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A Review of traditional uses, phytoconstituents and pharmacological activities of *Ipomoea obscura*

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Abstract
The genus Ipomoea belonging to the family Convolvulaceae this family comprises overall 2000 species in the world. There are all species in that Ipomoea obscura having many medicinal uses also previously whole plant used traditionally and folk medicine. This plant is a slender, twinning perennial herb it looks like pale yellow flowers with deep purple throats adorn with vine like vigorous smell, heart shaped leaves. Phytochemical investigation of *Ipomoea obscura* observed different phytoconstituents such as Flavonoids, tannin, alkaloids, glycosides, phenols, phlobatannins and saponin, Steroids, terpenoids, protein, polyphenol, coumarins and sesquiterpene-rich volatile oil. *Ipomoea obscura* exhibited different activities like antimicrobial, antifungal, hepatoprotective, antioxidant, nephroprotective, cytotoxic/anti-tumour, anti-inflammatory and anti-diarrhoeal activity. This review expressed about different phytoconstituents that containing whole plant i.e. seeds, leaves, stem and flower aerial part by using different solvent like 70% methanol, ethanol, petroleum ether, ethyl acetate, water, acetone and chloroform and also expressed different activities having major role for curing the diseased condition.

Keywords: ipomoea obscura, phytoconstituents, pharmacological activities and extracts

1. Introduction
Family Convolvulaceae is recognized as morning glory family. Nearly 2000 species of 58 genera spreaded overall the world. In India family Convolvulaceae constituted about 20 genera and 158 species in southern and western regions [1]. It is native to India, Africa, Asia, pacific islands and Sri Lanka. Plant grows usually in rainy season from June-august and flowering time of the plant is from October to January [2]. *Ipomoea obscura* commonly known as obscure morning glory or small white morning glory, *Ipomoea luteola*, Pugali, Bokadi [3]. Synonym of *I. obscura* are convolvulus obscures, *Ipomoea fragilis choisy*, *Ipomoea acanthocarpa(Choisy*) Asch ad Schweinf., *Ipomoea inconspicua Bak., Ipomoea luteola*, *Ipomoea insuavis Blume*. Hindi name it's called as pan-bel, Laksmana, Vachagandha [1].

Ipomoea obscura (L.) Ker-Gawl is a slender, twinning perennial herb found all over India [4], *Ipomoea obscura* (L.) commonly known as ''Laksmana'' in Ayurveda belonging to the family Convolvulaceae. It is small climbing vine, with small cordate leaves and acuminate apex. Corolla composed of five fully fused petals.

Ayurveda has recognized many medicinal properties of this plant used against dysentery, applied to open sores and pustules. Paste of leaves applied on ulcers, haemorrhoids and swelling. Seeds and fruits are used as cleansing agent to improve difficulty in breathing, relieve pain, also has ornamental value as climber with attractive flowers. Whole plant used as Stomach ulcer, cough, asthma, cold, pain, induces conception, rheumatoid arthritis, wound, sprain and stomach-ache [5].

Fig 1: *Ipomoea obscura*

2. Phytochemical constituents

Table 1: Bioactive compounds from *Ipomoea obscura*

Part of plant	Extract used	Phytocostituets / structures		References
Leaves	Aqueous	Flavanoids, tannin, alkaloids, glycosides, phenols, phlobatannins and saponin, Steroids, terpenoids, protein, polyphenol and coumarins		6,10
	Petroleum ether	Alkaloids, coumarins, flavanoids and phenolics Saponins, cardioglycosides		6 R. Saravana *et al* 2014
	Chloroform	Alkaloids, steroids, coumarins, phenolics Flavanoids, terpenoids, carbohydrate		6 R.Saravana *et al* 2014
	Acetone	Alkaloids, steroids, triterpenoids, coumarins		6
	Alcohol/ 70% methanol	Alkaloids, steroids, coumarins Tannins, saponin, flavaoids, terpenoids, triterpenoids, carbohydrate, protein, anthraquinone, polyphenol and glycoside		6 10
	Ethanol	Alkaloids, saponin, steroids, cardioglycosides, oils and fats, aminoacids and proteins and carbohydrate		17
	Ethyl acetate	Alkaloids, tannin and phenolics compound, cardioglycosides, oils and fats, aminoacids and carbohydrate		17
Stem	Aqueous	Alkaloids and flavonoids		6
	Petroleum ether	Alkaloids and flavonoids		6
	Chloroform	Alkaloids, phenolics and coumarins		6
	Acetone	Steroids, triterpenoids, coumarins and phenolics		6
	Alcohol	Alkaloids, steroids and coumarins		6
Seed	Aqueous	Alkaloids		6
	Petroleum ether	Alkaloids, steroids, coumarins and phenolics		6
	Chloroform	Steroids, coumarins and phenolics		6
	Acetone	Alkaloids, steroids and triterpenoids		6
	Alcohol	Steroids		6
	Methanolic	Indole alkaloids (Ipobscurine –B, Ipobscurine- C, Ipobscurine-D		18
Flowers aerial part	using gas chromatography equipped with a flame ionisation detector GC-FID	Major	Minor	4
		A-bulnesene (23.8%), α-humulene (13.7%) and seychellene (11.2%)	A-guaiene (8.3%), B-caryophyllene (7.1%), γ-terpinene (4.2%), hexadecanoic acid (3.0%) and β-elemene (2.7%).	

