using carrageenan model. There was significant reduction (P<0.05) in paw diameter in the group received high dose (500 mg/kg bw) of methanolic extract of *Hibiscus sabdariffa* from 0.566±0.023 to 0.414±0.009 as compared with the untreated group(18). The effects of the extracts from *Hibiscus sabdariffa* calyces on nociceptive response were studied using writhing,

hot plate and formalin test in mice, the antipyretic activity in yeast-induced fever in rats and anti-inflammatory activity on carrageenin-induced paw edema in rats. Oral administration of the ethanol extract at the dose of 800 mg/kg significantly decreased the number of contortions and stretchings induced by acetic acid in mice. Neither the ethanol nor aqueous extract had an effect in the formalin and hot plate tests in mice. The ethanol and the vacuum dried extract of *Hibiscus sabdariffa* calyces (200-800 mg/kg, po) decreased the yeast-induced fever in rats, while, *Hibiscus sabdariffa* extract had no effect on carrageenin induced paw edema in rats(148). The antinociceptive and anti-inflammatory of the ethanolic calyx extract of *Hibiscus sabdariffa* were studied in mice. The antinociceptive activity of the extract was evaluated by using the acetic acid-induced writhing test. The anti-inflammatory effect of the extract was tested by using the xylene-induced ear edema model in mice. In acetic acid-induced writhing test, the extract inhibited writhing in mice significantly compared with control ($P<0.01$). The extract showed significant inhibition of ear edema formation in xylene-induced ear edema model in mice in a dose-related manner compared with control ($P<0.01$)(142). The aqueous extracts of *Hibiscus sabdariffa* were tested for anti-inflammatory, analgesic and antipyretic activities in animal models. The extract had no effect on paw edema but had an inhibitory effect on yeast induced pyrexia and showed significant effect on the hot plate reaction time(149).

Immunological effects

The immunomodulatory activity of the total crude leaf extract of *Hibiscus sabdariffa* (125, 250 and 500 mg/Kg bw, daily for 14 days) was studied in Wistar albino rats. All the doses caused an increase in mean red blood cell counts as compared to control group. The mean percentage of neutrophils, monocytes, basophils and eosinophils was increased with dose, while the opposite was true for percentage of lymphocytes. The mean hemagglutination antibody (HA) titers for the herb were higher than control though no statistical difference ($P\geq0.05$) was observed. Similar effects were observed with neutrophil adhesions response as that of HA titers. For delayed-type hypersensitivity (DTH), the highest footpad thickness (175.2% increment) was observed at a dose of 500 mg/Kg bw, after 12 h and was statistically significant ($P\leq0.05$) as compared to control(23). The immunomodulatory properties of two fractions of the aqueous alcoholic extract of the dried calyx of *Hibiscus sabdariffa* were studied in experimental animals. Immunomodulatory activity was evaluated using red blood cell-induced immunostimulation. The ethyl acetate soluble fraction (EAC) exhibited a significant dose-dependent immunostimulation ($P<0.05$), higher than that observed for levamisole (positive control). The residual water-soluble fraction exhibited immunostimulatory activity at 100 mg/kg bw. The two fractions caused a significant reduction in production of tissue necrosis factor-alpha and an increase in interleukin 10(150).The immunomodulatory activity of water and alcohol extracts (including its fractions) of the dried calyx of *Hibiscus sabdariffa* was evaluated in mice. The ability of the extracts to inhibit or enhance the production of two cytokines, [tumor necrosis factor-alpha (TNF- alpha) and interleukin-10 (IL-10)], implicated as proinflammatory and antiinflammatory interleukins were also evaluated. The extracts at doses of 50 mg/kg were found to possess higher immunostimulatory activities in comparison with levamisole (positive control), with significant effects when compared with the vehicle-treated group ($P<0.01$). Increased activity was observed with increase in doses of the 50% ethanol and absolute ethanol extracts. The insoluble fraction exhibited a significant dose-dependent immunostimulatory activity ($P<0.05$), while the residual water-soluble fraction exhibited activity at 100 mg/kg bw(151). The aqueous extract of *Hibiscus sabdariffa* promoted the production of IL-6 and IL-8 and decreases the concentration of MCP-1 in a dose-dependent manner. This effect was not due to a concomitant increase in the antioxidant capacity of plasma. The mechanisms involved a direct inhibition of inflammatory and/or metabolic pathways responsible for MCP-1 production, and may be relevant in inflammatory and chronic conditions in which the role of MCP-1 was well established(152).

