World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

AN *IN VITRO* STUDY OF *IPOMEA CARNEA* AND *PIPER BETEL* LEAF EXTRACT MIXTURE ON ANTIDIABETIC AND BACTERIAL ENDOTOXIN NEUTRALIZATION

Saranya S.*[1], Ramachandran A. M.[2], Manju M.[3] and Harsha Karunakaran[4]

[1,3,4]Post Graduate and Research Department of Microbiology, Dr. N.G.P Arts and Science College, Coimbatore-641048, Tamil Nadu, India.
[2]Assistant Professor, Department of Microbiology, Dr. N.G.P Arts and Science College, Coimbatore-641048, Tamil Nadu, India.

Corresponding Author: Saranya S.
Post Graduate and Research Department of Microbiology, Dr. N.G.P Arts and Science College, Coimbatore-641048, Tamil Nadu, India.

Article Received on 11/06/2020 **Article Revised on 01/07/2020** **Article Accepted on 22/07/2020**

ABSTRACT

The main objective of this study was to analyse the rate of glucose transport across cell membrane in yeast cell system in the presence of the ethanolic extract of *Ipomoea carnea*and *Piper betel* leaves and to neutralize the bacterial endotoxin which causes wounds to the diabetic patient. *Ipomoea carnea* and *Piper betel* leaves has anti diabetic property and collected from kurumbapalayam. The ethanolic extract of leaves was prepared to analyse the phytochemical and antidiabetic activity done with the yeast cell system method. The lipopolysaccharide (LPS) from *E.coli* was separated using chloroform-methanol solvents. The LPS was treated with the leaf extract and monitored the neutralization of the *E.coli* toxin by SDS - PAGE method. The amount of glucose uptake was high in mixture of both leaf extract. The leaf extract of both *Ipomoea carnea* and *Piper betel* mixture was neutralized the LPS of *E.coli*. From this study we can reduce the adverse effect of allopathic medications and also acquire a permanentrecovery.

KEYWORDS: *Ipomoea carnea, Piper betle*, Diabetis, Bacterial endotoxin, Lipopolysaccharide.

INTRODUCTION

*Ipomoea carnea*contain variety of bioactive components such as phenolic acid, alkaloids, flavonoids, coumarins and sterols. Also having immense biological and pharmacological activities as an anti inflammatory, antioxidant activities anti diabetic, antimicrobial, wound healing, immune modulatory, antifungal and hepatoprotective (Kamal *et al.*, 2017).

Piper betle L. Piperaceae, a dioecious, annual creeper, climbing by many small adventitious rootless, grows to a height of about one m, generally grown in hotter and damper parts of the country. It is extensively found in damp forests and is propagated in India, Southeast Asia, Vietnam, and China. *P. betle L.* contains a various biologically active compounds and the variety of the plant has different concentrations, season, and climate. The pharmacological profile has shown antiplatelet, anti-inflammatory effects as well as immune modulatory, gastroprotective, and anti diabetic activity. The leaves are given for gastric and lung disorders in children and applied to purulent ulcers. They have a high content of potassium nitrate (0.26–0.42%). Glucose, fructose, maltose, and sucrose sugars are identified in *betel* leaves. Extracts of *P. betle L.* are used for the treatment of various ailments since ages due to its essential properties such as antibacterial, antioxidant, anticancer, andantiallergic. Theaqueousandalcoholicextract of *Epipremnumaureum*was determined to anti diabetic activity using alloxan-induced diabetic rats. Flavonoids might be producing the hypoglycemic effect by a mechanism independent of insulin secretion, e.g., by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. Hence, this study was taken up to investigate the antidiabetic and antioxidant activities of the *P. betle L.* in induced diabetic fish. This is the first report to antidiabetic activity of *P. betle*L. extract in induced fish model (Perumal and Saravanabhavan, 2018).

Diabetes Mellitus is an established non - communicable disease and often described as fourth or fifth leading cause of mortality in high income countries. According to world health organization the global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025 majorly in the developing countries (Pitchaipillai and Ponniah, 2016).

Basically diabetes mellitus is classified into two types, insulin dependent diabetes (type 1) and non- insulin

dependent diabetes (type 2). Type 1diabetes is an autoimmune disease characterised by a local inflammatory reaction in and around islets that is followed by the selective destruction of insulin secreting β – cells. Type 2 diabetes is characterised by peripheral insulin resistance and impaired insulin secretion. In modern medicine, there is still no satisfactory effective drug or therapy to cure diabetes. However, there are many synthetic drugs available as oral hypoglycaemic agents and as to treat diabetes but continuous use of synthetic drug cause severe side effects and highly expensive (Shettar and Vedamurthy, 2016).