Following structures were found plant part of seed using methanolic extraction [18]

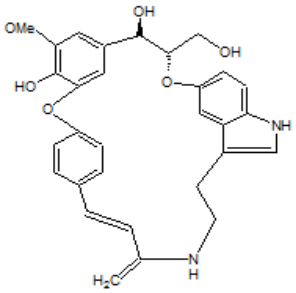

Fig.a

Fig a: Ipobscurine C

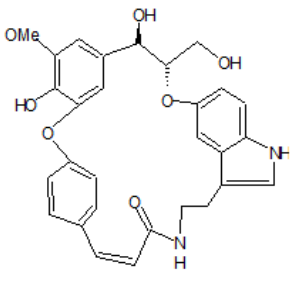

Fig.b

Fig b: Ipobscurine D

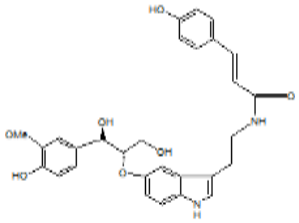

Fig.c

Fig c: Ipobscurine B

3. Pharmacological profile

Ipomoea obscura has wide range of biological activities. Various studies disclosed the confirmation of its developing acceptance in different ailments basically in Ayurveda, siddha, Unani and folk system of medicine that is used in form of extract.

3.1. Antimicrobial activity

Recent study showed that *Ipomoea obscura* has antimicrobial activity that is screened against six bacterial strains by using different type of extract like petroleum ether, chloroform, acetone, ethanol, water. It has been studied that preliminary phytochemical screening, antibacterial activity of various extracts of different part of plant that is seed, stem, leaf so it was found that presence of different phytoconstituents like tannin, flavonoid, coumarins, emodin, anthraquinones, anthocyanidins.

Antibacterial activity was carried out using different extracts

and modified agar well diffusion method against human and plant pathogenic bacteria i.e. staphylococcus aureus, Bacillus subtillis, Rhodococci sp., Bacillus stearothermophilus (Gram +ve); Escherichia coli, Proteus vulgaris, Salmonella sp., Pseudomonas sp. (Gram –ve). So it was observed that leaf, stem and seed extract of *Ipomoea obscura* showed inhibition of bacteria, stem sample was observed that have more capacity as compared to leaf and seed samples. Mainly all three samples in case of Salmonella sp. inhibit greater range of zone of inhibition diameter and Salmonella is causative agent for liver disorder so it can be proven that this plant have hepatoprotective activity [6].

In this study, *Ipomoea obscura* plant extract tested for antimicrobial activity, in that different extracts were used i.e. Petroleum ether, Methanol, Water extract against four different bacterial (E. coli, Salmonella typhi, Bacillus subtilis, Bacillus steartothermophillus) strains using cup plate method and zone of inhibition was calculated for each strain. It was observed that all three-extract showed moderate antibacterial against four microbial strains, the diameter of zone of inhibition was measured if diameter is less than 12 so considered no antibacterial activity. So hence it's proved that this plant shows antimicrobial activity [7].

3.2. Antifungal activity

In this study, different extracts of plant used i.e. Petroleum ether, Methanol, Water extract for evaluation of antifu*ngal* activity, here they evaluated against two fungal strains (Candida albicans, Aspergillus Flavus) strains using cup plate method and zone of inhibition was calculated for each strain It was observed that all three extract showed moderate antifungal activity against two fungal strains, diameter of zone of inhibition was measured if diameter is less than 12 so considered as no anti-fungal activity [7].

