



EVALUTATION OF ANTIOXIDANT POTENTIAL OF HEPATOPROTECTIVITY OF HERBAL DRUG

Yogesh Rahangdale, *Punam Bhange, Madhuri Nandgave, Pooja Bamne, Samer Ukey,

Pallavi Nikhade, Pyal Patle, Pyal Machhirke, Rohit Rinayat.

Manohar Bhai Patel Institute Of Pharmacy Kudwa Gondia Maharashtra 441614

Abstract.

The present study indicate that the ethanolic extracts of leaves of *A. paniculata* and *Z. officinale* exhibited the potential hepatoprotective activity against paracetamol induced hepatotoxicity and validate the traditional use of this plants in hepatocellular jaundice and other hepatic diseases and disorders. Further studies are required to isolate and characterize the active principles, which are responsible for the hepatoprotective activity/ efficacy of this valuable plants. The results presented in this report will also provide a suitable guide in choosing natural plant by the medical practitioners as natural oxidants treating and controlling liver diseases and also some other chronic disease conditions. With the potential of using natural antioxidants as medicines and as food additive the antioxidant research has attracted a prominent place at present.

Key words: kalmegh extract, hepatotoxicity, Ginger,

1. INDRODUCTION

ANDROGRAPHIS PANICULATA (KALMEGH)

Andrographis paniculata (burn. F) wall.ex nees (ap) also called Kalmegh or “King of Bitters” belongs to family Acanthaceae. It has been used for centuries in Asia to treat gastro-intestinal tract and upper respiratory infections, fever, herpes, sore throat, and a variety of other chronic and infectious diseases. Indian Pharmacopoeia narrates that it is a predominant constituent of at least 26 Ayurvedic formulation. In Traditional Chinese Medicine (TCM), *Andrographis* is considered as the herb possessing an important “Cold Property” useful to treat the head of body in fevers, and to dispel toxins from the body. In Scandinavian countries it is commonly used to prevent and treat common colds.

Table no. 1. TAXONOMICAL CLASSIFICATION

Kingdom	Plantae, plants
Sub-kingdom	Tracheobionta, seed plants
Division	Angiosperma
Class	Dicotyledonae
Sub class	Gamopetalae
Series	Bicarpellatae
Order	Perispermiales I
Tribe	Acanthaceae
Genus	<i>Andrographis</i>
Species	<i>Paniculata</i>

Botanical Description:

Andrographis paniculata is an annual, branched, herbaceous plant erecting to a height of 30-110 cm in moist shady places with stem acutely quadrangular, much branched, easily broken fragile texture stem. Leaves are simple, opposite, lanceolate, glabrous, 2–12cm long, 1–3cm wide with margin acute and entire or slightly undulated and upper leaves often bracti form with short petiole. Inflorescence of the plant is characterized as patent, terminal and axillary in panicle, 10–30 mm long; bract small; pedicel short. The flowers possess botanical features of calyx 5-partite, small, linear; corolla tube narrow, about 6 mm long; limb longer than the tube, bilabiate; upper lip oblong, white with a yellowish top; lower lip broadly cuneate, 3-lobed, white with violet markings; stamens 2, inserted in the throat and far exerted; anther basally bearded. Superior ovary, 2-celled; style far exerted. Capsule of the plant is erect, linear-oblong, 1–2 cm long and 2–5 mm wide, compressed, longitudinally furrowed on broad faces, acute at both ends, thinly glandular-hairy. Seeds are very small, sub-quadrate. (1-5)

Plant parts used: The aerial parts of the plant (leaves and stem) are used to extract the active phytochemicals and thus used for its medicinal importance. Very rarely roots are also used. (6)

Synonyms:

Arab: Quasabhuva; Bengali: Kalmegh; English: The Creat, King Of Bittes;

Gujarathi: Kariyatu; Hindi: Kirayat; Kannada: Nelaberu; Malayalam: Kiriyaattu. (6)

Phytochemistry:

The characteristic secondary metabolites encountered in the plant have considerably enhanced its importance in the arena of medicinal. It is specifically in the rated very high in therapeutic action in curing liver disorders and common cough and cold in humans z. A number of Diterpenoids and Diterpenoid glycosides of similar carbon skeleton have been isolated from *Andrographis*, mainly the most bitter compounds among them are andrographolide, neoandrographolids, deoxyandrographolids. Other such phytochemicals amassed by the plant are 14-deoxyandro-graphide, 14-deoxy- 11,12-didehydro andrographolide, andrographiside. (6) The leaves of *Andrographis*

contain the highest amount of Andrographolide (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest. (7)



Fig 1: ANDROGRAPHIS PANICULATA (8)

Pharmacology:

Researches conducted in past decades have confirmed that Andrographis, if properly administered, has a surprisingly broad range of pharmacological effects.