Effect on reproductive systems

The sub-chronic effect of *Hibiscus sabdariffa* (HS) calyx aqueous extract (1.15, 2.3, and 4.6 g/kg for 12-weeks) on testis was investigated in rats. Three test groups received different doses of 1.15, 2.30, and 4.60 g/kg based on the LD50. Results did not show any significant ($P>0.05$) change in the absolute and relative testicular weights, but there was a significant ($P<0.05$) decrease in the epididymal sperm counts in the 4.6 g/kg group, compared to the control. The 1.15 g/kg dose group showed distortion of tubules and a disruption of normal epithelial organization, while the 2.3 g/kg dose showed hyperplasia of testis with thickening of the basement membrane and 4.6 g/kg dose group, showed disintegration of sperm cells(153).

The potential adverse effects of the cold and hot *Hibiscus sabdariffa* calyx extracts (200 mg/kg bw, for 4 weeks, orally) on sperm morphology and testicular ultrastructure were studied in albino mice. The results revealed that aqueous extracts of dried calyx of *Hibiscus sabdariffa*, either cold or boiled, altered normal sperm morphology and testicular ultrastructure and adversely influenced the male reproductive fertility in albino mice(154). The effects

of *Hibiscus sabdariffa* (HS) on the development of the male reproductive tract following in utero exposure were investigated in rats. Pregnant rats received 250 or 500 mg/kg of HS extract from gestational day 12 until day 21 of lactation. Both doses of HS increased the body weight of male offspring at weaning, without compromising the puberty onset parameters. At puberty, there was a significant increase in the vas deferens absolute weight and a significant reduction in the relative weight of kidney at higher dose. At adulthood, the highest dose significantly reduced the sperm production in relation to controls and both doses provoked a reduction in the relative sperm number in the epididymis without affecting the sperm morphology(155).The effects of different concentrations of aqueous extracts of *Hibiscus sabdariffa* calyces (10%, 15% and 20%) in drinking water for 10 consecutive weeks, and its anthocyanins (50, 100, 200 mg/kg for 5 days, orally) were investigated in male and female rats, on the weight and histology of the testis, and on some biochemical constituents in testicular homogenates, as well as on plasma concentrations of testosterone, luteinizing hormone and estradiol. The possible presence of an estrogenic effect of the extract and anthocyanins on the uteri of immature female rats was also tested. Neither the *Hibiscus sabdariffa* extract nor the anthocyanins significantly altered either testicular weight and histology, or uterus weight. Plasma concentrations of the three hormones, the testicular concentrations of protein, reduced glutathione and total cholesterol, and superoxide dismutase activity were all insignificantly affected by either the extract or the anthocyanins(156). *Hibiscus sabdariffa* consumption caused delayed puberty of the offspring either the mothers consumed it during pregnancy or during lactation periods(157-159).Furthermore, consumption of aqueous extract of HS during the juvenile-pubertal period decreased fluid and food consumption, increased weight gain and delayed puberty onset in rats(160).

