The Pharmaceutical and Chemical Journal, 2017, 4(6):68-86

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Assessment of *In-vitro* and *In-vivo* Bioactivities Potential of Some Egyptian Wild Plants as Anti-Ulcerogenic Effect on male albino rats

Nermien Z. Ahmed[1], Omar A. Ahmed- Farid[2]

Molecular Drug Evaluation Department[1], Physiology Department[2]
National Organization for Drug Control and Research (NODCAR), Giza 12553, Egypt

Abstract Background: There is an abundance of herbal medicines that may introduce to cure the human patient for different syndromes with mild side effect. This study aimed to investigate *In-vitro* and *In-vivo* bioactivities potential of some Egyptian wild plants as anti-ulcerogenic on male albino rats. Polyphenols constitute a distinct group of natural compounds of medicinal plants as *Monsonia nivea*; *Savignya parviflora*; *Heterocaryum subsessile*, and *Trigonella anguina* ethanolic extracts exhibit a wide range of multi-physiological activities such as antioxidant, anti-inflammatory, and anti-ulcerogenic activities. 1st experiment *In-vitro* antioxidant properties DPPH radical assay; OH•; FRAP; TP; TF, NO, and flavonoid concentrations. Also, determine the Fe++/Ascorbate on mitochondria and microsomal fractions. Acid phosphatase; β-galactosidase; β-N-acetylglucosaminidase, and β- glucuronidase were determined in liver lysosomes against CCl4. the 2nd experiment carried out the *In-vivo* study, focusing the antioxidant, anti-inflammatory and anti-ulcerogenic activities of herbal cocktail ratio due to the best constituent results from the *In-vitro* study. Methods: Thirty-six male albino rats divided into six groups; 1st group: control. 2nd herbal cocktail group (100 mg/kg b.wt/day o.p) from Mn, Sp, Hs, and Ta extract with ratio 1:2:1:3 respectively, along with the experiment. The 3rd group treated with acetylsalicylic acid (500mg/kg b.wt/day o.p) for three successive days (positive group). The 4th group treated with herbal along 2 weeks then induction with ASA (protective group). The 5th group treated with ranitidine (27 mg/kg b.wt/day o.p) after induction with ASA (curative ranitidine group). The 6th group treated with herbal extract (27 mg/kg b.wt/day o.p) after induction with ASA (curative herbal cocktail group), the experiment lasted for 30 days. Results: Revealed data showed that the ASA induces a significant increase in blood leucocytes, neutrophils and stomach MDA, GSSG, NO, TNF-α, IL-6, and myeloperoxidase. On the other hand, ASA induces a significant decrease in blood RBCs and stomach GSH. Protective and curative effect of HC extract showed ameliorated effect for parameters near to control group. Conclusion: Obtained data concluded that individual wield herbal extract have *In-vitro* antioxidant, anti-inflammatory and *In-vivo* antioxidant, anti-inflammatory and anti-ulcerogenic properties due to reached flavonoids of the mixture.

Keywords DPPH, Fe^{++}/Ascorbate, NO, TAC, FRAP, TP, TF, MDA, GSH, IL-6, TNFα, HPLC, MPO, GSSG, herbal cocktail, peptic ulcer, MDA.

1. Introduction

Plants not only provide man with food and fiber but also cured him of several fatal diseases. Medication by herbs was the sole way against a lot of diseases, in recent days attention is re-directed towards the drug plants for having fewer side-effect troubles, the preliminary results of a study on behalf of WHO has shown that the number of individuals using medicinal plants is large and on the increase, even among young people [1].

Phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, tannins) are one of the main free radical scavenging molecules in plants [2]. Epidemiological studies have shown that many of these compounds possess anti-inflammatory, antioxidant, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities [3]. Numerous physiological and biochemical processes in human body may produce oxygen entered free radicals and other ROS as by-products, the overproduction of such free radicals can cause oxidative damage to biomolecules as lipids, proteins, and even DNA, eventually leading to many chronic diseases, such as atherosclerosis, cancer, aging, and other degenerative diseases in humans [4]. The available synthetic antioxidants have been suspected of causing or promoting negative health effects, so strong restrictions encountered their application and there is an urgent trend to substitute them with naturally occurring antioxidants [3] while the intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, and other diseases associated with age which have the advantage of being almost devoid of side effects [5].

Monsonia nivea (MN) belongs to the family Geraniaceae among the Egyptian wild plants was reported under the names of Dahma. The name Nivea means white snow as the plant is silvery green. Preliminary phytochemical screening of the aerial parts of MN was carried out and revealed the presence of carbohydrates and/or glycosides, flavonoids (Apigenin, Quercetin, and so on), saponins, tannins, unsaturated sterols and/or triterpenes. The anti-oxidant activity from all compounds isolated from the different extracts of MN exhibited a definitive free radical scavenging activity as compared to ascorbic acid. The isolated flavones and flavonols from the same plant showed similar results [6].

Savignya parviflora (SP) belongs to family Cruciferae, aerial parts of this plant gave yellow color oil in 0.13% yield (v/w) on a fresh weight basis by steam distillation. Gas chromatography-mass spectrometry (GC/MS) analysis of the oil resulted in the identification of 14 components representing 89.8% of the oil. The major constituents of the oil were palmitic acid (40.9%), Phytol (8.5%), eicosane (6.4%) and tetratetracontane (5.9%) [7].

Heterocaryum subsessile (HS) is annual medium herbs with narrow leaves and has long thickened pedicels.

Trigonella anguina (TA) belongs to family Leguminosae, fresh aerial parts on steam distillation gave yellow color oil in yield 0.04% (v/w) on a fresh weight basis, twelve constituents representing 91.1% of the oil were identified through Gas chromatography-mass spectrometry analysis. The major components were palmitic acid (38.4%), tetradecanoic acid (15.9%), linolenic acid methyl ester (11.3%), phytol (7.6%) and decanoic acid (7.3%) [8]. Trigonella has been shown to suppress inflammation, inhibit proliferation, and induce apoptosis in a variety of tumor cells. It down-regulate TNF induced expression of NF-κB-regulated gene products involved in cell proliferation (cyclin D1, COX-2, c-myc), antiapoptosis (IAP1, Bcl-2, Bcl-xL, Bfl-1/A1, TRAF1, and cFLIP), and invasion (MMP-9) [9]. It also inhibits STAT3 signaling pathway leading to suppression of proliferation and chemosensitization of human hepatocellular carcinoma. 4-hydroxyisoleucine, an unusual amino acid isolated from Trigonella seeds was characterized in type II diabetes [10].

The peptic ulcer is a common disorder of the gastrointestinal system. Originally, all peptic ulcers were believed to be caused by the aggressive action of HCL, pepsin on the mucosa and the infection with H. pylori increases the risk of gastric ulcer [1]. Recently, there has been interesting in alternative therapies and use natural products, especially those derived from plants [12]. Several wield plants have been used to treat gastrointestinal disorders including peptic ulcer. Studies have revealed that medicinal plants contain various classes of bioactive secondary metabolites such as polyphenols, tocopherol alkaloids, and flavonoids. Flavenoids particularly are known to produce pharmacological properties like antimicrobial, anti-inflammatory, anti-depression and anticarcinogenic effects [13, 14]. This study aimed to investigate *In-vitro* antioxidants, anti-inflammatory and *In-vivo* antioxidants, anti-inflammatory and anti-ulcerogenic bioactivities potential of some Egyptian wild plants on male albino rats.

2. Materials and Methods

Plant Materials

Leaves of MN; SP; HS, and TA which belong to different families (Geraniaceae, Cruciferae and Boraginaceae, Leguminosae respectively) were collected from Experimental Station of Faculty of Agriculture Cairo University, Egypt.

Extraction of Plant Materials

The collected leaves of MN; SP; HS, and TA were washed several times with distilled water; cut into small pieces, and dried in lypholizer. The dried leaves of the plant were grinded and extracted by soaking in ethanol 70%, then the solvent was filtered and then evaporated under reduced pressure and lyophilized (-50 ºC) [15, 16]. The residual materials was kept and stored at -20 ºC until used for further study.

***In-vitro* study**

Chemical

All chemicals, solvents and reagents used were of analytical and pure grade. DPPH was purchased from Fluke chemical company (Switzerland). Rutin hydrate as antioxidant standard was purchased from Sigma chemical Co., St. Lewis, USA.

Determination of Phenolic Compounds by HPLC

The mobile phase contains gradient elution with 0.01 M phosphoric acid–methanol. The flow rate was adjusted to 0.6 ml/min, the column was thermostatically controlled at 40^0C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent B to solvent A. The gradient elution was changed from 10 % to 40% B in a linear fashion for duration of 28 min, from 40 to 60 % B in 39 min, from 60 to 90 % B in 50 min. The mobile phase composition back to initial condition (solvent B: solvent A: 10: 90) in 55 min and allowed to run for another 10 min, before the injection of another sample. HPLC chromatograms were detected using a UV detector 285 nm wave length. Each compound was identified by its retention time and by spiking with standards under the same conditions according to [17].

Determination of Total Phenolic Content

The concentration of total phenolic contents in *Monsonia nivea; Savignya parviflora;* HS, *and* TA *ethanolic* extracts was determined as described by [18].

Determination of Total Flavonoids Content

For the assessment of Flavonoids, colorimetric method introduced by [19] was performed to determine the amount of flavonoids.

Test for Tannins

Ferric chloride ($FeCl_3$) test is rather a test for phenolics in general. Tannis produce different colors with ferric chloride (either blue, blue black, or green to green-black) according to the type of tannis [20, 21].