Some pharmacological effects are as follows:

Abortifacient (can abort pregnancy); although

Vermicidal (kills intestinal worms)

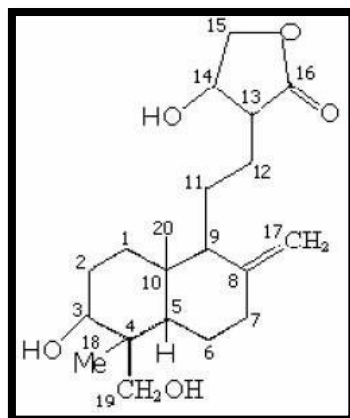


Fig. 2. Andrographolide (the main carbon skeleton) (9,10,11)

Uses:

Common Cold: Some researchers show that taking a specific andrographis extract combination with Siberian ginseng by mouth improves symptoms of the common cold when started within 72 hours of feeling sick.

Reducing the fever and sore throat due to tonsillitis: Some research shows that high dose andrographis works about as well as acetaminophen after 3 to 7 days of treatment.

Inflammatory bowel diseases: Research suggests that taking andrographis extract daily for 8 weeks reduces symptoms of inflammatory bowel disease as well as the drug mesalamine.(12)

ZINGIBER OFFICINALE (GINGER)

Ginger (*Zingiber Officinale*) is a flowering plant whose Rhizome, ginger root or ginger, is widely used as a spice and a folk medicine. It is a herbaceous perennial which grows annual pseudo stems (false stems made of the rolled bases of leaves) about a meter tall bearing narrow leaf blades. The inflorescences bear pale yellow with purple flowers and arise directly from the rhizome on separate shoots. Ginger is in the Family Zingiberaceae, to which also belong Turmeric (*Curcuma longa*), Cardamom (*Elettaria Cardamomum*), and Galangal. Ginger originated in the tropical rainforests from the Indian Subcontinent to Southern Asia where ginger plants show considerable genetic variation. As one of the first spices exported from the Orient, ginger arrived in Europe during the spice trade, and was used by Ancient Greeks and Romans. The distantly related dicots in the genus *Asarum* are commonly called wild ginger because of their similar taste.(13)

Botanical Description of Ginger:

Ginger is herbaceous rhizomatous perennial, reaching up to 90 cm in height under cultivation. Rhizomes are aromatic, thick lobed, pale yellowish, bearing simple alternate distichous narrow oblong lanceolate leaves. The herb develops several lateral shoots in clumps, which begin to dry when the plant matures. Leaves are long and 2 - 3 cm broad with sheathing bases, the blade gradually tapering to a point.

Inflorescence solitary, lateral radical pedunculate oblong cylindrical spikes. Flowers are rare, rather small, calyx superior, gamo-sepalous, three toothed; open splitting on one side, corolla of three sub equal oblong to lanceolate connate greenish segments.

Phytoconstituents are present in Ginger:

The constituents of ginger are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry but to summarize the major components that have been implicated in the pharmacological activities of the crude drug. The primary pungent agents (phenyl-alkyl-ketones or vanillyl ketones) of ginger are Gingerol, with other Gingerol analogues such as the shogaols, paradol and zingerone also found in high levels in rhizome extracts. The major pharmacological activity of Ginger appears to be due to Gingerol and shogaol. Phenylalkylketones or Vanillyl ketones of ginger include 6-gingerol 8- gingerol and 10-gingerol, 6-shogaol, 8- shogaol, 10-shogaol and zingerone, 6-paradol, 6- and 10- dehydrogingerdione and 6 and 10- gingerdione have also been identified. (14)



Fig 3: Ginger (14)

Uses:

Evidence that ginger helps alleviate nausea and vomiting resulting from chemotherapy or pregnancy is inconsistent. It is not recommended for any clinical uses or for nausea. There is no clear evidence of harm from taking ginger during pregnancy, although its safety has not been established. Ginger is not effective for treating dysmenorrhea, and there is no evidence for it having analgesic properties. (15)

DRUG INTERACTION:

The following discussion on ginger and its drug interactions is drawn from a text we highly recommend; it was written by a naturopathic physician, herbalist, and medical doctor working in concert. This treatise does not cover nearly enough herbs (largely because of a lack of information about most herbs) but when an herb is included in this text, as in the case of ginger, the beneficial and negative herb–drug interactions are discussed authoritatively and thoroughly. As a result, we highly recommend our readers to go to the source rather than relying on our brief summary. In TCM, ginger is added to formulas to moderate the potentially toxic effect of strong plants such as aconite and pinellia. In Western and Ayurvedic Medicine, ginger is often added to formulas to enhance, synergize, and promote absorption of the formulas herbal ingredients. Pharmacologic and animal studies do show that ginger, on occasion, enhances absorption (sulfaguanidine) and on occasion, increases the accumulation of a drug (daunorubicin). (17)

MATERIALS AND METHODS

Materials:

Herbal plant drug powder, drug extract, ethanol,

Plant material collection:

The powder of Ginger were purchased from Tulsi Ayurvedic Shop, Gondia.

The plant of Kalmegh was collected from the forest of Tigaon village (locality) and powdered coarsely for extraction.

Authentication:

We have done the authentication of *Andrographis paniculata* by DR. S. M. BHUSHKUTE SIR, Principal of the BHAWBHUTI MAHAVIDYALAYA AMGAON,

DIST. GONDIA on the date of 29th April, 2019 [field book no. (**Authentication no.**

701).

Process of Plant Extraction (SOXHLET EXTRACTION):

The dried powder (500 g) were successively extracted in a Soxhlet apparatus using solvents of increasing polarities: petroleum ether (40-60°C), chloroform and 95% ethanol for 72 h of each solvent. Solvent removal was carried by evaporation under reduced pressure at 40°C yielding semisolid residues of each herbal drugs *A. paniculata* and *Z. officinale*.

POWDER ANALYSIS AND PHYSICOCHEMICAL PARAMETER:**Physicochemical Parameter: Ash Value determination:**

1. Total ash
2. Acid insoluble ash
3. Alcohol soluble extract
4. Water soluble extract

Morphological Evaluation:

1. Colour
2. Odour
3. Taste
4. Appearance

EVALUATION OF THE DRUG EXTRACTS:**GENERAL TESTS FOR IDENTIFICATION CONSTITUENTS OF HERBAL DRUGS****A. Test for the phenolic compounds****1. Alkaloids****a. Test for the phenolic compounds**

These result in the production of amorphous or crystalline precipitates of various colors, in which the precipitating reagent is added to a neutral or slightly acidic aqueous solution of the alkaloidal salts. The reagents used contain heavy metals such as Hg, Pt, Bi and form double salts with most alkaloids.

b. Color reactions

These reactions are usually performed by the addition of color reagents to the solid free bases not to their salts to produce characteristic colored solutions. The reagents used generally contain concentrated sulphuric acid and an oxidizing agent. They give colors with most alkaloids, or may be specific for one alkaloid or a group of related alkaloids.(15)

2. Flavonoids:

The ethanol extract (5 ml) was added to a concentrated sulphuric acid (1 ml) and 0.5g of Mg. A pink or red coloration that disappear on standing (3 min) indicates the presence of flavonoids.(15)

3. Tannins:

Two methods were used to test for tannins.

a. About 1 ml of the ethanol extract was added in 2 ml of water in a test tubdrops of diluted ferric chloride solution was added and observed for green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration.

2 ml of the aqueous extract was added to 2 ml of water, a 1 to 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins. (15)

B. Test for saponins:

To 1 ml of aqueous extract was added few volume of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min. (15)

C. Test for emodols:

Evaporate 3 ml of etheric extract. Dissolve the dry residue in 1 ml of concentrated NH_4OH and treating the solution with the reagent Borntrager. A test is revealed by the appearance of a bright color ranging from orange red to purple. (15)

D. Test for anthracenosids:

Eight ml (8 ml) of the ethanolic solution treated with the reagent Borntrager, a positive test is revealed the appearance of a bright color change from orange red to purple. (15)

E. Test for anthocyanosids:

The presence of anthocyanoside is revealed by a color change as a function of pH due to titration of the acidic aqueous solution with a solution of NaOH. If the solution turns a red color, the pH is less than 3, if against a blue color; the pH is between 4 and 6.(15)