Endotoxin ac as a heat-stable, cell-associated material isolated from Vibrio cholerue which induced toxic reactions in guinea-pigs. This material to be clearly distinguishable from the heat-labile exotoxins which are secreted by bacteria. Endotoxin consists of polysaccharides and a lipid part and, therefore, was termed lipopolysaccharide (LPS). Both terms endotoxin and LPS are used for the same molecule and thus represent synonyms. Lipopolysaccharides (LPS; endotoxin) are cell wall constituents of most Gram-negative bacteria. Despite being essential for bacterial survival, as they shield the bacterium from cellular host defense strategies, bile acids and hydrophobic antibiotics, LPS play a prominent role in the infected host during severe infections, trauma, and shock. The presence of LPS in the bloodstream, as observed during severe Gram- negative, bacterial infections (notably after application of antibiotics) or possibly caused by translocation of enterobacteria from the gut, leads to various pathophysiological reactions such as fever, leukopenia, tachycardia, tachypnoea, hypotension, disseminated intravascular coagulation and multi-organ failure. The resulting septic shock syndrome has a mortality rate of 20-50% and causes approximately 100,000 deaths annually in the USA. On the other hand, low doses of LPS are thought to be beneficial for the host, e.g. by causing immune stimulation and enhanced resistance to infections and malignancy. The harmful as well as the beneficial host responses to LPS are mediated by endogenous mediators, cytokines, which are released by various cells, e.g. monocytes/macrophages, vascular cells, polymorphonuclear cells and T cells, the most important cytokine- producing being the cells of the monocyte/macrophage lineage (Holst *et al.*,996).

MATERIALS AND METHODS

Sample Collection

The leaves of *Ipomoea carnea*and *Piper betle*collected from kurumpapalayam, Coimbatore district, Tamil Nadu, India. The plant was identified and authenticated by Botanical Survey of India. The water sample collected from ukkadam lake, coimbatore district, Tamil Nadu, India.100 ml water sample was collected and transferred it into disposable sterilized test tubes. The pH of water was tested by ph paper. After collection of sample the test tubes were tightly closed to avoid other contamination.

Extraction preparation

The ethanolic extract leaves of *Ipomoea carnea*and *Piper betle*was used for in vitro analysis. Leaves were cleaned, dried in a hot air oven (50° C) and then grinded into a fine powder in a grinder (Redfern*et al.*, 2014). The powdered plant material was subjected to sequential extraction by soxhlet extraction method using soxheltapparatus.

Phytochemical analysis

Alkalods test (mayer test)

1 ml of sampleuse added to a few drop of mayer's reagent. Appearance of white pale yellow color precipitate indicates the presence of alkaloids in the sample.

Flavonoids Test (H2So4)

To the sample 5 ml of dilute water was added in 2ml of ammonium hydroxide ammonia solution and those were added to a portion of the solution and 5ml of conc. amyl alcohol aqueous filtrate also added. The samples were made up to addition to concentrated H2SO4. A yellow mark and left to react for 30 mins for colouration observed in each extracts indicated the development. This was measured at 505 nm, presence of flavonoids.

Proteins (Biuret Test)

1% of NaOH use added to 1 ml of extract and few drop of 1% CuSo4 were then added. Blue purple or violet pinkish color indicates the presence of proteins.

Tannins Test

To 1 ml of the extract, 2ml of 5% FeCl3 is added which gives dark blue or greenish black color and a positive tannin test.

Steroids Test (Salvski Test)

1 ml of test sample was dissolved in 1 ml of chloroform and equal amount of conc. H2So4. Formation of bluish red to cherry color in chloroform layer shows the presence of steroids.

Saponin Test (Foam Test)

A small amount of extract was shaken with water and observed for the presence of foam.

Anthocyanin Test

1 ml of plant extract was treated with 1 ml of 2N NaOH then heated. Formation of bluish green color indicated the presence of Anthocyanin.

Starch Test

To 1ml of iodine solution is mixed in 1 ml of extract, formation of blue color indicated the presence of starch in the extract.

Glycosides Test

To 1 ml of plant extract, 1 ml FeCl3(5%) , and equal amount of acetic acid is added, then few drops of H2sSo4 is added to the mixture Greenish blue color indicates the presenceofglycosides.