Determination of Free Radical Scavenging Assay (DPPH)

The free radical scavenging effect of the plant extracts was assessed by the discoloration of a methanolic solution of DPPH according to [22].

Determination of hydroxyl radical scavenging assay (OH•)

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the hydroxyl radicals generated from the Fenton reaction in Fe^{+3}/Ascorbate/EDTA/H_2O_2 system. The scavenging capacity for hydroxyl radical was determined according to [23].

Determination of Total Antioxidant Capacity (TAC)

The determination of the antioxidative capacity is performed by the reaction of antioxidants in the samples with a defined amount of sulphoric acid, ammonium molybdate and sodium phosphate. The antioxidants in the sample eliminate a certain amount of the provided radicals. The residual was determined colorimetrically using *Ascorbic acid* as standard at wave length 695nm [24].

Determination of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay is based on the reducing power of antioxidants by the reduction of the ferric ions to the ferrous ions, which form a green colored according to the method described by [25, 26]. The FRAP reagent was freshly prepared and the absorbance was measured at 700 nm after 10 min incubation at room temperature, using rutin hydrate as standard.

Determination of Nitric Oxide (NO) Contents

Nitric oxide (NO) content was measured by the determination of total nitrate and nitrite concentrations in the samples according to [27].

Animal Ethics

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (USA). The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments according to National Organization for Drug Control and Research (NODCAR).

Isolation of Mitochondria and Microsome from Rat Liver for Lipid Peroxidation

Ten male albino rats weighing between 200-250g were used to separate liver mitochondria and microsomes (*In-vitro*). The rats were obtained from the animal house of NODCAR, Egypt. The animals were kept under standard laboratory conditions of light/dark cycle (12/12h) and room temperature.

Mitochondrial preparation was performed by using the method of [28], rats were killed by decapitation after fasting for 24h and their liver tissues were quickly removed. The liver tissues were cut into small pieces in 0.25 M sucrose buffer (pH 7.4) at 4ºC and then homogenized with nine volume of sucrose buffer using Teflon homogenizer (CAT R_{18}). The protein of the isolated mitochondria and microsome was measured by the method of [29].

Lipid Peroxidation Assay (Fe^{++}/Ascorbate)

Lipid peroxide formation was determined as thiobarbituric acid reactive substances according to the method of Fe^{++}/Ascorbate induce lipid peroxidation in mitochondria and microsomal fractions [30].

Preparation and Isolation of Lysosomal Fraction from Rat Liver

Male albino rats weighing 170-200g were injected with CCl_4 (100µl/100g rat) and after 24hrs rats were decapitated and prepared to separate the lysosomal fractions from the liver according to the method of [31].

Incubation of Lysosomes with Different Plant Extracts

In a tube a mixture of 1.0 ml of lysosomal fraction and 1.0 ml of each extract solution at concentration of (10.0 and 5.0 mg/ml), the total volume was completed to 3.0ml by the addition of the sucrose buffer solution (0.25M). In order to avoid lysosomal membrane rupture by sudden elevation of temperature, ice-cold lysosomal preparation was warmed to room temperature 2 minutes before incubation at 37ºC. The tubes were incubated in a shaking water bath at 37ºC for 60 & 120 minutes of incubation; tubes of each concentration were removed and centrifuged at 19.000 r.p.m. for 20 minutes. The resulting supernatant was subjected to enzyme assay to determine the activity of the released enzymes [32].

Enzyme Substrates

1- For Orthophosphoric monoester phosphohydrolases (acid phosphatase) [EC.3.1.3.2], the substrate was p-nitrophenyl phosphate (sodium salt) from Koch-Light-U.K.

2- For ß-galactosidase [EC.3.2.1.23], the substrate was p-nitrophenyl-ß-D-galactopyranoside from Sigma Chemical Company - U.S.A.

3- For N-Acetyl- ß-glucosaminidase [EC.3.2.1.30], the substrate was p-nitrophenyl-2-acetamido-2-deoxy- ß-D-glucopyranoside from Sigma Chemical Company -U.SA.

4- For ß-glucuronidase [EC.3.2.1.31], the substrate was phenolphthalein glucuronic acid (sodium salt) from Sigma Chemical Company -U.S.A.

Methods of Enzyme Assay

The activity of four lysosomal acid hydrolases has been measured according to the method described by [33] with slight modifications by [34]. The principle of this method is identical for the first three lysosomal enzymes (Acid phosphatase, ß-galactosidase and β-N-Acetyl-glucosaminidase) depending on the reaction of each of the three lysosomal acid hydrolases with the appropriate substrate liberating p-nitrophenol (yellow color) which can be measured colourimetrically at 420 nm, together with the corresponding standard. While the method used for measuring ß-glucuronidase depends on the reaction of this enzyme with the substrate phenolphthalein glucuronide

liberating phenolphthalein (pink color) that can be measured colourimetrically at 542 nm together with the corresponding standard. The lysosomal enzymatic activities expressed as mean ± S.D as nmole/ml/hr.

***In-vivo* study**

Chemicals and Drugs

All chemicals were obtained from Sigma Company; Santa Lewis, USA, they obtained in analytical and purified grade.

Animals

Experiments were performed using male albino rats (170-200 g) supported from the animal house of NODCAR, Egypt. Animal were kept in a room at a constant temperature 22±1◦C with 12h light-dark cycles and had free access to diet and tap water.

Experimental Deigns

Thirty-six male albino rats divided into six groups: 1st group served as normal control (CG) rat received distilled water (1ml/rat o.p). 2nd group served as herbal mixture extract rat received herbal cocktail group (HCG) from Mn, Sp, Hs and Ta with ratio 1:2:1:3 respectively (100 mg/kg b.wt/day o.p), along with the experiment. 3rd group served as positive group rat received acetylsalicylic acid (ASA) (500mg/kg b.wt/day o.p) for three successive days **[35].** 4th group rat received HCG along 2 weeks then induction with ASA (protective group) (PHCG). 5th group rat received ranitidine (27 mg/kg b.wt/day o.p) after induction with ASA (curative ranitidine group) (CuRG) **[36].** 6th group rat received HCG (27 mg/kg b.wt/day o.p) after induction with ASA (curative herbal cocktail group) (CuHCG). The experiment lasted for 30 days then slaughtered, then blood samples were collected with heparine RBCs, leucocytes and neutrophils analysis. Stomach were isolated and washed with cold sterile physiological saline, blotted between two damp filter papers and stored at -80 ºC for further biochemical analysis. Parts of the stomach were isolated in formalin and used for the histopathology.

Red blood cells (RBCs), white blood cells (WBC's)

Red blood cells (RBCs), leukocytes and neutrophils of leukocytes were counted according to [37].

Determination of MDA by HPLC

MDA standard was prepared by dissolving 25 µl 1,1,3,3 tetraethoxypropane (TEP) in 100 ml of water to give a 1 mM stock solution. Working standard was prepared by hydrolysis of 1 ml TEP stock solution in 50 ml 1% sulfuric acid and incubation for 2 h at room temperature. The resulting MDA standard of 20 nmol/ml was further diluted with 1% sulfuric acid to yield the final concentration of 1.25 nmol/ml to get the standard for the estimation of total MDA [38].

The samples were analyzed on an Agilent HP 1100 series HPLC apparatus (USA), the analytical column Supelcosil C18 (5 µm particle and 80 A^o pore size) (250 x 4.6 ID). Mobile phase consists of 30 mmol KH_2PO_4 and methanol (65%-35%, H_3PO_4 by pH 4), and the mobile phase at a 1.5 ml/ min flow rate, wavelength 250 nm according to the method of [39].

Determination of GSSG and GSH by HPLC

The thiols compounds of oxidized and reduced glutathione were detected by HPLC using the method of [40]. Glutathione (oxidized and reduced) reference standard purchased from Sigma Chemical Co. Dissolved in 75% methanol in stock 1mg/ml and diluted before application to HPLC.

The HPLC system of Agilent consisted of quaternary pump, a column oven, Rheodine injector and 20µl loop, UV variable wavelength detector. The report and chromatogram taken from Chemstation program purchased from Agilent. Synerji RP Max column 3.9 at wavelength 210 nm with flow rate 2ml/min was used. Pot. Phosphate buffer - acetonitrile at pH 2.7 was used as an isocratic mobile phase.

Determination of Nitrites and Nitrates by HPLC

Nitrites and nitrate was determined according to the method of [41] by HPLC. Sodium nitrite and sodium nitrate used for the reference standard preparation with stock concentration 1mg/ml. A standard mixture of nitrite and nitrate was used to determine the retention times and separation of the peaks. Nitrite and nitrate concentrations were equal in the mixture solution.

The samples were analyzed on an Agilent HP 1100 series HPLC apparatus (USA). The analytical column was anion exchange PRP-X100 Hamilton, 150 x 4.1 mm, 10 µm. The mobile phase was a mixture of 0.1 M NaCl - methanol, at a volume ratio 45:55. The flow rate of 2 mL/min, wavelength adjusted to 230 nm.

Determination of Tissue PGE2, TNF- *α*, IL-6 and MPO (pg/ g tissue)

The PGE2, TNF-α, IL-6 and myeloperoxidase (MPO) levels were measured using an enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions. All of the samples and standards were assayed in duplicate, as suggested by the manufacturer (Rat ELISA KIT KOMA BIOTECH INC, Korea) [42].

Determination of Total Genomic DNA Extraction and Apoptosis Detection

DNA extraction and detection of apoptosis (DNA fragmentation assay) were done according to "Salting out extraction method" of [43] with some modifications of [44].