F. Test for coumarins:

Evaporate 5 ml of ethanolic solution, dissolve the residue in 1-2 ml of hot distilled water and divide the volume into two parts. Take half the volume as a witness and to add another volume of 0.5 ml 10% NH₄OH. Put two spots on filter paper and examined under UV light. Intense fluorescence indicates the presence of coumarins.(15)

G. Test for sterols and steroids:

Sterols and steroids were sought by the reaction of Liebermann. Ten (10 ml) ml of ethanolic extract was evaporated. The residue was dissolved in 0.5 ml of hot acetic anhydride; we added 0.5 ml of the filtrate chloroform. Treated with the reagent of Libermann Burchardt. The appearance, at the interphase, a ring of blue-green, showed a positive reaction.(15)

H. Test for the carbohydrates:**1. Reducing sugars**

Two methods were used to test for reducing sugars.

The ethanol extract (1 ml) was added to 1ml of water and 20 drops of boiling Fehling's solution (A and B) in a test tube was added too. The formation of a precipitate red-brick in the bottom of the tube indicates the presence of reducing sugars.

Added to 2 ml of aqueous solution, 5-8 drops of boiling Fehling's solution. A red- brick precipitate

showed the presence of reducing sugars.(15)

2. Starch:

The aqueous extract 5ml was treated with the reagent of the starch (iodine). Any shift to blue violet indicates the presence of starch.(15)

➤ Methods of Evaluation of Antioxidant activity:

1. DPPH for radical scavenging activity
2. Determination of phenol
3. Nitric oxide radical scavenging capacity assay
4. Reducing power assay
5. Hydrogen peroxide scavenging capacity assay
6. Total flavonoid content

➤ Methods of Evaluation Hepato-protective activity:

1. Determination of serum bilirubin
2. Determination of aspartate alanine transaminase (ALT)
3. Determination of aspartate amino transferase (AST)
4. Determination of gamma glutaryl transferase (GGT)
5. Determination of dinitro-phenyl hydrazine (DNPH)

EVALUATION OF ANTIOXIDANT ACTIVITY:

1. DPPH scavenging activity:

The molecule 1, 1-diphenyl-2-picrylhydrazyl (a,a-diphenyl-b-picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of

electron also gives rise to the deep violet color. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. According to Manzocco et al., 1998 the sample extract (0.2 mL) is diluted with methanol and 2 mL of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation as given below: % inhibition of DPPH radical = $[(A_{br} - A_{ar})/A_{br}] \times 100$ Where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place. (13)

2. Hydrogen peroxide scavenging (H₂O₂) assay:

Human beings are exposed to H₂O₂ indirectly via the environment nearly about

0.28 mg/kg/day with intake mostly from leaf crops. Hydrogen peroxide may enter into the human body through inhalation of vapor or mist and through eye or skin contact. H₂O₂ is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH) that can initiate lipid peroxidation and cause DNA damage in the body.

The ability of plant extracts to scavenge hydrogen peroxide can be estimated according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer.

Extract (20–60 µg/mL) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ scavenged (H}_2\text{O}_2) = [(A_i - A_t)/A_i] \times 100$$

Where A_i is the absorbance of control and A_t is the absorbance of test. (13)

3. Nitric oxide scavenging activity:

NO is generated in biological tissues by specific nitric oxide synthases, which metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction (David-1999; Ghafourifar and Cadenas-2005; Marletta-1989; Moncada et al.-1989; and Virginia et al.-2003). The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO. Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of

which can be determined using Griess reagent (Marcocci et al., 1994). Two (2) mL of 10 mM sodium nitroprusside dissolved in 0.5 mL phosphate buffer saline (pH 7.4) is mixed with 0.5 mL of sample at various concentrations (0.2–0.8 mg/mL). The mixture is then incubated at 25 °C. After 150 min of incubation, 0.5 mL of the incubated solution is withdrawn and mixed with 0.5 mL of Griess reagent [(1.0 mL sulfanilic acid reagent (0.33%) in 20% glacial acetic acid at room temperature for 5 min with 1 mL of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture is then incubated at room temperature for 30 min and its absorbance pouring into a cuvette is measured at 546 nm.