Protective effects

The effects of the water extract of the dried flowers of *Hibiscus sabdariffa* and *Hibiscus anthocyanins* (HAs) were evaluated in paracetamol-induced hepatotoxicity in rats. The water extract was given in drinking water for 2, 3 or 4 consecutive weeks, and the HAs were given orally at doses of 50, 100 and 200 mg/Kg for five consecutive days. The extract for 4 weeks (but not for 2 or 3 weeks) significantly improved some of the liver function tests, but did not alter the histology of the paracetamol-treated rats. At a dose of 200 mg/Kg, the hepatic histology and the biochemical indices of liver damage were restored to normal(161). Dried flower *Hibiscus sabdariffa* (HSE) extracts (1-5% for 9weeks) were tested for hepatoprotective effects against liver fibrosis induced by carbon tetrachloride (CCl4) in rats. HSE significantly reduced the liver damage including steatosis and fibrosis in a dose dependent manner. HSE also significantly decreased the elevation in plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and restored the decrease in glutathione content and inhibited the formation of lipid peroxidative products during CCl4 treatment(162). Pretreatment of rats with aqueous extract of *Hibiscus sabdariffa* resulted in significantly less hepatotoxicity than with Cd alone as measured by plasma ALT and liver ALT and AST activities. The extract also protected the rats against Cd-induced liver, prostate, and testis lipoperoxidation as evidenced by significantly reduced MDA values in these organs, as well as reduced prostatic acid phosphatase activity in the prostate, when compared to the Cd-only exposed rats(163).The hepatoprotective effect of the anthocyanin-rich extract of *Hibiscus sabdariffa* calyces (HSARE, 100 mg/kg/d for 4 weeks) was studied in thioacetamide (TAA)-induced hepatotoxicity in rats. Compared to the TAA-intoxicated group, HSARE significantly reduced the serum levels of alanine aminotransferase, aspartate aminotransferase and hepatic malondialdehyde by 37.96, 42.74 and 45.31%, respectively. It also decreased hepatic inflammatory markers, including tumour necrosis factor alpha, interleukin-6 and interferon gamma (INF-□), by 85.39, 14.96 and 70.87%, respectively. In addition, it decreased the immunopositivity of nuclear factor kappa-B and CYP2E1 in liver tissue, with an increase in the effector apoptotic marker (caspase-3 positive cells), restoration of the altered hepatic architecture and increases in the activities of superoxide dismutase and glutathione by 150.08 and 89.23%, respectively(164).The effect of *Hibiscus sabdariffa* extract (HSE) on acetaminophen (AAP)-induced liver injury was investigated in mice. Mice were fed orally with 200, 400 or 600 mg/kg HSE for 2 weeks and then injected with 1000 mg/kg AAP. Pretreatment with HSE decreased lipid peroxidation and increased catalase activity and glutathione level. It also decreased AAP-induced liver injury, accompanied by decreased expression of pJNK, Bax and tBid in the liver(165). The possible protective mechanism of the polyphenol extract of *Hibiscus sabdariffa* (HPE) against acetaminophen (AAP)-caused liver damage was studied in mice. Mice were orally fed with HPE (100, 200 or 300 mg/kg) for two weeks prior to an ip injection of 1000 mg/kg of AAP. The pretreating with HPE increased the level of glutathione (GSH), decreased the level of lipid peroxidation, and increased catalase activity in the liver. Histopathological evaluation showed that HPE decreased AAP-induced liver sterosis accompanied by a decreased expression of AIF, Bax, Bid, and p-JNK in the liver. An *in vitro* assay revealed that HPE

reduced AAP-induced death of BABL/c normal liver cells (BNLs), reversed the lost mitochondrial potency and improved the antioxidative status(166). CCl4 in rats elevated aspartate aminotransferase, alanine transaminase, alkaline phosphatase, total protein, globulin levels significantly ($P<0.05$) while albumin was reduced. CCl4 significantly reduced sperm count, viability and motility ($P<0.05$), while sperm head abnormality increased. However, administering of *Hibiscus sabdariffa* extract at the doses of 300 and 600 mg/kg caused the reversal of these effects significantly(167).The protective effect of extract of *Hibiscus sabdariffa* was studied against SGD-induced PC12 cells injury. Cells were pretreated with different concentrations of *Hibiscus sabdariffa* extract (HSE) for 2 hr, and then exposed to SGD condition for 6, 12 and 18 hr. SGD caused a major reduction in cell viability after 6, 12, and 18 hr as compared with control cells ($P<0.001$). Pretreatment with HSE (30-500 μg/ml) significantly increased cell viability following SGD insult for 6, 12 and 18 hr. A significant increase in cell apoptosis was seen in cells under SGD condition after 12hr as compared with control cells ($P<0.001$). Pretreatment with HSE significantly decreased cell apoptosis subsequent SGD condition after 12hr(168).Flavonoid-rich aqueous fraction of methanolic extract of *Hibiscus sabdariffa* calyx was evaluated for anti-hepatotoxic activities in streptozotocin-induced diabetic wistar rats. The ameliorative effects of the extract on STZ-diabetes induced liver damage was evident from the histopathological analysis and the biochemical parameters evaluated in the serum and liver homogenates. Reduced levels of glutathione, catalase, superoxide dismutase and glutathione peroxidase in the liver of diabetic rats were restored to a near normal level in the *Hibiscus sabdariffa* -treated rats. Elevated levels of aspartate amino transferase, alanine amino transferase and alkaline phosphatase in the serum of diabetic rats were also restored in *Hibiscus sabdariffa* -treated rats. Histologically, hepatic fibrosis and excessive glycogen deposition in the diabetic rats were ameliorated in the extract-treated rats(169).The ameliorative effect of co-administration of aqueous extract of *Hibiscus sabdariffa* (HS) and vitamin E was evaluated on sub-chronic carbamazepine (CBZ)-induced alterations in semen characteristics in rats. The result showed that mean sperm counts in the CBZ-treated alone group was lower than in groups with HS, Vitamin E, and HS and vitamin E ($P<0.05$) when compared to the control groups. There was significant decrease in mean progressive sperm motility with an increase in the means non-progressive motility and non-motile sperm cells of the CBZ-treated group as compare to the control group ($P<0.05$). While there were significant increases in mean progressive sperm motility with a decrease in non-progressive motility and non-motile sperm cell of the groups treated with CBZ in combination with HS, vitamin E, and HS and vitamin E, when compared to the CBZ-treated alone and control groups ($P<0.05$). There was no considerable statistically significant different in abnormal sperm cells in the treatment groups(170).The antigenotoxic property of *Hibiscus sabdariffa* dry calyx extracts was investigated in mice. The dried calyx extracts of *Hibiscus sabdariffa* were administered to male albino mice at doses of 50, 100, and 150 mg/kg bw, for 7 days followed by a single dose of interperitoneal injection of sodium arsenite (2.5 mg/kg bw). The calyx extract inhibited the DNA damage induced by sodium arsenite in a dose dependent manner(171).