Agarose Gel Electrophoresis

Gel electrophoresis is a method for separation and analysis of macromolecules and their fragments, based on their size and charge. 1% agarose gel was prepared according to the method of [45].

Statistical analysis

Statistical analysis of the obtained data was performed using the general linear model (GLM) produced by Statistical Analysis Systems Institute (SAS, 1989). Significant differences among means were evaluated using Duncann test Multiple Range Test at $p<0.05$. The following linear model was applied:

$Yij = \mu + \alpha i + \xi ij$

Yij= Observation measured

µ = Overall mean

αi= Effect of treatment .

ξIj = Experimental error assumed to be randomly distributed ($\sigma 2 = 0$).

% Change = Mean of treated / Mean of control × 100

3. Results

***In-vitro* study**

The results obtained in Table (1) appeared the presence of quercetin in high amount in TA by (1.698 µg/g rowder) as anti-ulcer followed by MN (1.547 µg/g powder); SP appeared the presence of Gallic acid in high amount by (4.118 µg/g powder) as anti-ulcer followed HS (2.112 µg/g powder); MN appeared the presence of caffeic acid in high amount by (1.337 µg/g powder) and Ferulic acid by (1.206 µg/g powder) as antioxidant and less amount of salycilic acid which is less used, and HS appeared the presence of coumaric acid in high amount by (0.428 µg/g powder) as ant-ulcer and caffeic acid by (0.890 µg/g powder) in high amount as antioxidant.

Table (2) showed that, each extract is reached in specific component which is essential for in vivo study. Individual extract of SP is a highly reached with gallic tannis and catechol tannis, MN, HS then TA respectively in comparing with rutin hydrate reference antioxidant standard. On the other hand HS is a most potent for total flavonoids and phenolic/g sample then SP, TA and MN respectively in comparing with rutin hydrate reference antioxidant standard.

Table (3) showed three concentrations of each extract were used "50, 100, and 200 µg/ml". The antioxidant activity of HS then TA, the lowest activity was observed for MN as compared to rutin hydrate at the high concentration of (200µg/ml), this can be attributed to the higher phenolic content.

Table (4) revealed that, the percentage of ·OH radical scavenging inhibition of rutin hydrate and comparison with each plant extract at the concentrations of 50, 250, 500 and 1000 µg/ml. The percentage of ·OH scavenging effect at 1000µg/ml was found to be higher in MN and TA as compared to Rutin hydrate, the percentage of inhibition for other extracts was decreased in the order of MN and TA, HS then SP respectively.

Table (5) showed the effect of the four herbal extracts as compared to rutin hydrate and ascorbic acid (as standards), respectively on TAC and FRAP, HS has higher TAC by (4.33±0.33, 38.24%), while TA has the greatest of FRAP by (1.43±0.02 µg/g dry plant, 65.71%).

Results in Table (6) show the effect of the four ethanolic plant extracts on non-enzymatic peroxidation (Fe^{++}/Ascorbate model system induced lipid peroxidation), it appears that the inhibition of lipid peroxidation was

increased by concentrations from 0.5, and 1.0 mg/ml in mitochondrial and microsomal fractions for all extracts. The percentage inhibition of lipid peroxidation was varied according to the cellular particles different as compared to rutin hydrate as standard. The microsomal fraction appeared to be high % of inhibition than the mitochondrial fraction. The higher inhibition were for TA and SP followed by HS then MN in microsomal fraction, while the higher inhibition in mitochondrial fraction appeared for SP followed by TA and HS then MN.

Tables (7&8) revealed the effect of the four ethanolic plant extracts under investigation on the four lysosomal enzymatic activities in CCl_4- induced hepatotoxicity groups as compared to positive control group. This study was performed to investigate the effect of the four ethanolic extracts of *Trigonella anguina*, *Savignya parviflora*, *Heterocarum subsessille*, MN by two concentrations (5.0 and 10.0 mg/ml) on the four lysosomal enzymatic activities ACP; β-GAL; β-NAG, and β-GLU in rat liver after 60 and 120 minutes of incubation. An significant decrease ($p<0.05$) values in the activities of ACP; β-GAL; β- NAG, and β-GLU were observed after 120 more than after 60 minutes of incubation, these percentage values were dependent on the concentration and each enzyme.

Tables 1: Concentration of phenolic compounds of MN, SP; HS, and TA ethanolic leaves extracts by HPLC

Components	Phenolic compounds concentration µg/g powder			
	MN	**SP**	**HS**	**TA**
Ratios	1	1	1	3
Caffeic acid	1.337	0.565	0.89	0.699
Cinnamic acid	0.833	ND	ND	0.647
Coumaric acid	0.241	0.178	0.428	0.184
Ferulic acid	1.206	0.438	0.673	ND
Gallic acid	1.586	4.118	2.112	1.936
Salycilic acid	0.393	0.951	ND	0.624
Quercetin	1.547	0.426	0.751	1.698

Table 2: Gallic tannis, Catechol tannis, Total phenolic and total flavonoid contents of four ethanolic plant extracts as compared to rutin hydrate

Extract	Parameters			
	Gallic tannis	**Catechol tannis**	**% of total flavonoids**	**% of total phenolic**
Rutin hydrate	----------	----------	100%	100%
HS	+ve	+ ve	25.76%	45.56%
TA	+ve	+ ve	25.55%	36.58%
SP	+++ve	++ ve	26.58%	42.19%
MN	++ve	+ ve	15.17%	25.96%

The results are given as mean of six measurements of percentage and expressed as mean ±S.D. (QE) [a]: Querestin equivalents and (GAE) [b]: Gallic acid Equivalents.

Table 3: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical scavenging activity for ethanolic extracts as compared to rutin as a reference antioxidant standard

Extractions	Yields %	% of inhibition for DDPH		
		50 (µg/ml)	**100 (µg/ml)**	**200(µg/ml)**
Rutin hydrate	---------	80.0%	85.0%	90.0%
HS	14.25%	26.4%	38.4%	58.2%
TA	12.84%	27.3%	31.0%	56.0%
SP	9.51%	28.0%	29.4%	55.0%
MN	17.51%	23.4%	32.0%	45.0%

N. B.: (%): It was expressed as the absorbance of sample versus the absorbance of control, the highest % I for high antioxidant power as compared against *Rutin hydrate*.

Table 4: Hydroxyl (OH•) Radical-Scavenging Activity of four ethanolic plant extracts as compared to rutin hydrate

Extractions	*Hydroxyl radical (OH•) scavenging activity "I %"*			
	50 (µg/ml)	***250 (µg/ml)***	***500 (µg/ml)***	***1000 (µg/ml)***
Rutin hydrate	61.17 %	58.44 %	50.0 %	***49.76***
HS	74.94 %	62.10 %	54.59 %	47.18 %
TA	68.51 %	60.36 %	59.48 %	***52.32 %***

SP	87.91 %	61.61 %	57.48 %	45.15 %
MN	75.31 %	55.56 %	52.93 %	***52.57 %***

N. B.: (%): It was expressed as the absorbance of sample versus the absorbance of control, the highest % of inhibition for high antioxidant power according to high dose as compared to *Rutin*.

Table 5: Total Antioxidant Capacity (TAC) and Ferric Reducing Antioxidant Power (FRAP) of ethanolic extracts in comparing to rutin hydrate for FRAP and Ascorbic acid for TAC

Extractions	**[a] TAC U/min**	**[b] FRAP (µg/g powder)**
Rutin hydrate	----------	2.18 ±0.07
Ascorbic acid	11.33 ± 0.331	----------
HS	4.33 ± 0.182	1.15 ± 0.032
TA	2.45 ± 0.112	1.43 ± 0.021
SP	0.54 ±0.044	0.98 ± 0.001
MN	0.44 ± 0.067	1.12 ±0.012

[a,b] Values are means ± S.D. of six measurements, Data are significantly different ($p < 0.05$).

[b] Higher absorbance indicated higher reducing power.

Table 6: Fe^{++}/Ascorbate model system induced lipid peroxidation *In-vitro* in rat liver mitochondria and microsomal fractions of four ethanolic extracts in comparable against rutin hydrate (standard)

Extractions	**Fe^{++}/ Ascorbate in rat liver (% of I)**			
	Mitochondrial fraction		**Microsomal fraction**	
	0.5 (mg/ml)	1.0 (mg/ml)	0.5 (mg/ml)	1.0 (mg/ml)
Rutin hydrate	77.7	95.1	60.6	98.0
HS	67.1	70.4	65.7	89.6
TA	66.8	70.5	63.5	98.0
SP	65.3	93.7	95.9	97.9
MN	33.7	39.7	59.9	81.4

N.B: the highest % of inhibition for high antioxidant activity as compared to rutin hydrate.

Table 7: Anti-inflammatory Effect of plants under investigations by two doses (5.0 and 10.0 mg/ml) after injection of CCl_4 (100µl/100g rat) in comparable against *Rutin* as standard *In-vitro* on the four marker lysosomal enzymatic activities "ACP, β-GAL, and β-NAG in rat liver lysosomes after 60 minutes of incubation.