The amount of nitric oxide radical inhibition is calculated following this equation:

$$\% \text{ inhibition of NO radical} = [A_0 - A_1]/A_0 \times 100$$

Where A_0 is the absorbance before reaction and A_1 is the absorbance after reaction has taken place with Griess reagent. (13)

4. Reducing power method (RP):

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples (Jayaprakash et al., 2001). In the method described by Oyaizu (1986) 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) are added to

1.0 mL of sample dissolved in distilled water. The resulting mixture is incubated at 50 °C for 20 min, followed by the addition of 2.5 mL of Trichloro acetic acid (10% w/v). The mixture is centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL), mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1%, w/v). The absorbance is then measured at 700 nm against blank sample. (13)

5. Estimation of total phenolic compounds:

Total phenolic content was determined by the Folin Ciocalteu method by Folin *et al* 1927. To 0.5 ml of 1-5 mg/ml of herbal preparation made up with 0.5 ml of distilled water, 0.5 ml of Folin Ciocalteu reagent was added and gently mixed. After 2 minutes

0.5 ml of 100mg/ml sodium carbonate was added. The contents were mixed and allowed to stand for 2 hours. The optical density of the blue coloured samples was measured at 765 nm spectrophotometrically.

Standard gallic acid of concentration 100-500µg/ml was used. The concentration of total phenolics is

expressed as milligram of gallic acid/g of mixture. All determinations were carried out in triplicate.(14)

6. Estimation of flavonoids:

The method used by Chang *et al* 2002 with slight modifications in total volume of reagents used; was followed for estimation of flavonoids. 0.5 ml of concentration 100- 500µg/ml of herbal preparation was mixed with 1 ml aluminium trichloride in ethanol (20g/l) and diluted with ethanol to 25 ml. The absorbance was read after 40 minutes incubation at 37°C spectrophotometrically at 415nm. Rutin (a citrus flavonoids glycoside) of concentration 0.5mg/ml, 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml and 2.5 mg/ml was used as a reference compound and absorbance was measured under the same conditions. All determinations were carried in triplicate. The amount of flavonoids in herbal preparation was calculated as milligram of rutin/g of mixture.(14)

EVALUATION OF HEPATOPROTECTIVE ACTIVITY:

A. Determination of serum Bilirubin

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form pink colored azobilirubin with absorbance directly proportional to Bilirubin concentration. Direct Bilirubin, being water directly reacts in acidic medium. However indirect or in conjugated Bilirubin is solubilized using a surfactant and then it reacts similar to Direct Bilirubin.

Procedure:

Total bilirubin/Direct bilirubin

In different tubes, Pipetted 500µl of working reagent into marked test tubes as Blank, Standard and Test and too this add 25µl distilled water calibrator and serum respectively.

Mix well, incubated for 5 minutes at 37⁰ C for total Bilirubin and Direct Bilirubin. Read absorbance at 546/630 nm against reagent blank using semi auto analyzed by endpoint method. The units are expressed as mg/dl. Was calculate by following formula.

Total Bilirubin = Absorbance of Test × 23(factor) Direct Bilirubin = Absorbance of Test ×17 (factor)

Determination of Aspartate alanine Transaminase (ALT): Procedure:

In different tubes Pipetted 1000µl of working into marked test tubes as Blank and Test and to this added 100µl working reagent and Serum respectively. Mix well, incubated for 5 minutes at 37⁰ C and read the absorbance at 340nm. A decrease in the absorbance against Reagent blank was measured for 180 seconds at

an interval of 30 seconds using semi auto analyzer by kinetic method. The Units were expressed as IU/L

and the result was calculated by following formula,

$$\text{ALT activity [IU/L]} = \Delta A / \text{min.} \times \text{Factor (1768)}$$

B. Determination of Aspartate amino Transferase (AST): Procedure:

In different marked test tubes as Blank and Test, Pipetted 100µl working reagent in both the tubes and 10µl Serum to the tube marked as test. Mixed well, incubated for 5 minutes at 37 °C and read the decrease in absorbance at 340nm, against reagent blank for 180 seconds at an interval of 30 seconds using semi auto analyzer by kinetic method. The Units are expressed as IU/L. The result was calculated by the following formula.

$$\text{AST activity [IU/L]} = \Delta \text{Absorbance} / \text{min.} \times \text{Factor (1768)}$$

C. Determination of Gamma – Glutamyl Transferase (GGT): Procedure:

In different tubes marked as Blank and test added 1000µl working reagent and 10µl Serum respectively. Mixed well and read the increases in the absorbance against Reagent blank at 405 nm for 180 seconds at an interval of 30 second using semi auto analyzer by kinetic method. The units are expressed as IU/L. The $\Delta A / \text{min}$ was calculated by the following formula:

$$\text{Activity of GGT at } 37^{\circ}\text{C (IU/L)} = (\Delta \text{Absorbance} / \text{min}) \times \text{factor (1158)}.$$