Phenols Test
1 ml of plant extract, when treated with few drops of FeCl3 solution it gives blue green color and confirms the presenceofphenols.

Terpinoids Test
2 ml of chloroform and 3 ml of conc. H2So4 was added in 1ml of plant extract. A reddish brown precipitate at the interface, confirmed the presence ofterpinoids.

Redusing Sugar Test (Benedic Test)
1 ml of benedic reagent was added to the 1 ml of plant extract. Then the mixture was shaken well and placed in water both for 10-15 minutes formation of reddish precipitate indicates the presence of sugar in thesample.

In Vitro EvalutionOf Glucose Uptake By Yeast Cells
Bakers yeast was repeated washing by the use of centrifugation (3,000xg, 5 min) method in distilled water until the supernatant fluids were clear and 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1-5 mg/ml) were added to 1 ml of glucose solution (5, 10 and 25 mM) and further incubated for 10 min at 37 °C. Reaction was started by adding 100µl of yeast suspension, vortex and further incubation at 37 °C for 60 min the tubes were centrifuged (2,500x g, 5 min) and glucose was estimated in the supernatant. Metformin was taken as standard anti diabeticdrug used. The percentage of increase in glucose uptake by yeast cells was calculatedby the following formula:

$$\text{Activity\%} = \frac{[(\text{Abscontrol}-\text{Abssample})/\text{Abscontrol}]\times 100}{(\text{Increase in glucoseuptake})}$$

Where, Abs control is the absorbance of the control (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. Absorbance was measure at 540 nm and all experiments were carried out in triplicates.

Processing Of Water Sample
The water samples are streaked on prepared Eosin Methylene Blue medium and incubate for 24 hours at 37°C. After the incubation microbial growth is present in the plate Gram staining should be performed. Then Biochemical tests (Hemraj*et al.,* 2013) are should be performed for confirmation of organism.

Extraction Of Lippopolysaccharide (Lps) By Chloroform – Methanol Method: (Kalambhe*et al.,* 2017)
Lipopolysachharide (LPS) was extracted by Chloroform-methanol method. In brief, 100ml overnight grown broth culture of E. coli was centrifuged at 3000 rpm at 40C for 30 minutes. The supernatant was discarded and the pellet was re-suspended in two ml of 95% alcohol by vortexing and then was centrifuged for 10 min at 2000 rpm. The step was repeated four times. Finally the supernatant was discarded and pellet was dried by placing the tube inside hood to completely evaporate alcohol. The dried pellet was re-suspended in one ml of 10 % EDTA (quantity sufficient to dissolve a pellet) and was sonicated for 15 min. One ml of saturated methanol/chloroform (1:2 ratios) was added to bacterium- EDTA solution. The tube lid was covered by paraffin and kept on shaker for two hours following 10 min centrifugation at 2000 rpm. Three layers were formed; methanol, left biomass including cell lysate and chloroform layer from top to bottom. The biomass layer with cell lysate was discarded and chloroform and methanol layers were separated and poured into 50ml glass beaker to permit complete and quick evaporation of methanol and chloroform layers. The dried pellet thus obtained LPS. LPS pellet was weighed and reconstituted in sterilized PBS (quantity enough to dissolve a pellet completely) and stored at -200C until furtherused.

SDS PAGE
Proteins are separated by SDS PAGE method described in Roy and Kumar (2012).

RESULTS

In this study investigated the In vitro study of *Ipomoea carnea*and *Piper betel* leaf extract mixture on Antidiabetic and Bacterial Endotoxin Neutralization. The Plants *Ipomoea carnea*(BSI/SRC/5/23/2020/Tech/529) and *Piper betel* (BSI/SRC/5/23/2020/Tech/528) authenticated Botanical Survey of India, South Regional Centre, T.N.A.U. Campus, Coimbatore.

Phytochemical analysis
The Phytochemical analysis should be performed for aqueous extract and ethanol extract.