Groups	**Doses**	**The lysosomal enzymatic activities expressed as mean ± S.D by nmole/ml/hr**			
		ACP	**β-GAL**	**β-NAG**	**β-GLU**
Control positive	**CCl_4**	2880.0±0.033	2120.44±0.034	3620.0±0.019	1058.0 ± 0.004
	Relative % change	100%	100%	100%	100%
Rutin + CCl_4	**L.D. 5.0 mg/ml**	2110.88 ±0.021	1990.0±0.014	3231.0±0.018	338.7 ± 0.022
		73.3%*	93.8%*	89.3 %*	32.01%**
	H.D. 10.0 mg/ml	2331.0±0.018	2011±0.013	3310.0±0.014	374.5 ± 0.018
		80.9 %*	94.8 %*	91.4 %*	35.40%**
TA + CCl_4	**L.D. 5.0 mg/ml**	1850.92±0.022	1830.10±0.017	3420.72±0.007	648.6 ± 0.018
		64.27 %**	86.3 %*	94.5%*	61.30%**
	H.D. 10.0 mg/ml	2470.89±0.033	1950.31±0.028	3520.77±0.024	734.7 ± 0.022
		85.8 %*	92.0 %*	97.26%*	69.44%**
SP + CCl_4	**L.D. 5.0 mg/ml**	1570.75±0.022	1350.21±0.017	3350.21±0.012	717.9 ± 0.016
		54.5 %**	63.7 %**	92.5%*	67.85%**
	H.D. 10.0 mg/ml	2510.64±0.024	1920.49±0.014	3600.44±0.020	809.7 ± 0.025
		87.2 %*	90.6 %*	99.46%*	76.53%**
MN + CCl_4	**L.D. 5.0 mg/ml**	1700.89±0.010	1800.28±0.013	3540.93±0.006	573.6± 0.018
		59.1 %**	84.9 % *	97.8%*	54.22%**
	H.D. 10.0 mg/ml	2140.08±0.036	2080.45±0.015	3600.08±0.008	592.4 ± 0.017
		74.3 %*	98.11 %*	99.50%*	56.0%**
HS + CCl_4	**L.D. 5.0 mg/ml**	1304.38 ± 0.032	1456.63±0.016	2984.24±0.020	747.9 ± 0.028
		45.3%**	68.7%**	82.4%*	70.69%**

	H.D. 10.0 mg/ml	1560.31 ±0.020 54.2%**	1784.47±0.019 84.2%*	3296.68±0.017 91.1%*	755.4 ± 0.017 71.40%**

*Significant from Control group at P < 0.05, **: Highly significant at P< 0.05.

Table 8: Anti-inflammatory Effect of plants under investigations by two doses (5.0 and 10.0 mg/ml) after injection of CCl_4 (100µl/100g rat) in comparable against *Rutin* as standard *In-vitro* on the four marker lysosomal enzymatic activities "ACP, β-GAL, and β-NAG in rat liver lysosomes after 120 minutes of incubation

Groups	**Doses**	**The lysosomal enzymatic activities expressed as mean ± S.D by**			
		ACP	**β-GAL**	**β-NAG**	**β-GLU**
Control	**CCl_4**	1665 ± 43.9	2880.2 ± 79.7	3760.2 ± 101.5	1993 ± 53.2
Rutin + CCl_4	**L.D. 5.0 mg/ml**	1576.9 ± 43.2	1824.6 ± 49.7	2896.6 ± 82.6	593.8 ± 16.5
	H.D. 10.0	1601.7 ± 43.6	2040.3 ± 54.1	2328.4 ± 63.3	469.7 ± 12.8
TA + CCl_4	**L.D. 5.0 mg/ml**	1064.9 ± 29.6	1881 ± 51.2	2040.4 ± 58.2	342.7 ± 9.3
	H.D. 10.0	1393.1 ± 38.5	1896.6 ± 50.5	2760.6 ± 78.2	538.8 ± 14.3
SP + CCl_4	**L.D. 5.0 mg/ml**	984.6 ± 27.6	984.9 ± 26.3	2056.5 ± 55.3	407.7 ± 10.9
	H.D. 10.0	1112.7 ± 30.3	1640.9 ± 44.4	3180.6 ± 87.4	450.8 ± 12.4
MN + CCl_4	**L.D. 5.0 mg/ml**	1040.3 ± 28.6	1008.8 ± 27	2568.4 ± 73	210.1 ± 5.7
	H.D. 10.0	1168.1 ± 31.3	1072.7 ± 29.1	3036.6 ± 85.5	261.3 ± 7
HS + CCl_4	**L.D. 5.0 mg/ml**	912.2 ± 24.7	1176.5 ± 31.1	3676.5 ± 103.5	219.6 ± 6
	H.D. 10.0	1001.2 ± 27	1528.2 ± 41.9	3862.8 ± 110	245.5 ± 6.9

***In-vivo* study**

Table (9) showed that, prophylactic and curative effects of wiled herbal cocktail extract on hematological parameters (RBCs, Leucocytes and neutrophils) in blood sampling of rats treated with acetylsalicylic acid (ASA).

Table (10) revealed the prophylactic and curative effects of wiled herbal cocktail extract on oxidative stress markers (MDA, GSH, GSSG and NO) in stomach tissue of rats treated with acetylsalicylic acid (ASA), data show decrease in GSH contents but increased in MDA concentration GSSG and NO contents after induction with ASA in the third group, while this results was altered in the protective and curative groups after pretreated or treated with HCG, also in protective than in curative groups.

Table (11) showed Prophylactic and curative effects of wiled herbal cocktail extract on inflammation parameters (TNF-α, IL-6 and MPO) in stomach tissue of rats treated with acetylsalicylic acid (ASA). Data represent increase in TNF-α, IL-6 and MPO but decrease in PGE-2, while after pretreated or treated with the HCG the effects were altered. It shows decrease in inflammation, it may be due to the active compounds in HCG.

Table 9: Prophylactic and curative effects of wiled herbal cocktail extract on hematological parameters (RBCs, Leucocytes and neutrophils) in blood sampling of rats treated with acetylsalicylic acid (ASA).

Groups	**Parameters**		
	RBCs x 10^6/mm^3	**Leukocytes**	**Neutrophils %**
CG (Negative group)	5.18 ± 0.064	5.51 ± 0.120	32.68 ± 0.885
HCG	5.19 ± 0.050	5.55 ± 0.118	30.94 ± 0.855
ASA (Positive group)	4.05 ± 0.041a	6.33 ± 0.142a	22.36 ± 0.622a
PHCG	4.23 ± 0.044a	6.27 ± 0.095a	26.54 ± 0.681a
CuRG	4.33 ± 0.042ab	6.24 ± 0.161a	27.95 ± 0.736ab
CuHCG	4.25 ± 0.055a	5.96 ± 0.108b	29.18 ± 0.775ab

Data are expressed as Mean ± S.E.M for 6 rats /group. a significant difference from CG group at the same column with one way ANOVA at P < 0.05. b significant difference from ASA at the same column with one way ANOVA at P < 0.05. CG: control group; HCG: herbal cocktail extract group; ASA: acetylsalicylic acid group; PHCG: protective herbal cocktail group; RG: curative ranitidine group and CuHCG: curative herbal cocktail group.

Table 10: Prophylactic and curative effects of wiled herbal cocktail extract on oxidative stress markers (MDA, GSH, GSSG and NO) in stomach tissue of rats treated with acetylsalicylic acid (ASA).

Groups	Parameters			
	MDA	**GSH**	**GSSG**	**NO**
	ng/g tissue	**µg/g tissue**	**µg/g tissue**	**µg/g tissue**
CG (Negative group)	39.01 ± 1.194	3.07 ± 0.203	0.59 ± 0.064	0.46 ± 0.038
HCG	39.85 ± 0.956	3.21 ± 0.297	0.63 ± 0.072	0.48 ± 0.011
ASA (Positive group)	60.62 ± 2.372a	1.91 ± 0.188a	1.14 ± 0.108a	1.01 ± 0.046a
PHCG	52.06 ± 0.680ab	2.29 ± 0.232ab	0.85 ± 0.080b	0.61 ± 0.035ab
CuRG	50.43 ± 1.814ab	2.12 ± 0.203ab	0.78 ± 0.059b	0.54 ± 0.041ab
CuHCG	44.56 ± 2.214ab	2.66 ± 0.229ab	0.62 ± 0.087ab	0.51 ± 0.047b

Data are expressed as Mean ± S.E.M for 6 rats /group. a significant difference from CG group at the same column with one way ANOVA at $P < 0.05$. b significant difference from ASA at the same column with one way ANOVA at $P < 0.05$. CG: control group; HCG: herbal cocktail extract group; ASA: acetylsalicylic acid group; PHCG: protective herbal cocktail group; RG: curative ranitidine group and CuHCG: curative herbal cocktail group.

Table 11: Prophylactic and curative effects of wiled herbal cocktail extract on inflammation parameters (PGE-2, TNF-α, IL-6 and MPO) in stomach tissue of rats treated with acetylsalicylic acid (ASA).

Groups	Parameters			
	PGE-2	**TNF-α**	**IL-6**	**MPO**
	pg/mg protein	**pg/g tissue**	**pg/g tissue**	**pg/g tissue**
CG (Negative group)	46.32 ± 1.51	64.04 ± 1.03	24.77 ± 1.27	9.94 ± 0.80
HCG	43.84 ± 1.38	66.55 ± 1.50	25.47 ± 0.96	9.46 ± 0.75
ASA (Positive group)	22.58 ± 0.69a	108.15 ± 2.32a	39.34 ± 2.13a	27.70 ± 1.01a
PHCG	25.62 ± 0.81ab	97.21 ± 2.04ab	35.76 ± 1.20ab	13.73 ± 1.17ab
CuRG	30.21 ± 0.96ab	81.16 ± 1.20ab	32.15 ± 1.69ab	12.66 ± 0.79ab
CuHCG	27.89 ± 0.77a	89.84 ± 2.20ab	29.46 ± 2.07ab	9.95 ± 0.72ab

Data are expressed as Mean ± S.E.M for 6 rats /group. a significant difference from CG group at the same column with one way ANOVA at $P < 0.05$. b significant difference from ASA at the same column with one way ANOVA at $P < 0.05$. CG: control group; HCG: herbal cocktail extract group; ASA: acetylsalicylic acid group; PHCG: protective herbal cocktail group; RG: curative ranitidine group and CuHCG: curative herbal cocktail group.