3.3. Antioxidant activity

According to the recent study aqueous extract of *Ipomoea obscura* fresh and dry leaves was investigated for phytoconstituents, anti-inflammatory, antioxidant activity and flavonoids, total phenols, alkaloids, tannins were evaluated.

According to the recent study aqueous extract of fresh and dry leaves was investigated for phytoconstituns anti-inflsmmatory.

Antioxidant activity was performed by using DPPH radical scavenging activity, nitric oxide radical scavenging, hydrogen peroxide assay and reducing power assay so result observed that DPPH radical scavenging activity of the dry leaves extract found more potent than fresh leaves extract at various concentrations. (The Butyl hydrated anisole and ascorbic acid used as standard). Hydrogen peroxide assay showed that both dry and fresh leaves extract show high activity than ascorbic acid. Nitric oxide radical scavenging activity showed that fresh leaves found more potent than dry leaves extract and reducing power assay in that fresh leaves showed greater than dry leaves extract and butyl hydrated anisole and ascorbic acid at different concentrations. So, it concluded that fresh leaves showed more antioxidant activity than dry leaves extract [8].

In these study, free radical scavenging activity of *Ipomoea obscura* evaluated by using whole plant different extracts i.e. (petroleum ether, methanol and water). Estimation of total phenolic content and vitro antioxidant activity using 2,2'-diphenyl-2-picryl hydrazyl (DPPH), 2,2'-azino-bis(3-ethyl-benzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), hydrogen peroxide, nitric oxide, superoxide, hydroxyl radicals by P-nitroso dimethyl aniline (p-NDA) and deoxyribose assay and IC5 0 value were calculated. The phenolic compound of extracts was expressed as gallic acid equivalent in mg/g of extracts. And observed that methanolic extract has highest phenolic compound as compared to water (methanol has 18.15mg/g and water has 9.12 mg/g) but petroleum ether has no phenolics compound.

Antioxidant activity were carried out by using seven methods so observed that in methanolic extract showed good antioxidant activity in ABTS, DPPH, hydrogen peroxide, and nitric oxide radical scavenging method with IC50 values of 53±0.33, 108.40±2.15, 107.90 ±1.20 and 424± 2.90 µg/ml. In water extract showed moderate activity in ABTS, DPPH, hydrogen peroxide and nitric oxide radical scavenging method with IC50 values of 103.60±0.57, 224±5.30, 215.83±1.50 and 505.12±3.30 µg/ml. And petroleum ether showed that moderate to low activity in ABTS, DPPH, hydrogen peroxide and nitric oxide radical scavenging methods with IC50 values of 203.33±1.66, 250.00±4.80, 235±1.44 and 534.40±3.66 µg/ml. All three-extract failed to express antioxidant activity in scavenging of superoxide radical by deoxyribose and and p-NDA method [9].

3.4. Hepatoprotective activity

Traditionally *Ipomoea obscura* used to treat liver disorders. In recent study identification of the phytochemicals, histochemical, fluorescence, GCMS analysis and hepatoprotective activity of *Ipomoea obscura* leaves. Extracts were used aqueous and 70% methanol. Histochemical study was performed by treated with pholoroglucinol and conc. Hcl so it showed presence of phytochemicals like lignin, flavanoid, alkaloids and tannins. Florescence study can be investigated by day light (245nm) and under U.V. light (365nm) in that plant leves was carried out in different reagent like methanol,H2SO4,HCl, HNO3,NaOH, acetone, hexane, chloroform, and distilled water so observed that ultraviolet light produces fluorescence in different products as compared to day light.

Phytochemical screening was carried out so observed that presence of different phytoconstituents in water and 70% methanol extract, aqueous extract showed presence of tannin, carbohydrate, saponins, terepenoids, phenolics, phlobatannins, protein flavanoids, glycosides, and steroids but triterpenoids, anthraquinone were absent. In case of methanolic extract presence of alkaloids, steroids, saponins, triterepenoids, phenolics, anthraquinones, flavanoids, tannin, protein, carbohydrate but phlobatannin was absent.

Vitro hepatoprotective activity performed by using CCl4 induced hepatotoxicity model in that 5 groups were divided first group received normal vehicle, second group received CCl4, third group received CCl4+100mg leaves of I. obscura, fourth group received CCl4+ 250mg leaves extract and last fifth group received CCl4+ 500 mg leaves extract. Assessment of biochemical estimation that is AST/GOT (aspartate aminotransferase) and ALT/GPT(alanine amonitransferase), determination level of SOD, CAT and GPx (reduced glutathione) also MDA (malondialdehyde it's an secondary product of lipid peroxidation) result observed that elevation level of enzymes that indicate hepatocellular injury in group of second as compared to other groups.