Aqueous Extract
Table 5.1: Phytochemical analysis for aqueous extract of *I. carnea*, and *P. betle.*

Compounds	***Ipomoea carnea***	***Piper betle***
Alkaloids	Present	Present
Flavonoids	Present	Present
Saponins	Present	Absent
Phenols	Present	Present
Glycosides	Absent	Present
Protein	Present	Present

Reducing Sugar	Absent	Absent
Anthocyanins and Beta cyanins	Absent	Absent
Coumarins	Present	Present
Terpenoids	Present	Present
Steroids	Absent	Present

Eathanol Extract

Table 5.2: Phytochemical analysis for ethanol extract of *I. carnea*and *P. betle.*

Compounds	***Ipomoea carnea***	***Piper betle***
Alkaloids	Absent	Absent
Flavonoids	Present	Present
Saponins	Absent	Absent
Phenols	Present	Absent
Glycosides	Present	Present
Protein	Absent	Present
Reducing Sugar	Absent	Absent
Anthocyanins and Beta cyanins	Absent	Present
Coumarins	Present	Absent
Terpenoids	Present	Absent
Steroids	Absent	Absent

The rate of glucose transport across cell membrane in yeast cells system it was evidenced in Figure 5.1 – 5.3. After the treatment of the yeast cells with the ethanolic extract of *Ipomoea carnea* and *Piper betle*, the glucose uptake was observed. The highest concentration of *Ipomoea carnea*and *Piper betle*mixture sample (5 mg/ml) exhibited maximum activity at all glucose concentrations and showed the maximum increase (86.12%) in the presence of at 25 mM glucose.

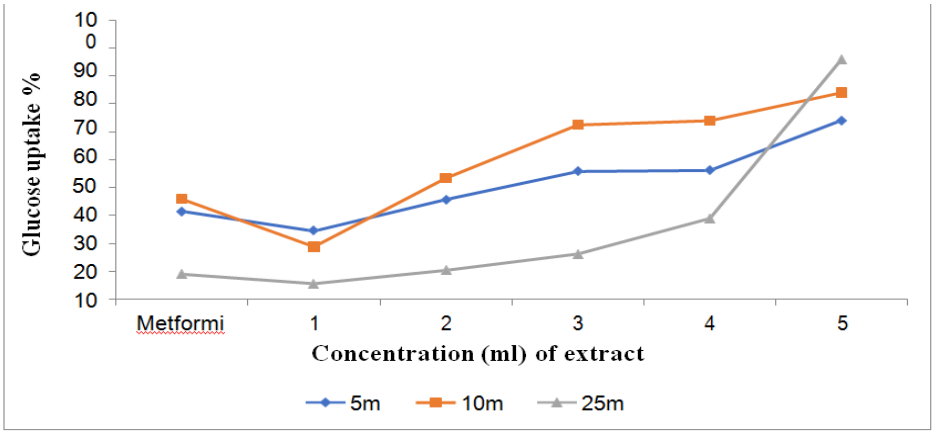

Fig. 5.1: Effect Of Extract Mixture On Glucose Uptake By Yeast Cells.

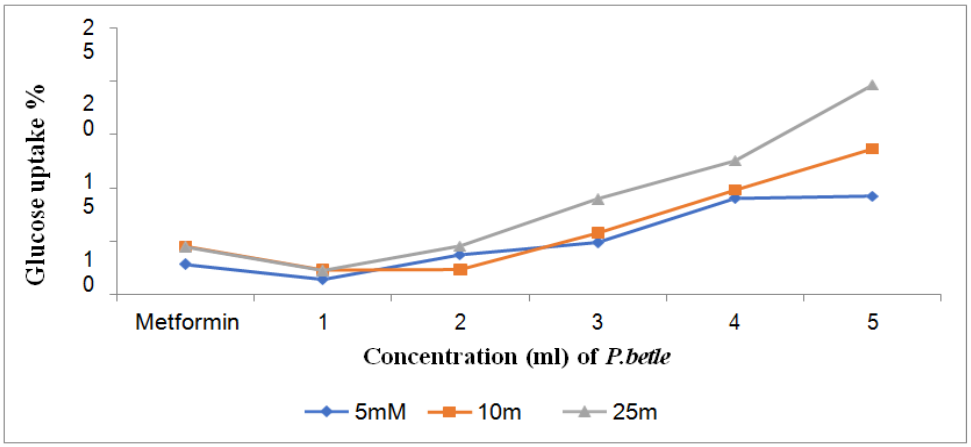

Fig. 5.2: Effect Of *P. betle* Extract On Glucose Uptake By YeastCell.