4. Discussion

In vitro study

The most common methods to pheolic compound and determine antioxidant activity in a herbal extraction was HPLC, DPPH radical assay; OH•; FRAP; TP; TF and NO. The main phenolic compoundswhich have biological and pharmacological activity are gallic acid, caffeic acid, and quercetin, Rutin contains quercetin as flavonoids [46]. The close correlation between antioxidant activity and phenolic content obtained from various natural sources has been demonstrated by [47]. Rutin hydrate exhibited a highly scavenging efficiency toward DPPH radicals which is in agreement with [48]. The ·OH scavenging activity of rutin hydrate and other extracts were concentration dependent, these results are similar to those reported by [49]. Ferric Reducing antioxidant power and total phenolic contents showed the different correlations depending on plant species, but it might not always correlate with the amount of phenolics [50]. The reducing capacity of a compound serves as a significant indicator of its potential antioxidant activity. To measure reductive ability, it was investigated the $Fe^{+3} \rightarrow Fe^{+2}$ transformation in the presence of Rutin samples using the method of [51], the reductive capacity of Rutin (as indicated by the absorbance at 700nm) compared to other extracts. Higher absorbance of the reaction mixture indicated greater reducing power; the reducing power was in a concentration-dependent manner. These results suggest that rutin hydrate and other extracts have a remarkable potency to donate electron to reactive free radicals, converting them into more stable non-reactive species [48]. These results indicated that the percentage inhibition of lipid peroxidation was cellular

fractions and concentration-dependent, and these findings are in agreement with the results of [48]. It has been suggested that oxygen free radicals generated during cell toxicity or damaging effect and may also be responsible for the damage through the release of lysosomal enzymes [52]. The increased release of acid hydrolases from lysosomes alters the metabolism of glycoproteins and glycosaminoglycans which are involved in lysosomal structure, these enzymes are involved in the destruction of structural macromolecules in connective tissue due to the enzymatic destruction of proteoglycans [53]. These release of enzymes also stimulate the inflammatory mediators like oxygen radicals, prostaglandins etc. which stimulate tissue disruption [52]. The increase in lysosomal enzymes produces a reduction in membrane integrity and the leakage of the enzymes from the enclosed sacs lead to intracellular dysfunction and disruption of potential substrates and organelles such as mitochondria [54]. This behavior may be due to the hydroxyl radical is very reactive as it combines with almost all molecules found in living cells. Proteins; lipids; carbohydrates, and DNA in living cells represent oxidizable substrates. The secondary events include changes in membrane structure permeability and fluidity, lysosomal destabilization and stimulation of apoptosis. Lipid peroxidation finally leads to loss of membrane function and integrity leading to cell necrosis and death [23]. Hydroxyl radicals can also react with bases in the DNA and cause mutations. Phenolics, vitamin E and some drugs such as non-steroidal anti-inflammatory can stabilize the membranes by decreasing their permeability and they also have an ability to bind free fatty acids [55].

***In vivo* study**

Acetylsalicylic acid (ASA) is a potent analgesic and anti-inflammatory that is used for the treatment of rheumatoid arthritis and prevention of cardiovascular thrombotic diseases. The mode of action of ASA mainly started with inhibiting synthesis of prostaglandins by COX1 and COX2 pathway. Revealed data showed hematological disorders as a result of ASA ingestion which associated with anemia accompanied by leucocytosis, are some of the most frequently reported adverse effects of ASA [56]. These results are in agreement with the published reports in rats [57]. ASA altered the iron uptake from the GI tract and increase blood loss due to peptic ulcer or erosion which cause iron deficiency or anemia [58]. Also there is increase in leucocutes following hemorrhage which is consisted with result of [59]. This increase may be due to the hemopoitic activity as a result of the hemolysis of RBCs. Regarding the neutrophils, there was a relative decrease in this parameter which might have been occurred due to stress leading to demargination in circulation. There was a relative decrease of neutrophil in blood stream and increase of neutrophil infiltration into ulcerated gastric tissue with release of ROS and micro vascular injures which is considered as the prime event in gastric mucosal damage [60].

In addition ASA produce peptic ulcer with significantly increase in oxidative stress markers and inflammatory mediators as compared with control. The oxidative stress resulting from the increase production of oxygen derived free radicals [61]. The increase in MDA, GSSG, NO and decrease of GSH may be due to exhausted of GSH and the other endogens antioxidant defense mechanism attributed to ROS generation during mucosal damage. Inflammatory mediators and MPO concentration is a biochemical indicator of the mucosal neutrophil infiltration, increases with the development of gastric mucosal injury. Arachidonic acid released from phospholipids' membrane which is a chemical signal in the inflammation process. One of them is the prostagladins synthesis through COX and the other one is the leukotrienes synthesis through 5-LOX. LTB4, a by-product of 5-LOX and cPLA2, is the major chemotactic factor for leukocytes which development of peptic ulcers [62]. In the present study, the obtained data were in parallel line with these previous data, ASA significantly reduced gastric mucosal PGE2 level in comparing with control group this reduction may be attributed to elevation of COX-2 activity .

Since gastric ulcer does not necessarily involve high gastric acidity, many gastric ulcer drugs do not act via inhibition of acid secretion, rather their ameliorative effects is through the inhibition of H. pylori, the causative agent of the disease. Although our extract, based on the findings of this study did not inhibit acid secretion, it could still be useful as an anti-gastric ulcer agent because of its proven effect on H. pylori **[63].** Due to the reported side effects of available antiulcer drugs, we must have focused and shifted towards natural products as the new sources of antiulcer agents. The role of cinnamic and coumaric acid after induction of peptic ulcer may increase mucus and bicarbonate secretion and inhibition of acid secretion. With the increasingly growing interest in natural medicine,

various plants have been studied based on the traditional knowledge of their pharmacological properties and confirmed to be useful in treating and managing ulcer [64]. One's due of synergetic herbal cocktail in peptic ulcer to ameliorate the imbalance between aggressive factors and the maintenance of the mucosal integrity was shown through the stimulation of endogenous defense mechanism. The enhancement of defense mechanism via herbal mixture may be due to caffeic, coumarins and ferulic acid which inhibit H+, K+-ATPase and histaminergic pathway of the gastric acid secretory mechanism [65]. Results of these studies indicate that the antiallergenic effects of herbal cocktail may be due to its antioxidant and free radical scavenging properties. The structural characteristics by enrichment with phenolic compound which make herbal mixture a potent inhibitor of lipid peroxidation by decreasing MDA, NO and GSSG. The most antioxidant phenolic compound in herbal mixture is gallic acid which increases enzymatic and nonenzymatic (GSH) antioxidant defense mechanism. Gallic acid acts as a potent agent to scavenging ROS, superoxide anions and hydroxyl radicals [66]. In another hand, the decreasing of MDA, GSSG and NO were observed in curative group with herbal mixture more than protective group and ranitidine group. Decrease of oxidative stress markers by herbal cocktail treated group may be due to caffeic acid concentration which in turn decrease LPO and MDA and increase GSH.

The ability of herbal cocktail to ameliorate RBCs level may be due to its role in ulcer healing which prevent haemorigic bleeding and hematopoietic activity. In addition, Flavonoid QUS and FA present in herbal cocktail has been reported to protect RBCs from haemolysis by preventing the generation of free radicals and maintaining the membrane integrity of the RBCs thus preventing the haemolysis and protect its for damage [67].

Synergetic effect for selected ratio of herbal mixture may be due to the enrichment of MN with caffeic acid and ferulic acid, SP with Gallic acid, HS with coumaric acid and TA with qurecetin. Generally, increase of endogenous antioxidant defense mechanism (GSH), decrease oxidative stress markers and decrease inflammatory mediators such as PGE2, IL-1, TNF- α and MPO may be due to blocking inflammatory pathways downstream of cytokine release and subsequent by reducing macrophage production of proinflammatory factors [68]. Another constituent of herbal mixture is gallic acid which acts as antisecretory activity, enhancement of the mucosal barrier through the increase production of PGE2. MPO is an enzymatic catalyst stored in macrophages and hallmark for neutrophil infiltration during stressful condition. The release of proinflammatory mediators caused activation of neutrophils, which elevates the MPO level. Presented data are consisted with [69] who found that the FA inhibit the production of IL-1β, and TNF-α via the promotion of NF-κB. In addition enrichment of mixture with QUE showed anti-inflammatory properties via inhibition TNF-α and IL-1β. The effect of QUE on the pro-inflammatory cytokines occurred through blocking of ERK signaling pathway and regulation of NF-kB activation, indicating a protective role of QUE against inflammation generated by oxidative stress [70]. Worthily [71] showed that the gallic acid is a most potent phytoextract acts as anti-inflammatory which inhibits inflammatory mediators in some animal models of inflammation.

Biochemical results were confirmed with histopathological examination on the gastric mucosa revealed that ASA administration induced a peptic mucosal ulceration and treated groups nearly back to normal.