Other antioxidant status showed that SOD CAT, GPx indicated alone ccl4 treated group contains more antioxidant level as compared to other groups, so overall its concluded that leaves of I. obscura is source of phytochemicals and possess hepatoprotective activity that can be important in oxidative stress diseases like diabetes, cancer, arthritis [10].

This study carried out for assessment of hepatoprotetive activity on the basis of preliminary phytochemical screening of *I. obscura*, here antibacterial and phytochemical screening both are evaluated. In that antibacterial test were carried out using different extracts and modified agar diffusion plate method against both human and pathogenic bacteria it showed that leaf and stem extract shows more inhibit bacterial growth so confirmed antibacterial activity. Mainly in phytochemicals screening used that different part of plant i.e. leaf, stem, seed and various extracts like petroleum ether, chloroform, acetone, alcohol, water so observed that phenolics compound, emodins, anthocynidins, anthocynanins, anthracene derivative, anthraquinone, saponins these were found on the basis of different Rf values.

So plant contains different phytoconstituents and antibacterial activity so it concluded that this plant have hepatoprotective activity [8].

3.5. Anti-inflammatory acitivity

Ipomoea obscura aqueous plant extracts showed anti-inflammatory activity of the fresh leaves ad dried leaves, by performed in vitro acitivity using Human red blood cell membrane stabilization method and inhibition of albumin denaturation method, in that antioxidant acitivity showed HRBC stabilizaittion activity and albumin denaturation method of dry leaves material was found higher than fresh leaves extracts as also compared to diclofenac [8].

Recent study showed that methanolic extract of plant I. obscura was used for vivo and vitro anti-inflammatory activity, plant extract 10mg/kg b.w. was given both chronic and acute model. models used that are acute a)caraggenan induced model b) dextran induced model and formalin induced model (Chronic) also determination of effect of plant extract on proinflammatory cytokines, NO, CRP (C-reactive protein) and IL-1β, TNF-α and IL-6, determination of effect of plant extract on TNF- α produced by microphages in vitro, determination effect of plant on serum CRP and proinflammatory cytokines levels in LPS, also determine iNOS, COX-2 and proinflammatory cytokines gene expression in LPS- activated macrophages of iNOS, Cox-2, IL-1β, IL-6,TNF- α by using PCR.

So result observed that subplanter injection carageenan induced more swelling of paw volume in control and vehicle control group after that plant extract treated animals showed no effect upto 1 hr after that 2hr., extract showed significant reduction in paw edema, 55.5% produced inhibition of paw thickness. In another case when dextran injected maximum paw thickness was observed, on 3rd day treated with plant extract showed reduction in paw thickness and inhibition of paw edema (42.06%) also inhibition in paw edema in plant extract treated group in case of chronic inflammatory model using formalin, inhibition of paw thickness was 65% was observed on the second day when compared to control.

Effect of i. obscura plant extract on serum proinflmmatory i.e. significant reduction of level of TNF-α, IL-1β and IL-6 as compared to control group. Also inhibit serum level CRP and NO level during inducing inflammation.

In vitro techniques checked effect of plant extract on TNF-α by macrophages, here L929 cells were treated with culture supernatant that is collected from macrophages culture of LPS (lipopolysaccharide) alone treated animals so it produces 100% cytotoxicity. After that culture cells treated with LPS along with I. obscura extract so observed that 12% cytotoxicity shown so it's clear that plant can inhibit cytotoxicity that is induced by LPS. Effect of plant extract on NO and CRP levels in LPS stimulated animals there is increase level of CRP and NO in LPS treated animals as compared to normal after when treated with plant extract significantly reduction in CRP and NO level as compared to alone LPS treated animals. Proinflammatory cytokines levels such as IL-1β, IL-6 and TNF- α so this level of cytokines level gets down when treated with plant extract (I. obscura) as compared to LPS alone treated animals. Determination of iNOS, COX-2 and gene expression in LPS-activated macrophages in that RT-PCR technique was used, when LPS induced there were detect amount of of iNOS and COX-2 levels increased. And when treated with I. obscura there was no total inhibition of levels but only 29.95% and 35.8% expression of iNOS and COX-2. The proinflammatory cytokines such as IL-1β, IL-6 and TNF- α was clearly expressed in LPS administered animals, but mRNA expression of IL-1β, IL-6 and TNF- α significantly inhibited when treated with I. obscura [11].