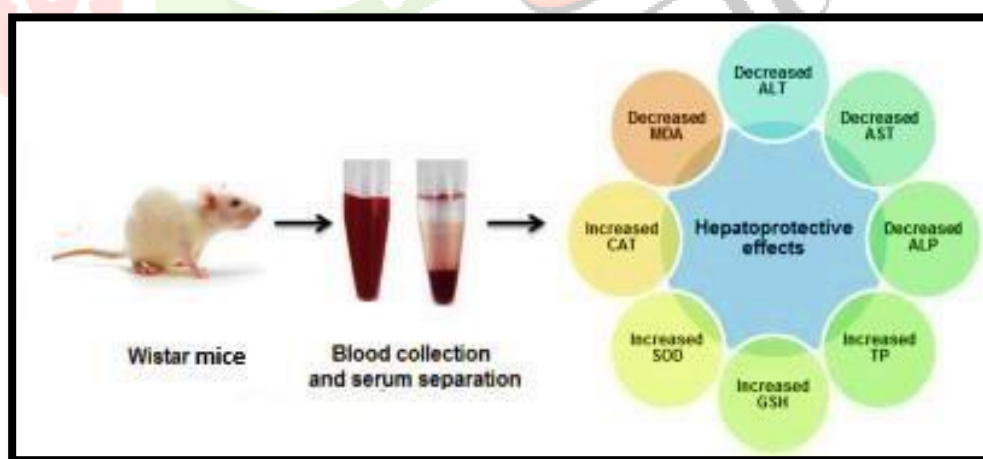


Fig.4. Evaluation of Hepatoprotective effect of Selected Drug extracts

RESULT AND DISCUSSION:**Table 2. Ash Value Determination:**

TEST	RESULT	
	Kalmegh	Ginger
Total ash %	11.600 %	1.036 %
Acid insoluble ash %	1.400 %	0.320 %
Alcohol soluble extract %	12.918 %	4.661 %
Water soluble extract %	0.921 %	20.013 %

Morphological Evaluation: KALMEGH**5. Colour and Odour**

By visual examination,

Dark green colour with characteristic odour

6. Taste : Bitter**7. Appearance : Solid after drying****GINGER****A. Morphological evaluation:****1. Colour and Odour**

By visual examination,

Yellow colour with characteristic odour

2. Taste : Bitter**3. Appearance : Solid after drying**

Table 3 . Total sugar content (g/L) of aqueous, ethanolic extracts of ginger and kalmegh

Solvent \ Substrate	Ginger (%)			Kalmegh (%)		
	1	5	10	1	5	10
Water	0.12	1.28	5.5	0.18	1.1	11.2
	(0.12)	(0.26)	(0.6)	(0.18)	(0.22)	(1.1)
Ethyl alcohol (50%)	0.9	6.4	10	0.7	5.4	8.8
	(0.9)	(1.3)	(1)	(0.7)	(1.08)	(0.9)

Table 4: Total alcohol (%) content of ginger and kalmegh extracts

Conc. (%)	Observations	
	Kalmegh extract	Ginger extract
10	12.51	12.50
20	12.54	12.50
30	12.54	12.53

Table5: Total phenols (mg GAE/100 ml) content of ginger and kalmegh extracts

Conc. (%)	Observations	
	Kalmegh extract	Ginger extract
10	396	386.4
20	444.1	442.1
30	447.9	454.3

Table 5: Tannin contents (mg/100 ml) of ginger and kalmegh extracts

Conc. (%)	Observations	
	Kalmegh extract	Ginger extract
10	4.05	3.92
20	4.53	4.08
30	4.56	4.14

Table 6: Total Flavonoids (mg/ ml) content of ginger and kalmegh extracts

Conc. (%)	Observations	
	Kalmegh extract	Ginger extract
10	0.096	0.089
20	0.104	0.095
30	0.099	0.103

➤ **Effect of *A. paniculata* and *Z. officinale* on hepatic marker enzymes in Paracetamol induced hepatotoxicity in rats:**

Treatment	ALT (IU/L)	AST (IU/L)	Bilirubin		GGT (IU/L)
			Total (mg/dl)	Direct (mg/dl)	
Vehicle Control	71.5±3.5	74.20±3.45	0.45±0.05	0.38±0.05	60.45±0.25
Paracetamol treated	202.55±3.20	164.01±2.65	2.95±0.07	2.45±0.045	80.22±0.15