As shown in Figures (2) genomic DNA fragmentation of stomach tissues on 1% agarose gel electrophoresis, the DNA from control group was found to be intact (undamaged) as seen in lane (1), also in group was taken plant extract only (HCG) show the same result of control group as seen in lane (4). The DNA from other groups indicated the presence of damage. The administration of ulcer with treatment (lane 5,6) was the best treatment which induced moderated DNA damage associated with apoptotic pattern compared to ulcer induced group without treatment (lane 2) which induced sever necrotic pattern observed as a late stage of apoptosis of DNA damage. Group treated with reference drug (lane 3) showed moderated necrotic damage in DNA when compared to the induction of ulcer model. DNA fragmentation; was to reflect upon their significance in apoptosis or necrosis induced by oxidative stress under GSH depletion [72]. High intracellular GSH levels have been related to apoptosis resistance [73], while GSH depletion has been shown to induce apoptosis [74].

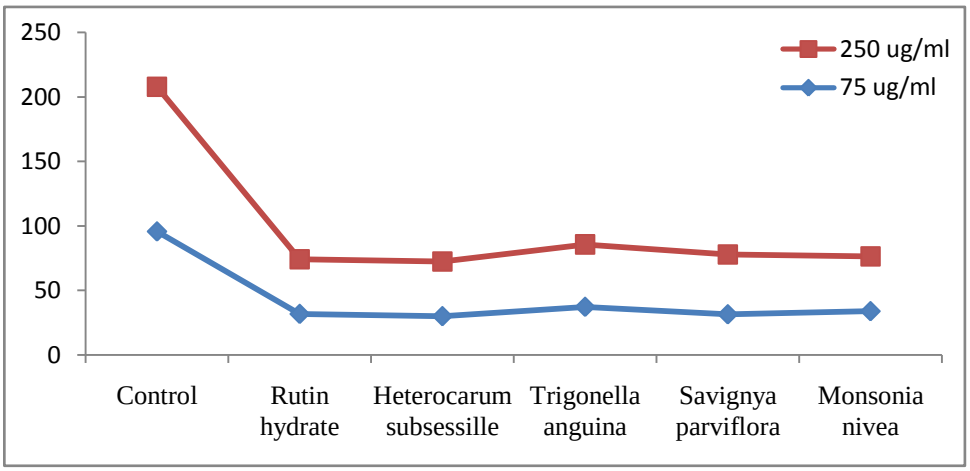
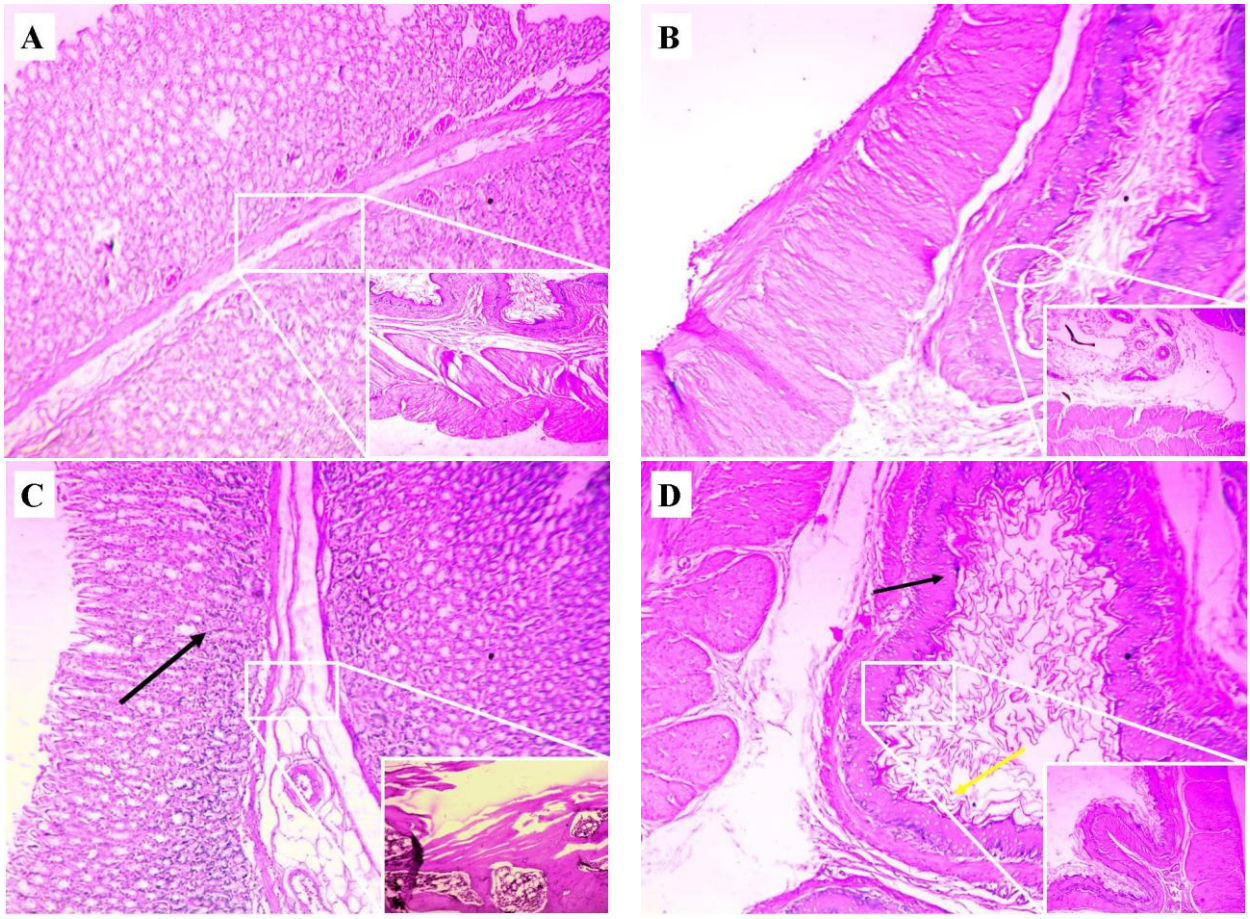


Figure 1: The effect of the four herbal extracts as compared to rutin hydrate (as standard) on nitrite and nitrate contents, Heterocarum subsessille and SP showed the most inhibition followed by MN then TA according to the amount of phenolic compounds against rutin hydrate as compared to the control group (100% toxicity).



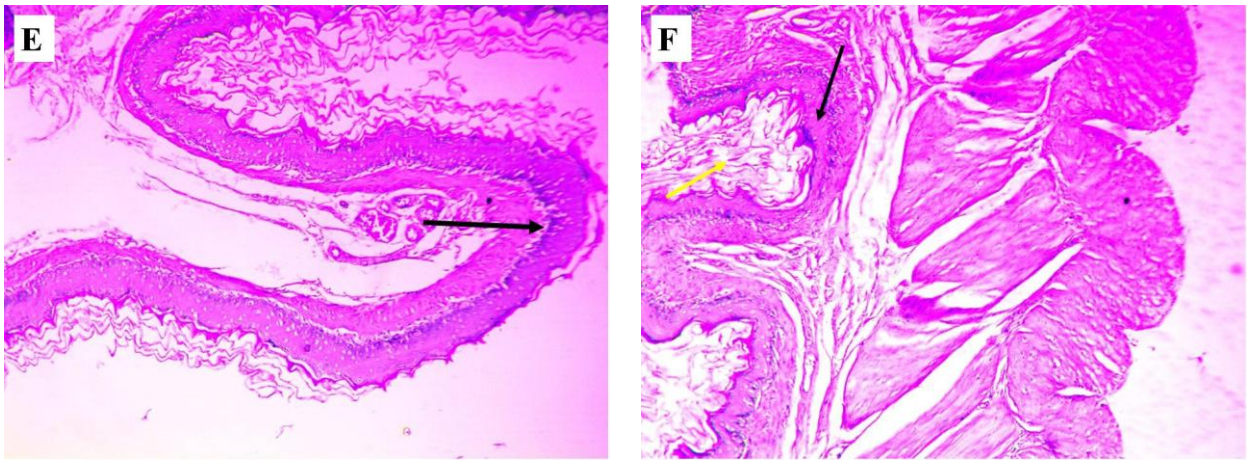


Figure 2: Photomicrograph of stomach for different rat: Light micrograph of rat stomach (A) control shows normal stomach and mal gastric mucosa. Light micrograph of rat stomach (B) ***HCG*** *shows normal stomach and mal gastric mucosa. Photomicrograph of stomach rat in group ASA inducted with acetylsalicylic acid showing inflammatory necrosis, massive leukocyte cells inflatration in lamina properia and congestion in blood vessels in sub mucosa (H and E * 40). Photomicrograph of stomach rat in group* ***PHCG*** *inducted with ASA and protected with herbal cocktail 2 weeks before induction showing mild inflammation in sub mucosal cells and increase furrows of mucosal cells (H and E * 40). Photomicrograph of stomach rat in group* ***CuRG*** *inducted with ASA and treated with ranitidine 2 weeks after induction showing mild congestion in sub mucosal cells and mild furrows of mucosal cells (H and E * 40). Photomicrograph of stomach rat in group* ***CuHCG*** *inducted with ASA and treated with herbal cocktail 2 weeks after induction showing mild congestion in sub mucosal cells and mild edema of mucosal cells (H and E * 40).*