This study I. obscura plan ethanolic extract used as anti-inflammatory its evaluated by; carrageenan induced paw oedema, cotton pellet granuloma in rat's Preliminary phytochemical investigation showed the presence of glycosides, saponins, flavonoids, tannins and steroids. These observations prove scientifically the anti-inflammatory activity. in carrageenan (0,1ml of 1% suspension was injected sub planter region right hind paw) and ethanol extract (250 and 500 mg/kg body weight) was administered before 1hr of carrageenan administration so paw volume measured by plethysmometer so result observed that greater percentage inhibition and paw volume decrease as compared to normal control animals in case of I. obscura and indomethacin. And another method cotton pellet granuloma in that cotton pellet 20 mg was introduced subcutaneously into rats under anaesthesia. They treated orally with the ethanol extract of I. obscura (250 and 500 mg/kg body weight) for six days. In case of Control animal's 1 ml/kg tween 80, and indomethacin (5 mg/kg p.o.) was administered after last day animals undergo anaesthesia cotton pellet were removed and weighed. So, result observed that reduction in the post implantation weight of the cotton pellets as compared to control group [12].

3.6. Antidiarrheal activity

In this study ethanolic extract of *I. obscura* plant used for antidiarrheal activity, model were used castor oil induced diarrhoea and charcoal meal test in mice.in castor oil induced diarrhoea plant extract *I. obscura* (250 and 500 mg/kg body weight) given to mice and normal control group received tween 80 (1ml/100g) and reference used as loperamide (2mg/kg p.o.) And 1 hr later castor oil was given (1ml/100g). So, result observed that no of faeces reduced when treated with *I. obscura* and loperamide as compared to control group.

And another gastroinstinal motility test was carried out on mice were divided into 4 groups, 6 animals each. Administration of ethanol extract of *I. obscura* (250 and 500

mg/kg. p.o.). Control animals given 10-ml/kg tween 80; loperamide (2 mg/kg p.o.) was used as reference drug. 1 mL of 10% charcoal suspension in 5% acacia solution was given to each mouse orally after 30 min. After 20 min animals were sacrificed and the abdomen was opened. The small intestines removed. Distance measured that travel charcoal meal from the pylorus to ceacum. The entire length of the small intestine was also measured. So result observed that both doses of I. obscura showed less distance travelled by charcoal meal to gastrointestinal tract.

So, it concluded that ethanolic extract of I. obscura have antidiarrheal activity [12].

3.7. Nephroprotective activity

Recent study proved that curative and preventive effect of I. obscura in nephrolithiasis induced in rats. It carried out by dividing 5 groups i.e. 1st group as normal, second, third fourth, fifth group received 1% ethylene glycol in drinking water for 28 days and group third and fourth received 100mg/kg and 200mg/kg b. w. of plant extracts and last group received cystone i.e. standard (200mg/kg of b. w.) evaluation parameters are body weight, urinary and serum data, urine collection done at 0, 14 and 28 days so result observed that the when individual treated ethylene glycol animals so there were increased excretion of creatinine, uric acid, calcium, phosphate, and protein in urine when administration of plant extract of (100mg/kg and 200mg/kg b. w.) significantly reduced the elevated level of creatinine, uric acid, calcium, phosphate, and protein in urine also kidney and also slowdown of kidney stone formation and body weight increases in plant extract treated animals as compared to control group. Treatment with extract increased reduced volume of urine also improvement of cellular integrity and reduced crystal deposition so it proved that they have curative ad preventive effect nephrolithiasis in rats [13].

Nephroprotective effect was observed of *Ipomoea obscura* plant extract by gentamycin inducing nephrotoxicity. Gentamycin causes inhibit protein synthesis in cells and increase level of serum urea, creatinine clearance, urea secretion. So gentamicin was used to induced nephrotoxicity, in that gentamycin was given at dose 40mg/100g b.w. i.p.ad another group received gentamycin + powder of I. obscura (100mg/100g body wt.), evaluation parameters are AST, ALT, phosphatases, transaminases, serum creatinine, blood urea, uric acid and Cystatin-c (if cystatin-c level is too high observed then kidney is not working properly) cystatin-c used mainly biomarker of kidney function and assessment of histopathological examination. So overall result observed that increase level of serum urea, creatinine clearance, urea secretion, ALT, AST and cystatin-c in gentamycin treated group, significantly reduced level of serum urea, creatinine clearance, uric acid, ALT and AST and cystatin-c when treated with I. obscura.