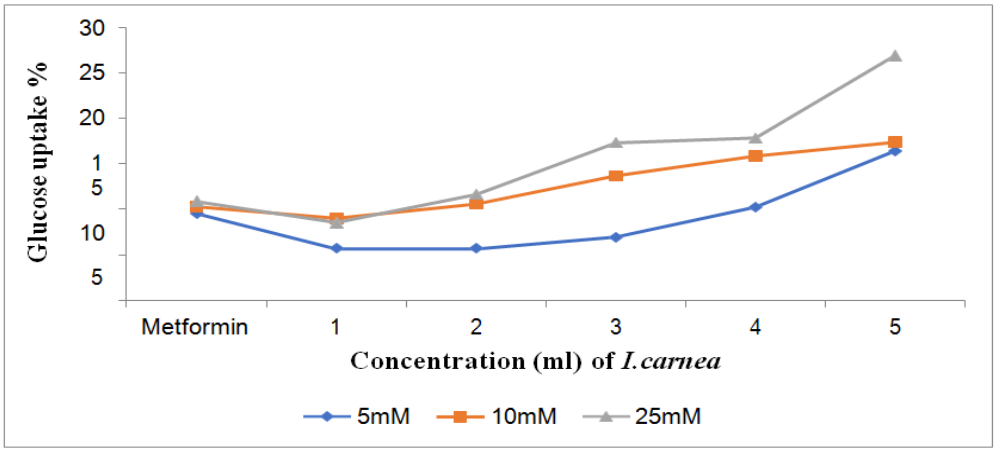

Fig. 5.3: effect of *I. carnea*on glucose uptake by yeast cells.

Processing of water sample

The organisms are identified and confirmed by Gram staining and Biochemical tests.

Table 5.3: The results of biochemical test analysis for *E.coli.*

TEST NAME	RESULTS
Gram staining	Gram negative
Shape	Rod
Spore	Non – Spore
Indole Test	Positive
Methyl Red Test (MR)	Positive
VogesProskaure (VP)	Negative
Citrate Test	Negative
Triple Sugar Iron Agar (TSI)	Acid / Acid, Gas Positive
Carbohydrate Fermentation Test	Ferment Lactose, Sucrose, Galactose

LPS Extraction

From the 100ml over night culture of E. coli the lippo polysaccharide was extracted using Chloroform Methanol Method. LPS pellet was weighed and reconstituted in sterilized PBS (quantity enough to dissolve a pellet completely) and stored at -200C until further used.

Extraction and Purification Of Lippopolysaccharide (LPS)

The amount of Lipopolysaccharide (LPS) obtained by Chloroform-methanol method was0.078g.

Sodium-Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-Page) Characterization Of Lippopolysaccharide (LPS)

Lipopolysaccharide extracted by methanol chloroform method, and the LPS was treated with *Ipomoea carnea*, *Piper betle*, and its mixture. The obtained results indicate that traditional application of *Ipomoea carnea*and *Piper betle*leaves extracts. The SDS PAGE analysis of the intraction between lippopolysaccharide and plant extracts. The lippopolysaccharide and plant extracts was treated with 1:1 ratio.

During electrophoresis endotoxin (LPS) separated into 10 kDa subunit. The *Ipomoea carnea*and *Piper betle*leaf extracts cause changes in the toxin structure. Both plants extract mixture cause the highest changes in the protein bands, and more amount of protein bands are disappeared when it treated with the mixture (Komiazyke *et al.*, 2019). Therefore, the plant extracts neutralize bacterial endotoxin when it treated with plant.

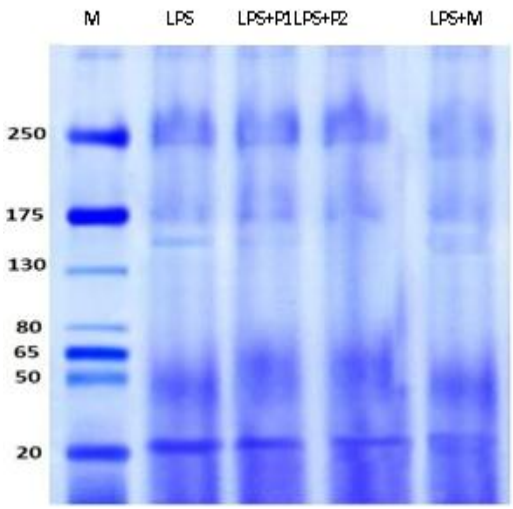

Fig. 5.4: SDS PAGE.

DISCUSSION

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as in vitro screening method for hypoglycaemic effect of various compounds/ medicinal plants (Maier *et al.*, 2002).