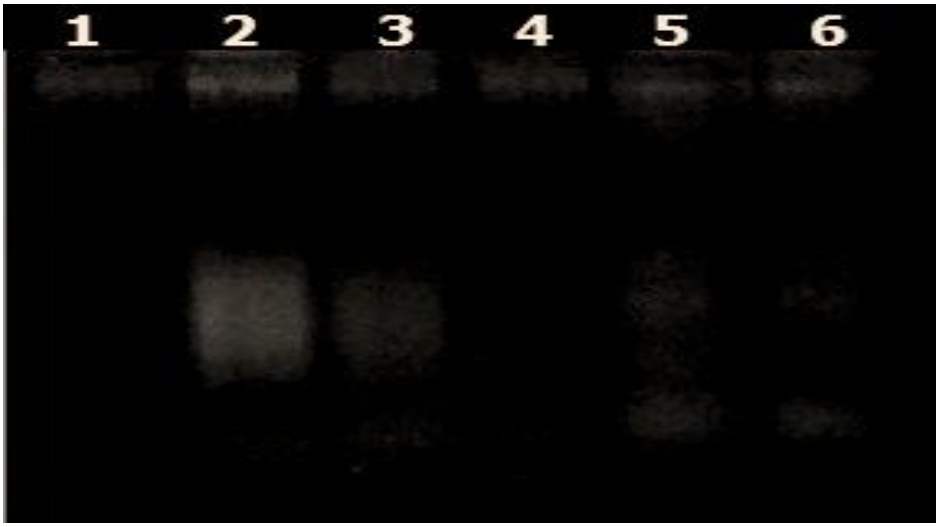


Figure 3: Genomic DNA fragmentation of Stomach tissues on 1% agarose gel electrophoresis. Lane 1: distilled water (untreated group), Lane 2 treated with ASA (positive group), Lane 3 Treated with ASA then Ranitidine (CuRG) (curative ranitidine group), Lane 4 treated with herbal cocktail group (HCG), Lane 5 Treated with HCG then ASA (PHCG)(protective ranitidine group), Lane 6 Treated with ASA then HCG (CuHCG) (curative herbal cocktail group).

5. Conclusion

Obtained data concluded that individual wield herbal extract have *In-vitro* antioxidant, anti-inflammatory properties due to reached phenolic, flavonoides and tannins content. *In-vivo* study concluded that the curative effect is most

potent treatment of peptic ulcer more than prophylactic or ranitidine treatment. Mixture of extracts enriches with qurecetin ameliorate ulcer via cytoprotective action, Gallic acid via antisecretory activity, enhancement of the mucosal barrier through the increase production of prostaglandin, Cinnamic and coumaric acid via increased mucus and bicarbonate secretion and inhibition of acid secretion, Caffeic and Ferulic acid via inhibition of H^+, K^+-ATPase and histaminergic pathway of the gastric acid secretory mechanism.

List of abbreviations

Monsonia nivea (MN); *Savignya parviflora* (SP); *Heterocaryum subsessile* (HS); *Trigonella anguina* (TA); 1,1,3,3 tetraethoxypropane (TEP); 1,1-Diphenyl-2-picrylhydrazyl (DPPH); acetylsalicylic acid (ASA); Acid phosphatase (ACP); Analysis of variance (ANOVA); antioxidant capacity (TAC); enzyme-linked immunosorbent assay (ELISA); Ferric chloride ($FeCl_3$); ferric reducing antioxidant power (FRAP); general linear model (GLM); myeloperoxidase (MPO); National organization of drug control and research (NODCAR); nitric oxide (NO); Red blood cells (RBCs); Statistical Analysis Systems (SAS); white blood cells (WBC's); β- glucuronidase (GLU); β-galactosidase (β-GAL); β-N-acetylglucosaminidase (β-NAG).

Acknowledgment

The authors are thankful to Dr. Rofanda M. Bakeer, Pathology Department, Medical division, National Research Centre, Egypt, for the kind help in immunohistochemistry shoots and comments with the interpretations of the findings.

Declarations

Ethics Approval and Consent to Participant

Rats were grouped and housed in a conventional clean facility according to the guidelines of the Institutional Animal Ethics Committee of NODCAR. All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals.

Consent to Participate

Not applicable

Consent for publication

Not applicable

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

The whole research was totally funded by the authors and we declare that was no other funding sources.

Authors' Contributions

All authors contributed equally in the study design, interpretation of the data and writing of the final manuscript.

References

1. Al-Sodany YM, Bazaid SA. and Mosallam HA. Medicinal Plants in Saudi Arabia: I. Sarrwat Mountains at Taif, KSA. *Academic J. of Plant Sci*. 2013; 6 (4): 134-145.

2. Cai YZ, Sun M, Corke H. Antioxidant activity of betalains fromplants of the Amaranthaceae. J Agric Food Chem 2003; 51: 2288-2294.
3. El-Helal A.A, Nevein M. Abdel-Hady A, Dawoud GM, Hamed AM, Morsy TA. Phenolic content, antioxidant potential and Aedes Aegypt ecological friend larvicidal activity of some selected Egyptian plants. *J. Egypt. Soc. Parasitol.*, 2013; 43(1): 215-234.
4. Poulson HE, Prieme H, Loft S. Role of oxidative DNA damage in cancer initiation and promotion. Eur. J. Cancer Prev. 1998; 7(1):9–16.
5. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004; 26(2):211–219.
6. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009; 2(5): 270–278.
7. Afifi MS, Madiha A, Naeim, H, El-Sayed WN. A pharmacognostical study of *Monsonia nivea* DECNE. WEBB. Growing in Egypt. Bull. Pharm. Sci., Assiut University, 2007; 30 (1): 23-37.
8. Al-Mazroa SA, Wahaibi LH, Mousa A.A, Al-Khathlan H.Z, Al-Sodany YM et al. Essential oil of some seasonal flowering plants grown in Saudi Arabia. *Arab. J. of Chem*., 2015; 8:212-217.
9. Shishodia S, Aggarwal BB. Diosgenin inhibits osteoclastogenesis, invasion, and proliferation through the down regulation of Akt, I kappa B kinase activation and NF-kappa B-regulated gene expression. *Oncogene*. 2006; 25:1463-1473.
10. Aggarwal BB, Prasad S, Reuter S, Kannappan R, Vivek R, Yadev B, et al. Identification of Novel Anti-inflammatory Agents from Ayurvedic Medicine for Prevention of Chronic Diseases: "Reverse Pharmacology" and "Bedside to Bench" Approach. *Curr Drug Targets*. 2011; 12(11): 1595–1653.
11. Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M. Helicobacter pylori infection induces gastric cancer in mongolian gerbils. Gastroenterology. 1998; 115:642–648.
12. Ekor M. (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2013; 4: 177.
13. Al-Mofleh I, Al-Sobaihani M, Alqasoumi S, Al-Said M, Al-Dosari M, Al-Yahya M, et al. Fennel" Foeniculum vulgare" treatment protects the gastric mucosa of rats against chemically-induced histological lesions. Int J Pharm. 2013; 9(3): 182.
14. Ahmeda RF, Abdel-Rahman RF, Abdallah HM, Saleha DO, Ahmed-Farid OA, Hessin AF. Antidepressant-like effect of resveratrol in a subchronic model of depression. The Arab Society for Medical Research. 2014; 9(2): 84-53.
15. Lee JC, Kim HR, Kim J, Jang, YS. Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. Saboten. *J. Agric. Food Chem.* 2002; 50: 6490-9496.
16. Ajith TA, Nivitha S, Usha S. *Zingiber officinalis* roscoe alone and in combination with α- Tocopherol protect the kidney against cisplatin-induced acute renal failure. *Food and Chemical Toxicology*. 2007; 45, 921-927.
17. Nogata Y, Ohta H, Yoza KI, Berhow M, Hasegawa S. High-performance liquid chromatographic determination of naturally occurring flavonoids in Citrus with a photodiode-array detector. *J. Chromatogr*. A 1994, 667, 59–66.
18. Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J. Agric. Food Chem.*, 2003; 51(22): 6509-6515.
19. Dewanto V, Wu X, and Liu R.H. (2002): Processed sweet corn has higher antioxidant activity. *J. Agric. Food Chem*., 50(17): 4959-4964.
20. Jonathan Y. Phytochemical analysis and antimicrobial activity of Scoparia dulcis and Nymphaea lotus. *Aust. J. Basic App. Sci.*, 2009; 3(4): 3975-3979.
21. Akinjogunla OJ, Yah CS, Eghafona NO, Ogbemudia FO. Antibacterial activity of leave extracts of Nymphaea lotus (Nymphaeaceae) on Methicillin resistant Staphylococcus aureus (MRSA) and

Vancomycin resistant Staphylococcus aureus (VRSA) isolated from clinical samples. *Ann. Biol. Res.*, 2010; 1(2): 174-184.