In case of histopathological examination normal architecture was observed in case of control group, gentamycin treated group showed that renal lesions including tubular and focal necrosis, inflammation and glomerular congestion. After lesions were reduced when treated with plant extract. So hence its proved that plant has nephroprotective activity [14].

This study showed that I. obscura have nephroprotective activity on cyclophosphamide induced Nephrotoxicity and urotoxicity. Here 4 groups were divided 1st group received normal saline I .p. for 5 days, another three group received CP (1.5mmol/kg b. w.), group three animals received CP along with I. obscura plant extract (10mg/kg b. w.) and last group was administered MESNA (sodium 2-mercaptoethane sulfonate) and after at the time of scarification animal weight, kidney and bladder weight recorded. Parameters were evaluated like biochemical investigation i.e. serum urine, total protein, serum creatinine and urea nitrogen, measurement of antioxidant status like GSH (reduced glutathione), SOD (superoxide dismutase), MDA (malondialdehyde) and CAT (catalase), histopathological examination were carried out in both kidney and urinary bladder.

Determination of TNF-α, IFN-γ, IL-2. Results were observed in both kidney and bladder that BUN(blood urea nitrogen), urine nitrogen levels and creatinine increased in group of alone treated cyclophosamide and when treated with plant extract elevated levels were decreased, In case of total protein level get decreased in CP alone treated animals when treated with I. obscura protein levels get elevated. In antioxidant activity SOD, CAT and GPx are significantly decreased as compared to normal, administration of I. obscura levels of antioxidant are increased.

Morphological examination observed for at 4hr, 24hr and 48 hr in that CP alone treated animals group urinary bladder get inflamed and red colouration due to haemorrhage where CP along I. obscura showed less inflammation and normal colouration, So better results were observed at 48hr. Histopathological examination on urinary bladder 4 hr after CP administration showed mild nuclear pleomorphism of epithelial cells and mild hyperplasia, papillary formation, scattered lymphocytes was found then after 24 hr metastatic epithelium observed mitotic activity, wall of bladder look like oedema, after 48 hr area of necrosis of the mucosa, infiltration of lymphocytes, few polymorphs and bladder of wall like oedema, epithelium replaced with necrotic tissue and cells structure get severe inflamed. After treatment with I. obscura effect observed after 4hr, 24, and 48 hr of CP administration bladder structure does not vary much as than normal. Histopathology of kidney in case of CP alone treated animals after 4hr oedema ad decreased cellularity, renal tubule shows hypodropic degeneration, interstitial cells shows necrosis. After 24 hr lymphocytes and necrosis observed, by 48 hr renal tubule observed oedema and vessels get congested haemorrhage when treated with I. obscura along with CP after 4, 24 and 48 hr glomeruli look normal, mild haemorrhage and few inflammatory cells [15].

3.8. Cytotoxic/anticancer and anti- tumour activity

Present study evaluated that cytotoxic activity of I. obscura plant three extract (petroleum ether, water, methanol) tested against three human cancerous and three normal cell lines by using MTT(3-(4, 5,-dimethyl-2-thiazolyl)-2,5-dipheyl-2H-tetrazolium bromide) and SRB (Sulforhodamine B) assays. Three normal culture cells (Vero-African green monkey kidney, BRL-3A-normal rat liver and MDCK-normal dog kidney) and three cancerous cell lines (e HeLa-human cervical cancer cells, HEp-2-human larynx epithelial cancer cells and A-549-human small cell lung carcinoma cells) were used to determine cytotoxicity. Cytotoxicity expressed as the concentration of test drug kills cells by 50% (CTC50). The water extract showed moderate cytotoxicity against all the cell lines and values ranging of CTC50 from 299.9 µg/ml to 368.06µg/ml. Petroleum ether

showed its nontoxic dose at all cell lines and methanol shows less toxic towards normal cell lines Vero, BRI-3 A ad MDCK when compared to cancerous cell lines. So overall it is concluded that any extract does not show any toxicity [7].

In this study effect of I. obscura observed for anti-tumour effect, methanolic plant extract was given i. p. to mice (10mg/kg b. w.) before inducing tumour. This methanolic extract was 100% toxic at concentration of 500 µg/ml for both Dalton's lymphoma ascites (DLA) ad Ehrlich ascites carcinoma (EAC) cells. Methods which are used in cytotoxicity determination of short-term cytotoxicity in that DLA and EAC were incubated with diff concentration of I. obscura (10-500 µg/ml) after incubation percentage of cytotoxicity was determined by using tryphan blue dye exclusion method.