Fig. 5. Effect of *A. paniculata* and *Z. officinale* extract on ALT, AST, GGT in Paracetamol induced hepatotoxicity in mice

Extracts Concentration (μ g/ml)	Antioxidant activity			
	DPPH assay (μ g/ml)	Nitric Oxide assay (in per cent)	Reducing power assay (in per cent)	Total Flavonoid assay (in per cent)
Methanol extract of Kalmegh	0.07 ± 0.0017	68.948 %	47.590 %	58.309 %
Methanol Extract of Ginger	0.05 ± 0.0018	63.735 %	40.755 %	40.816 %

The phytochemical analysis of the combination of four selected medicinal plants, ginger is rich in total phenolic compounds, flavonoids and flavonols. Tannins, polyphenols and flavonoids are reported to have significant antioxidant properties. Accordingly; these compounds have shown to have antioxidant activity. Total phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators hence it was reasonable to detect their amount in the herbal preparation. Flavonoids are the most widespread group of natural compounds and probably the most important natural phenolics. Total phenolics and flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties. Such property is especially distinct for flavonols. The medicinal effects of plants are often attributed to the antioxidant activity of phytochemical constituents mainly phenolics, flavonoids and flavonols. It is claimed that phenolic compounds are powerful chain breaking antioxidants. The scavenging activity of phenolic group is due to its hydroxyl group.