CNS effects

The neuropharmacological effects of the aqueous extract of *Hibiscus sabdariffa* (HS) calyx were studied in rodents. HS (100, 200 and 400 mg/kg, ip) caused a remarkable dose-dependent decrease in spontaneous motor activity in mice and increased the duration of pentobarbital (40 mg/kg, ip) induced sleep in rats. The extract (100-400 mg/kg, ip) significantly reduced the exploratory behaviour in mice. The extract significantly inhibited the intensity of apomorphine (1 mg/kg, sc) induced stereotypic behaviour and attenuated climbing in the mice dose-dependently(172). The nootropic acitivity of calyces of *Hibiscus sabdariffa* was studied in mice using elevated plus maze and passive avoidance paradigm to evaluate learning and memory parameters. The aqueous extracts of calyces of *Hibiscus sabdariffa* (100 and 200 mg/kg, po) significantly attenuated amnestic deficits induced by scopolamine (0.4 mg/kg, ip) and natural aging. *Hibiscus sabdariffa* (100 and 200 mg/kg) decreased the transfer latencies and increased step down latencies significantly in the aged mice and scopolamine induced amnesic mice as compared with piracetam (200 mg/kg, ip). Acetylcholinesterase activity in the whole brain was significantly decreased in mice which could be refere to the underlying mechanism of action(173).

Lactogenic effect

The lactogenic effect of ethanolic seed extract of *Hibiscus sabdariffa* was investigated by administering extract and metoclopramide in albino rats. The extracts were administered at varying doses (200, 400, 800 and 1600mg/kg) for six days orally. The ethanolic seed extract of *Hibiscus sabdariffa* possessed lactogenic effect. It caused significant increase ($P<.01$) in serum prolactin levels in a dose dependent manner. The doses of 800 and 1600 mg/kg seemed more effective with serum prolactin levels of 15.74 ± 0.8 and 17 ± 0.6 respectively, compared to control 6.68 ± 0.5 ng/ml(9).

Wound healing effect

The wound healing activities of water in oil cream of the methanol extract of *Hibiscus sabdariffa* was evaluated in rats with superficial skin excision wounds. Creams containing *Hibiscus sabdariffa* extract showed significant ($P<0.05$) and concentration dependent wound healing activities. There was also evidence of synergism with creams containing a combination of gentamicin and *Hibiscus sabdariffa* extract(174).