2ndly effect checked for tumour cell proliferation (^{3}H-Thymidine Incorporation Assay) in that cells were cultured with plant extract (1, 2 and 5 µg/ml) and vehicle used i.e. 0.1% DMSO later period of incubation at 6, 30 and 54 hr at 37°c in 5% CO_2 atmosphere. After ^{3}H- Thymidine added each well and continue incubation for additional 18 hr. Later incubation plates kept for centrifuge and culture supernatant removed and washed three times with PBS and treated with ice cold PCA lastly precipitate was collected and dissolved in 0.5N NaOH, adding to the scintillating fluid and radioactivity counted and percentage of inhibition was calculated.

Effect of I. obscura on tumour development in that 3 groups were divided 1st treated with *I. obscura* (10 mg/kg b. wt.) for 10 days group II and III kept as untreated and vehicle control (1% gum acacia) then solid tumour induced by injecting DLA S.C. to right hind limbs all groups, tumour was measured by using digital Vernier calliper at 3-days intervals for 1month and volume of tumour was calculated. last method survival tumour bearing animals in that animals were divided into 3 groups all animals were injecting EAC cells i.p. except normal group, this taken as day 0 and group I only treated with EAC then group II received I. obscura (10mg/kg b. wt.) for 10 days and lastly group III received 1% gum acacia served as vehicle control. Calculation of death pattern of animals due to burden and percentage of increasing life span was noted.

So overall result observed that first method I. obscura showed that 100% toxic at concentration of 500 µg/ml for both DLA and EAC cells. In tumour cell proliferation DLA and EAC cells showed very high rate of proliferation observed (100%) in vehicle control, when treated with I. obscura inhibition of proliferation of DLA and EAC cells of dose and time dependant manner. 5 µg/ml concentration of plant extract was very effective for 100% total inhibition of proliferation after 72 hr. In tumour development method reduction of solid tumour which is induced by DLA in I. obscura treated group as compared to normal group. volume of tumour also decreases in I. obscura treated group as compared to other, tumour volume of untreated and normal control group shows 4.92cm^3 and 4.53 cm^3 where in case of I. obscura tumour volume is 0.84 cm^3. And last method life span of EAC tumour bearing mice treated with I. obscura found to be increased as compared to another group. So, it was concluded that I. obscura have anti-tumour activity [11].

Anti- angiogenic activity of I. obscura determined by vitro angiogenesis and vivo angiogenesis assay. In vitro method carried out using human umbilical vein endothelial cells and vivo carried out using B16F10 melanoma cells. Vitro methods were MTT, HUVEC, Scrape wound assay, Trans well, Tube formation assay, Rat aortic ring assay, Gelatin zymography and Expression studies. Vivo methods are Capillary formation, Pro-and anti- angiogenic factors and Serum nitrite level. Vivo angiogenesis was induced by injecting B16F10 melanoma cells intradermally on the shaven ventral skin of mice and In vitro induced by using human umbilical vein endothelial cells (HUVECS). And also, determination of pro- and anti- angiogenic factor i.e. estimation of IL-1β, IL-6, TNF-α, GM-CSF, VEGF ad TIMP-1.

Result were observed that in MTT assay percentage viability observed after treatment with plant extract or IPO-A found non-toxic at below concentration 10 & 5 µg/ml (concentration are used 1,2, and 5 µg/ml for plant extract and IPO-A 0.05, 0.1and 1 µg/ml used.) Proliferation assay in that rate of proliferation was determined by 3H-thymidine incorporation & its expressed as radioactive count per minute (cpm) so HUVECS shows high rate of proliferation when stimulated with VEGF after that administration of plant extract show inhibition of rate of proliferation(concentration for plant extract at 5 µg/ml and IPO-A at 1 µg/ml showed inhibition effect). Another scrape wound assay in that checked motility/ migration of endothelial cells, plant extract and IPO-A showed inhibition of VEGF induced migration of endothelial cells at 5 and 1 µg/ml. In trans well assay observation of invasive property through collage matrix so HUVECS in that large no of cells observed below the surface of polycarbonate membrane.

After administration of IPO-A and extract significant inhibition of invasion at 1 & 2 µg/ml. Tube formation assay when treated with extract and IPO-A show inhibited of tube formation in dose dependant manner ad in case of HUVECS on matriged with VEGF formation of tube. and in case of rat aortic ring assay, untreated B16F10 cells show growth of micro vessel of aortic ring as compared to when treated with extract (5 µg/ml) and IPO-A (1 µg/ml) treated with B1610 melanoma cells so observed that inhibition of micro vessels outgrowth of aortic ring. Gelatin zymography and expression studies showed satisfied result.