Recent studies on the transport of non metabolizable sugars and glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers. It is reported that in yeast cells (*Saccharomyces cerevisiae*) glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient highlighting that the effective transport is only attained if there is removal of intracellular glucose (Ahamad and Uroo*jet al.*, 2010 and Teusink*et al.*, 1998).

The results of this study revealed that *Ipomoea carnea*and *Piper betle*and its mixture of ethanol extracts increased glucose uptake in yeast cells approximately (0–90%) at various glucoseconcentrations.

The positive rates of the GNB culture results in the acute ulcer wounds was mainly consisted 12.6% in *Escherichia coli*. The specimens from chronic ulcer wounds mainly had GNB (54.2%), of the infections. Comparing the acute ulcer wounds with the chronic ulcer wounds showed significant differences in the microbial composition, with *Pseudomonas aeruginosa*(16.6%) the most common GNB .35 specimens training result was positive, positive rate of 52.2%. There one case was identified to vancomycin resistant *Enterococcus faecalis*among the Enterococcus strains, and Enterococcus faecalis was most susceptible to tigecycline (100%) and ampicillin (100%), followed by vancomycin (96.6%), penicillin G (96.6%), and linezolid (86.2%). The susceptibilitie rate of Enterococcus faecium to antibiotics such as vancomycin, linezolid, and tigecyclinewere all100%.

The traditional and ethno medicinal literatures proved that the plant is very effective and safe for medicinal uses also. By using the reserve pharmacological approaches innatural drug can be investigated from the plant for various chronic diseases (Kumar and Priyanka, 2018). natural drug can be investigated from the plant for various chronic diseases.

*Ipomoea carnea*is a ethnic valuable plant and it is used in a number of activities such as Glycosidase Inhibitory Activities, Antioxidant Activity Anti Inflammatory Activity, Anti diabetic Activity, Wound Healing Activity Antimicrobial Activity, Immunomodulatory Activity, Cardiovascular Activity, abortifacient, Antifungal Activity, Anticancer and Hepatoprotective Activity. The Presence of many active chemical constituents which are responsible for various pharmacological medicinal uses. Hence*Ipomoea carnea*has a leading role for the development of noval efficacious drugs in future (Sexena*et al.*, 2017). The extract of *P. betel* was effective against both gram positive and gram negative bacteria which suggest that the plant extract may possess broad spectrum of antibiotic compounds or simply general metabolic toxin (Akter*et al.*, 2014).

Aqueous and ethanolic extract of leaves of *Piper betle*indicated that the ethanolic extract of this plant showed better antibacterial activity against *B. subtilis, S. aureus* and *E. coli* (Balaji*et al.*, 2011).

Ethanolic extract of Piper betle was more effective in antidiabetic and antioxidant assays. So these could used for the treatment of antidiabetic activity, for development of new pharmaceutical formulations. (Perumal and Saravanabhavan, 2017)

Bacically leaves compounds inhibit the toxins. Flavonoids content reduce blood glucose and sugar level and it enhance the humoral immunity. (Khan *et al.*, 2014)

This is one of the traditional method for treat the diabetic mellitus. In this study *Ipomoeacarnea*and *Piper betle*leaf extracts reduce the blood glucose level and it neutralize the bacterial toxin level. Flavonoids content is very essential for reduce blood glucose, phytochemical analysis was performed to identify the flavonoid content. When we treated with both *Ipomoea carnea*and *Piperbetle*leaf extracts mixture it was more effective. Yeast call glucose uptake method used for the identification of antidiabetic activity of leaf extracts. Choloroform methanol method was used to extract the LPS and SDS PAGE method was used to determine the toxin neutilaization property of leaf extracts.

CONCLUSION

The current epidemic of diabetes indicates the need of proper and effective medications that are limited in their potency to have many side effects. The analyzed plant extracts are potential complements to standard antidiabetic treatment, and the plant extracts were neutralize the lippopolysaccharide of the *E.coli* which is act as wound causing organisms indiabeticpatients.

ACKNOWLEDGEMENT

The authors are grateful to DST-FIST Scheme, DBT-Star Scheme, management, principal and Deans of Dr.N.G.P. Arts and Science college (Autonomous) for their extended support of this work. Communication number: DrNGPASC 2020-21 BS019.