Side effects and toxicity

The median lethal dose of the calyx extract in rats is estimated to be higher than 5 g/kg(15). LD50 of *Hibiscus sabdariffa* seed extract in albino rats was also found to be above 5g/kg(9). No acute toxicity was observed in mice after oral administration of the ethanol and aqueous extract of *Hibiscus sabdariffa* calyces at the dose of 15 g/kg(148).In acute toxicity study, all the doses (2, 4, 8 and 20 ml/kg bw) of petroleum ether extract of *Hibiscus sabdariffa* seeds were found to be non-toxic for rats. No animal mortality was observed after receiving petroleum ether extract up to the dose of 20 ml/kg bw(44).*Hibiscus sabdariffa* calyces extract, 0.6g, 1.2g and 1.8g in 100ml distilled water for rats, caused no significant difference in weight of the organs when compared with the control, but there were inflammations on the liver tissues. Kidney organs also showed no difference in their normal gross anatomical features size, color, and consistency(32). The effects of a 90-day oral administration of water and alcohol extracts of dried calyx of *Hibiscus sabdariffa* were evaluated in albino rats. Significant reductions in the erythrocyte count with no difference in total leucocyte count were observed. The activity of aspartate aminotransferase was enhanced by the administration of aqueous and 50% ethanol extract with a significant increase in its level at higher doses ($P<0.05$). Alanine aminotransferase and creatinine levels were significantly affected by all the extracts at the different dose levels. Aqueous extracts exhibited a significant increase in creatinine levels ($P<0.05$) at higher doses. No significant histopathological changes were observed, although there was a significant reduction in the weight of the spleen of the animals administered with ethanol and water extracts when compared with the control ($P<0.01$). Other organs were of the same relative weight(175).Acute and chronic toxicities of the water extract from calyces of *Hibiscus sabdariffa* were studied in rats. After 14 days of a single oral administration of the water extract 5g/ kg bw, no signs and differences of the weights or behaviour were observed compared to the control rats. The chronic toxicity was determined by oral feeding both male and female rats daily with the extract at the doses of 50, 100, and 200 mg/kg body weight for 270 days. The examinations of signs, animal behaviour and health monitoring showed no defects in the test groups compared to the control groups. Results showed no differences from the control groups in haematology, blood clinical chemistry, and microanatomy(176). The aqueous fraction of an aqueous-alcoholic extract of *Hibiscus sabdariffa* calyces was given to Wistar albino rats orally, to study the toxicity of the extract. Results of the study showed that the levels of serum aspartate aminotransferase and alanine amino transferase were significantly ($P<0.05$) increased in all the treatments compared with the control group. However, the serum levels of alkaline phosphatase, and lactate dehydrogenase were not significantly ($P>0.05$) affected. Only the group with 15 doses had their serum level of albumin significantly ($P<0.05$) increased. However, the results of histopathological studies showed that both the livers and hearts gave no pathological features for all the treatments(177). Clinical trials showed that ingestion of *Hibiscus sabdariffa* was well tolerated and there was no adverse effect during the trials(119,117,118). The health benefit effects of *Hibiscus sabdariffa* dried calyces beverage on some clinical, biochemical and hematological parameters were investigated in humans. A drink was prepared for 32 male volunteers. Each participant consumed 500 ml twice a day (in the morning and in the evening) as supplement beverage for two weeks. The anthropometrics (age, height, weight, body mass index (BMI)), clinical (systolic and diastolic blood pressure), hematological (RBC, Hb, PCV, MCV, MCH, MCHC, WBC, Lymphocytes, MID cells, Granulocytes, platelet and MPV) and biochemical (TC, HDL-C, LDL-C, TG, serum iron, blood glucose, creatinine, urea, ASAT and ALAT) parameters were determined in the blood on days 0 and at the end of each week. A significant increase of RBC, Hb, PCV, MPV, HDL-C, TG and creatinine and a significant decrease of WBC, MID cells, LDL-C and TC ($P<0.05$) were observed during the study period. Furthermore, there was no significant change on BMI, MCV, MCH, MCHC, lymphocyte, granulocyte, platelet, serum iron, blood glucose, ASAT, ALAT and urea levels. The authors concluded that *Hibiscus sabdariffa* dried calyces drink can be safely used. It also revealed good cholesterol lowering potential. No hepatoxicity and no kidney damage have been observed(178).

Dose

In clinical investigating of the hypotensive effect of plant, daily dose of dry calyx 10 g (equivalent to anthocyanin 9.6 mg) as an infusion in water, or total anthocyanin 250 mg per dose were used for 4 weeks. The pharmacokinetics in healthy volunteers revealed a half-life of 2.6 hours and a maximum excretion at 1.5 to 2 hours(89,179,180).

Conclusion

This review discusses the chemical constituent, pharmacological and therapeutic effects of *Hibiscus*

sabdariffa as promising herbal drug because of its safety and effectiveness.

Author's contribution

Al-Snafi is the single author of the manuscript, drafted the and approved the manuscript.

Conflict of interests

The author declared no competing interests.

Consent for publication

The manuscript didn't contain any individual persons data.

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Ethical considerations

The work is a review, the author didn't perform experimental and clinical work. Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the author.

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