In vivo angiogenesis capillary formation method carried out inducing tumour capillary formation and pro-inflammatory mediator analysis so observed that after treated with extract and IPO-A there is reduction of tumour formation. In pro- and anti-angiogenic factor, control group elevation of serum level IL-1β, IL-6, TNF-α, and GM-GSF, after treated with extract ad IPO-A decreased the serum levels of level IL-1β, IL-6, TNF-α, and GM-GSF. And levels of serum nitrite levels in control groups after induction of angiogenesis & level was decreased after administration of extract [16].

Conclusion

The plants of the genus ipomoea long used in used in folk medicine for treating various disease conditions. They have been long used for as anti-inflammatory, analgesics, colic disorder, constipation and digestive disorders, but recently scientist taken interest in ipomoea obscura species it is already reported different uses about *I. obscura* i.e. on dysentery, also paste of leaves applied on ulcer, haemorrhoids and swelling. Also whole plant used as cough, asthma, rheumatoid arthritis, wound and stomach-ache. This plant previously reported different pharmacological activities like Antimicrobial, antibacterial, antifungal, antioxidant, hepatoprotective activity, anti-inflammatory

activity, antidiarrheal, nephroprotective, cytotoxic/ anticancer and anti-tumour activity. Recently scientist or researcher focused on extract of plant so we can obtain different phytoconstituents like alkaloids, flavonoids, glycosides, phenols, saponins, polyphenols, steroids, terpenoids, coumarins and cardio glycosides present in different extracts like petroleum ether, methanol, ethanol, ethyl acetate and other extracts. Recently looking for future prospective different chemical structure groups or phytochemicals play major role in diseased condition. Each phytochemical showed different activities and used for prevention of anticancer, mainly show antioxidant activities i.e. mainly for prevention or slow down progression of free radicals so helpful for cure diseased conditions.

We can develop different phytomedicines for increase future purpose. Uses of synthetic drug can be chances of increased different reactions or toxicological reactions so better focused on medicinal plant that is safe for rodents and non-rodents, play major role for cure various disease. Further we can perform structure activity relationship of various phytoconstituents and isolation of active compound for deep features of intracellular pathway. Study may carry out of active constituents of Ipomoea obscura will lead to discovery of novel botanical drug. This plant also used traditionally in vivo and in vitro techniques for prevention or cure, we can implement different things for future prospective so this is an interesting plant for research.

List of Abbreviations

Table 2

2,2'-diphenyl-2-picryl hydrazyl	**DPPH**
2,2'-azino-bis (3-ethyl-benzo-thiazoline-6-sulfonic acid) diammonium salt	ABTS
P-nitroso dimethyl aniline p-NDA	(p-NDA)
Ug/ml	Microgram/millilitre
H2so4	Sulphuric acid
HCl	Hydrochloric acid
nm	Nanometre
CCl4	Carbon tetrachloride
SOD	Superoxide dismutase
CAT	Catalase
GPX	Glutathione peroxidase
ALT	Alanine transaminases
AST	Aspartate transaminase
I, obscura	Ipomoea obscura
b.w.	Body weight
IL-1β	Interleukin-1β
TNF-α	Tumour necrosis factor-α
IL-6	Interleukin-6
iNos	Inducible nitric oxide synthase
COX2	Cyclooxygenase-2
PCR	Polymerase Chain reaction
NO	Nitric oxide
CRP	C-reactive protein
Mg/kg	miligram/kilogram
p.o.	Per oral
MESNA	2-mercaptoethane sulfonate sodium
CP	Ceruloplasmin
MDA	Malondialdehyde
SRB	(Sulforhodamine B
MTT	(3-(4,5,-dimethyl-2-thiazolyl)-2,5-dipheyl-2H-tetrazolium bromide)
EAC	Ehrlich ascites carcinoma
DLA	Dalton's lymphoma ascites
HUVEC	Human umbilical vein endothelial cells
GM-CSF	Granulocyte-macrophage colony stimulating factor
VEGF	Vascular endothelial growth factor
TIMP-1	Metallopeptidase inhibitor 1
IPO-A	Ipobscurine-A
IPO-B	Ipobscurine-B

Conflict of Interest

Author declare no conflict of interest in this work.

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