REFERENCES

1. Amel M Kamal, Zeinab T Abdel Sherif R Abdel All, Amany A SleemEman G Haggag, Phtochemical and biological Investigation of *Ipomoea* carneaJacq.Grown in Egypt, International Journal of Pharmacognosy and PhytochemicalResearch, 2017;

9(2): 266- 281.
2. PrathasarathiPerumal, KavithaSaravananabhavan, Antidiabetic and Activitesof Ethanolic Extract of Piper betle L. Leaves in Catfish, ClariasGariepinus, Asian Journal of Pharamaceutical and Clinical Research, 2018; 11(3).
3. Revathi Pitchaipillai, Thirumalaikolundusubramanian Ponniah, In Vitro Antidiabetic Activity of Bruguiera Cylindrica L. Glucose Uptake by Yeast Cells Method, International Biological and Biomedical Journal, 2016; 2(4).
4. Arunkashivishwanathshettar, Ankalabasappavedamurthy, Studies on In Vitro Antidiabetic Activities of HopeaPonga and VitexLeucoxylon, International Journal of Pharmacy and PharmaceuticalSciences, 2017; 9(2).
5. Otto Holst, Artur J. Ulmer, Helmut Brade, Hans – Dieter Flad, Ernst Th. Rietschel, Biochemistry and Cell Biology of Bacterial Endotoxins, FEMS Immunology and MedicalMicrobiology, 1996; 16: 83-104.
6. James Redfern, Malcolm Kinninmonth, DarieBurdass, and Verran, Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential oils) for Their Antimicrobial Properties, Journal of Microbiology and Biology Education, 2014; 15(1).
7. D. G. Kalambhe, N. N. Zade and S. P. Chudhari, Evalution of Two Different Lippopolysaccharide Extraction Methods for Purity and Functionality of LPS, International Journal of Current Microbiology and AppliedSciences, 2017; 6(3).
8. Suvra Roy, Vikash Kumar, A Practical Approach on SDS PAGE for Separation of Protein, International Journal of Sciences and Researrch, 2014; 3(8).
9. Magdalena Komiazyk, MalgorzataPalczewska, and Patrick Groves, Neutralization of Cholera Toxin by Rosaceae Family Plant Extracts, BMC Complementary Medicine andTherapies, 2019; 19: 140.
10. Andreas Maier Bernhard Volker, Eckhard Boles , Fuhrman Gunter Fred, Characterisation of glucose transport in Saccharomyces cerevisiae with plasma membrane vesicles (countertransport) and intact cells (initial uptake) with single Hxt1, Hxt2, Hxt3, Hxt4, Hxt6, Hxt7 or Gal2 transporters, FEMS YeastResearch, 2002; vol 2, issue 4, 539-550.
11. Ahmed F, Urooj A, In vitro studies on the hypoglycemic potential of Ficusracemosa Stem bark, J Sci Food Agric, 2010; 90: 397-401.
12. BasTeusink,JasperA.DiderichHansV.Westerhoff,KarelvanDam,andMichael C. Walsh, Intracellular glucose concentration in derepressed yeast cells consuming glucose is high enough to reduce the glucose transport rate by 50%, JBacteriol, 1998; 180: 556-62.
13. PSaravanKumarandVanamPriyanka,AReviewOnPhytomorphologicalPhytochemical And Phytopharmacological Profile Of Ipomoea carnea And Future Perspective, Pharmaceutical Sciences, 2018; 5(1): 371-378.
14. Prasoon Kumar Saxena, Deepak Nanda, Ritu Gupta, Nitin Kumar, NidhiTyagi, A Review On Ipomoea carnea: An Exploration, International Research Journal of Pharmacy, 2017; 8(6).
15. KaziNahidAkter, PalashKawmakar, Abhijit Das, ShanmimaNasrinAnonna, SharminAkterShom and, Mohammad MafruhiSattar, Evalution Of Antibacterial and Anthelmintic Activities With Total Phenolic Contents Of Piper betle leaves, Avicenna Journal of Phytomedicine, 2014; 4(5): 320-329.
16. K Balaji T Lisa, TSK Sarnnia, B Mirza, Antibacterial Activity of Piper betlt leaves, International journal of Pharmacy Teaching and Pracices, 2011.
17. TasneefY.KhanRajinderRaina, Pawan k. Vermma, Mudasir Sultana, Mohinder K. Jyoti, Phytochemical Comsituents and Antidiabetic Potential of Ipomoea carneaJacq Leaves Extracts, Journal of Experimental Medicine, 2014; 4(2): 137- 142.