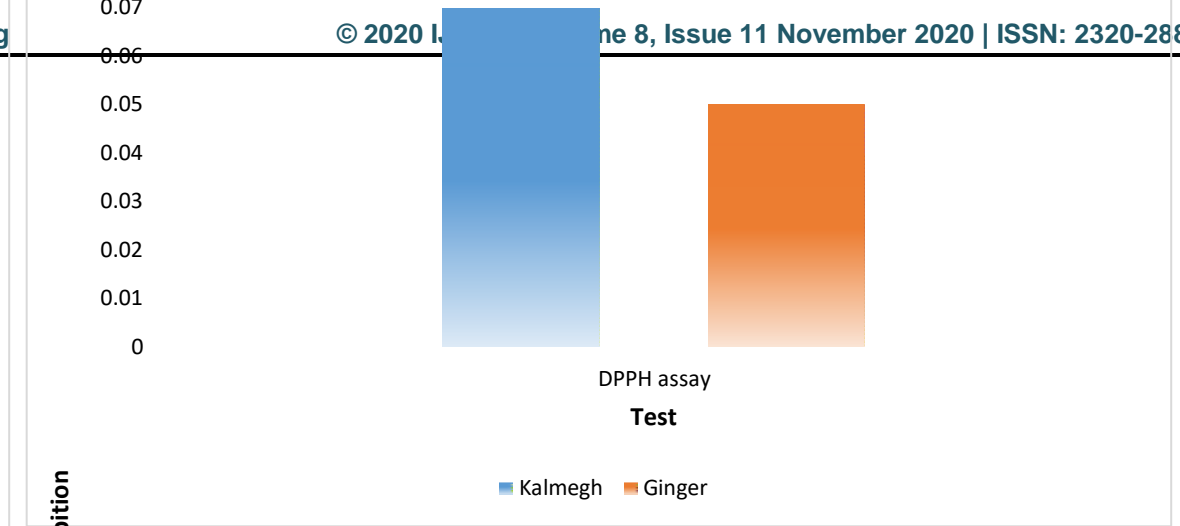


Fig. 6. Antioxidant activity evaluation (DPPH assay) of Kalmegh and Ginger

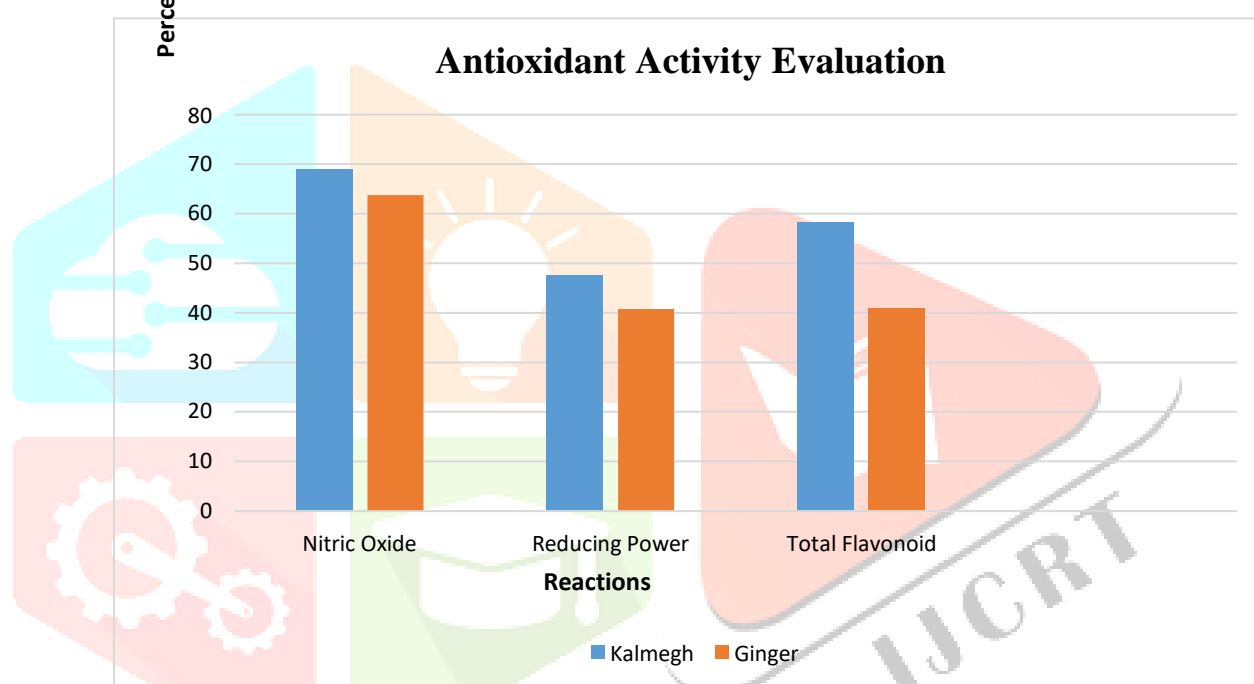


Fig. 7. Antioxidant Activity Evaluation of Kalmegh and Ginger

The antioxidant activity has been reported to be concomitant with the development of reducing power. Herbal preparation revealed synergistic effects both in DPPH scavenging and reducing power in comparison with the individual plant extracts selected for the study. The crude extracts of plants are pharmacologically more active than their isolated active principles due to the synergistic effects of various components present in the whole extract. A synergistic relationship amongst phytochemicals has been adduced to be responsible for the overall beneficial effects derivable from constituent plants. The synergy of phytochemicals may make up for the apparent low values for individual classes of phytochemicals. Preliminary phytochemical analysis of extract has shown the presence of flavonoids and phenolic

compounds, which have been known for their anti-oxidant and hepatoprotective activities. Anti-oxidant activity was assayed by in vitro DPPH scavenging activity. Scavenging of free radicals is one of the major anti-oxidant mechanisms to inhibit the chain.

The model DPPH free radical scavenging assay is an easy method to evaluate antioxidant activity in a relative short time compared to the other methods. DPPH is a relatively stable radical. The assay is based on the measurement of the scavenging ability of the antioxidants towards the stable radical DPPH which reacts with suitable reducing agent. The electrons become paired off and solution loses its color stoichiometrically depending on the number of electrons taken up. The color change can be quantified by its decrease of absorbance at wavelength 517nm. The antioxidants exert their DPPH free radical scavenging due to their hydrogen donating ability.

The plant extracts of *Andrographis Paniculata* has reported to have many medicinal uses as hepatoprotective, antibacterial, antifungal and cytotoxic activity. It is also used to relieve skin & blood diseases, abdominal pains, tumors, spleen enlargement. It is also widely studied for its activity against induce oxidative stress and hepatoprotective activity in the recent past and has exhibited promising results for the same.

CONCLUSION:

In conclusion the result of the present study indicate that the ethanolic extracts of leaves of *A. paniculata* and *Z. officinale* exhibited the potential hepatoprotective activity against paracetamol induced. With the reported undesirable effects of synthetic antioxidants and the high cost for such antioxidants, have also encouraged most of the people to use natural antioxidants. Through this investigation we have shown that both plants used in this investigation exhibits antioxidant activities with both of them namely to *Andrographis paniculata* and *Zingiber officinale* be having remarkable antioxidant activity with a good potential to be used in therapeutics.

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