22. Astudillo L, Schmeda-Hischmann G, Herrea J.P, and Cortes M. (2000): Proimate composition and biological activity of Chilean Prosopis species. *J. Sci. Food Agri*., 80: 567-573.
23. Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata)* (L.) seeds extracts. *J. Food Chem*. 2007; 101: 10-19.
24. Koracevic D, Koracevic G. Colorimetric method for determination of total antioxidant capacity kits. *J. Clin. Pathol.*, 2001; 54: 356-361.
25. Oyaizu M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese J Nutr., 1986; 44:307-315.
26. Uddin MK, Juraimi AS, Ali ME, Ismail MR. Evaluation of Antioxidant Properties and Mineral Composition of Purslane (*Portulaca oleracea* L.) at Different Growth Stages. Int. J. Mol. Sci., 2012; 13, 10257-10267.
27. Badami S, Moorkoth S, Rai S. R, Kannan E. and Bhojraj B. (2003): Antioxidant activity of Caesallpinia sappan heartwood. *Biol. Pharm. Bull.* 26(11): 1534-1537.
28. Yen GC, Hsieh CL. Antioxidant activity of extracts from Du-zhong *(Eucommia ulmoides)* toward various lipid peroxidation models *In-vitro*. *J. Agric. and Food Chem*., 1998; 46: 3952-3957.
29. Lowry, OH. Rosebrough, N. J, Farr, A. L. and Randall, R. J. J. Biol. Chem. 1951; 193, 265-275.
30. Nagababu, E, Lakshmaiah M. Inhibitory effect of eugenol on non enzymatic lipid peroxidation in rat liver mitochondria. *Biochem. Pharmacol*., 1992; 43: 2393-2400.
31. Tanaka K, Iizuka Y. Suppression of enzyme release from isolated rat liver lysosomes by non –steroidal anti-inflammatory drugs. *Diochem. Pharmacol*., 1968; 17: 2033 – 2032.
32. Robin CR, William BW. The temperature-dependence of the loss of latency of lysosomal enzymes. *Biochem. J.*, 1978; 172: 163-173.
33. Van Hoof F, Hers HG. The abnormalities of lysosomal enzymes in mucopolysaccharides. *European J. Biochem*., 1968; 7: 34 – 44.
34. Younan EA, Rosleff F. Changes of lysosomal enzymatic activities in human skin fibroblasts at various passages. *J. Drug Res., Egypt, 1974;* 6(3): 137-139.
35. Hemmati H, Rezvani A, Djahanguiri B. Prevention of aspirin-induced ulceration in rats with alphamethyl dopa and disulphiram. Pharmacology. 1973; 9:374-378.
36. Itoh M, Guth PH. Role of oxygen-derived free radicals in hemorrhagic shock-induced gastric lesions in the rat. Gastroenterology, 2008; 88:1162-1167.
37. Feldman BF, Zinkl JG, Jain NC. Schalm's Veterinary Haematology 5th Edition. 2010; 504-507.
38. Karatep M. Simulatenous determination of ascorbic acid and free malondialdehyde in human serum by HPLC-UV. Chromatographic Line, 2004; 12: 362-365.
39. karalas F, Karatepe M, Baysar A. Determination of free malondialdehyde in human serum by high performance liquid chromatography. Anal. Biochem., 2002; 311:76-79.
40. Jayatilleke E, Shaw S. A high performance liquid chromatographic assay for reduced and oxidized glutathione in biological samples. Anal. Biochem., 1993; 214(2): 452-457.
41. Papadoyannis LN, Samanidou VF, Nitsos ChC. Simultaneous determination of nitrite and nitrate in drinking water and human serum by high performance anion-exchange chromatography and UV detection. J. Liq. Chrom. Rel. Technol., 1999; 22(13): 2023-2041.
42. Sakamoto K, Arakawa H, Mita S, Ishiko T, Ikei S, Egami H, Hisano S, Ogawa M. Elevation of circulating interleukin 6 after surgery: factors influencing the serum level. *Cytokine*.1994; 6: 181-6.
43. Aljanabi SM, Martinez I. universal and rapid salt-extraction of high quality genomic DNA for PCR based techniques. *Nucleic Acids res*., 1997; 25(22): 4692-4693.

44. Hassab El-Nabi SE. Molecular and cytogenetic studies on the antimutagenic potential of eugenl in human lymphocytes culture treated with depakine and apetryl drugs. J. Egypt. Ger. Soc. Zool., 2004; 43(2): 171-196.
45. Awwad M.H. (2003): Molecular identification of Biomphalaria alexandrina and bulinus truncates using PCR-RELP of Actin gene. EGYPT *Acad. Soc. Environ. Develop*., 3(1): 39-52.
46. Nakamura Y, Ishimitsu S, Tonogai Y. Effects of quercetin and *Rutin* on serum and hepatic lipid concentrations, fecal steroid excretion and serum antioxidant properties. *J. Health Sci*. 2000; 46: 229-240.
47. Erkan N, Ayanci G, Ayranci E. Antioxidant activities *(Nigella Sativa L.)* essential oil, carnosic acid, rosmarinic acid, and sesamol. *Food Chem*., 2008; 110:76-82.
48. Yang J, Guo J, Yuan J. *In-vitro* antioxidant properties of *Rutin*. LWT. 2008; 41:1060-1066.
49. Caillet S, Yu H, Lessard S, Lamoureux G, Ajdukovic D, Lacroix M. Fenton reaction applied for screening natural antioxidants. *Food Chem*. 2007; 100: 542-552.
50. Scalzo J, Politi A, Pellegrini N, Mezzetti B, Battino M. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition*. 2005; 21: 207-213.
51. Athukorata Y. Lee-Kiwan Song-ChoomBok Ahn-ChangBum ShinTaiSun Cha-YongJun E. Shahidi and Jeon-Youjin 2003. Potential antioxidant activity of marine red alga Grateloupia filicina extracts. J. Fd. Lipids, 10: 251–65.
52. Kalra J, Prasad K. Oxygen free radicals and cardiac depression. *Clin. Biochem*. 1994; 27: 163-168.
53. Reddy GK, Dhar SC. Studies on carbohydrate moieties of glycoproteins in established adjuvant induced arthritis. Agents Actions. 1988; 25: 63-70.
54. Subashini R, Gnanapragasam A, Senthilkumar S, Yogeeta SK, Devaki T. Protective efficacy of nardostachys jatamansi (Rhizomes) on mitochondrial respiration and lysosomal hydrolases during doxorubicin induced myocardial injury in rats. *J. of Health Sci*., 2007; 53(1): 67-76.
55. Dorman H.J.D, Deans S.G, Noble R.C, and Surai P. (1995): Evaluation *In-vitro* of plant essential oils as natural antioxidants. *J. Essent. Oil. Res*.,7: 645-651.
56. Raybak MEM. Hematologic effects of Nonsteroidal antiinflammatory drugs. In: NASIDs a profile of adverse effects. Borda IT, Koff RS, eds. Philadelphia: Hanley & Belfus.Inc., 1992; 113-32.
57. Navratil L, Blehovaz A, Brbonlavova H. Effect of long, term administration of acetylsalicylic acid on haematological and haemocoagulation changes in the rat. *Boll. Chim. Farmaceutico.* 1992; 131:363-8.
58. Collins R, Langman M. Treatment with Histamine H2 antagonists in upper gastrointestinal hemorrhage. Implications of randomized trials. N Engl J Med 1985; 313:660.
59. Bush B. M. (1991): Interpretation of result in clinical medicine. Blackwell scinetific publication Pp. 35-40.
60. Kohtani T, Abe Y, Sato M. Protective effects of anti-neutrophil antibody against myocardial ischemia/reperfusion injury in rats. Eur Surg Res., 2002; 34:313–20.
61. Abdulla HM, Ali KA, Ahmed SM, Noor S, Ismail F. Evaluation of the anti-ulcer activities of Morus Alba extracts in experimentally-induced gastric ulcer in rats Biomed. Res., 2009; 20 (1):35-39.
62. Beck P.L, Xavier R, and Lu N. (2000): Mechanisms of NSAID-induced gastrointestinal injury defined using mutant mice. *Gastroenterology*. 119:699-705.
63. Kubo J. Lee JR, Kubo I. Anti-Helicobacter Pylori Agents from the Cashew Apple. J. Agric., Food Chem. 1999; 47(2):533-7.
64. Gregory M, Vithalrao KP, Franklin G, Kalaichelavan V. Anti-Ulcer (Ulcer-Preventive) Activity of Ficus arnottiana Miq.(Moraceae) Leaf Methanolic Extract. American Journal of Pharmacology and Toxicology, 2009; 4: 89-93.
65. Reyes-Chilpa R, Baggio CH, Alavez-Solano D, Estrada-Muñiz E, Kauffman FC, Mesia-Vela S. Inhibition of gastric H+,K+-ATPase activity by flavonoids, coumarins and xanthones isolated from Mexican medicinal plants. J. *Ethnopharmacol.* 2006; 21;105(1-2):167-72.

66. Priscilla DH, Prince PSM. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. Chem. Biol. Interact., 2009; 179: 118-124.
67. Patil S, Kanase A, Kulkarni PH. Antianaemic properties of Ayurvedic drugs, raktavardhak, punamavasav and navayas louh in albino rats during phenylhydrazine induced haemolytic anaemia. Ind. J. Exp. Biol., 2000; 38: 253-257.
68. Tripathi S, Maier KG, Bruch D, Kittur DS. Effect of 6-gingerol on pro-inflammatory cytokine production and co-stimulatory molecule expression in murine peritoneal macrophages. *J. Surg. Res*. 2007; 138, 209–213.
69. Song Y, Zhang C, Wang C, Zhao L, Wang Z, Dai Z. Ferulic Acid against Cyclophosphamide-Induced Heart Toxicity in Mice by Inhibiting NF-κB Pathway. *Evid Based Complement Alternat Med*., 2016; 126-127.
70. Peet GW. IκB Kinases α and β Show a Random Sequential Kinetic Mechanism and Are Inhibited by Staurosporine and Quercetin. J. Li, J. Biol. Chem. 1999; 274: 32655-32661.
71. Romay C, Ledo´n N, Gonza´lez R. Further studies on anti-inflammatory activity of phycocianin in some animal models of inflammation. *Inflamm. Res*. 1998; 47, 334-338.
72. Carson DA, Ribeiro JM. Apoptosis and disease. *Lancet*, 1993; 341: 1251-1254.
73. Cazanave S, Berson A, Haouzi D, Vadrot N, Fau D, Grodet A, et al. High hepatic glutathione stores alleviate fas-induced apoptosis in mice. *J. Hepatol*., 2007; 46(5): 858-868.
74. Friesen C, Kiess Y, Debatin KM. A critical role of glutathione in determining apoptosis sensitivity and resistance in leukemia cells. *Cell death Differ*. 2004; 11(1): 73-85.
1.