Chapter 4
RESULTS AND DISCUSSION

4.1 Plant material

In the present study, the selected plant material *Phyllanthus emblica* (fruit) and *Punica granatum* (fruit peel) were collected from the local market, *Hodgsonia heteroclita* (fruit plup) from the forest of Kokrajhar district and leaf of *Bambusa balcooa* (leaf) was collected from the Bambusetum, Bodoland University, Kokrajhar, BTR, Assam, India.

4.2 Yield of the extract

The percentage of yield of PHF-HOPE aqueous extract was found to be 3.25 % *(w/w).*

4.3 Organoleptic evaluation

Organoleptic evaluations, including texture, fragrance, flavour, colour, and powder microscopy, form the basis of the examination of powdered crude medicine. According to Patil et al., (2018), organoleptic qualities play a significant role in industrial production by maintaining or improving the formulation's consistency, increasing patient compliance, and determining the product's overall effectiveness. The organoleptic examination of plants was documented and summarized in table

Table 4.1: Organoleptic parameters of powdered of PHF-HOPE

Parameters	PHF-HOPE
Texture	Granular
Aroma	Grassy
Flavour/taste	Bitter
Colour	Fawn brown

4.1, which pertains to their scent, colour, texture, flavour/taste, and flavour.

4.4 Powder microscopy of PHF-HOPE

According to Venkateswarlu et al., (2018), microscopy is an essential pre-testing stage for identifying impurities in pharmaceuticals. The main method for identifying and authenticating plants for medicinal applications is by analyzing their structural and cellular characteristics in crude powder. The PHF-HOPE powder had a brownish colour (Figure 4.1). The lignified fibres were long and distinct, with thick or thin walls. Circular to oval-lignified stone cells were common and mainly used to reinforce or support soft tissues. Plants rely on their fibrous tissues for structural integrity and mechanical support (Aslam et al., 2019). We found the epidermis. A plant's epidermis prevents water loss and facilitates gas exchange within the cell.

4.5 Determination of physical characteristics of powder

In the pharmaceutical industry, the flow properties of powders have an effect not only on the quality of the final product, such as tablets or capsules, but also on the efficiency with which solid dosage forms are manufactured. In order to process these powders, which frequently involve multiple unit processes such as blending, aeration, fluidization, compaction, and storage in hoppers or bins (Leturia et al., 2014), it is essential to achieve flow characteristics that are either acceptable or exceptional. This is because all of these processes involve the flow of particulate solids. A further factor that influences the powder discharge from a process vessel is the form of the vessel, as well as the friction that occurs between the walls of the vessel. Due to the complexity of the situation, several methods for evaluating the flowability of powder have been devised, the majority of which are founded on empirical knowledge obtained. Among the preliminary pharmacopeia techniques that are applied the most frequently are the angle of repose, the Hausner ratio, and the flow through the orifice (Li et al., 2004). For instance, Gedart et al., (2006) and Abdullah et al.., (2010)

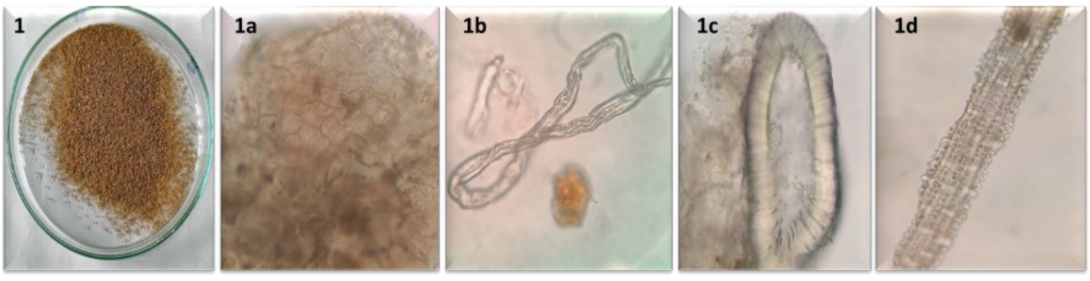


Figure 4.1: Photomicrographs of microscopic evaluation (400x) 1. PHF-HOPE powder, 1a. Epidermis, 1b. Fibre, 1c. Stone cells, 1d. Pitted vessels.

Table 4.2: Flow characteristics of the powdered PHF-HOPE

	Angle of repose	Bulk density (g/mL)	Tapped density (g/mL)	Hausner ratio	Carr's index
HOPE	39.64	0.36	0.536	1.46	31.71

Angle of repose = 30–40 Passable (Sharma et al., 2011); Hausner's ratio = 1.12–1.18 (Good), 1.19–1.25 Fair, 1.26–1.34 (Passable) and 1.35–1.45 (Poor) (Attia et al., 2014) Carr's index = 12–16 (Good), 18–31 (Fair to Passable) (Sharma et al., 2011).

investigated the flowability of powders by utilizing the Hausner ratio and angle of repose. Additionally, Abdullah et al., (2010) investigated the values of cohesion and the effect that particle size has on flowability. The assessment of the material's physical qualities, which were ascertained by the utilization of tap density, bulk density, angle of repose, Hausner ratio, and Carr's index, It was reported in Table 4.2 that the observations were made.

4.6 Heavy metal test

According to Rajinith et al., (2018), the absence of heavy metals in plants is a good indicator of the purity and impurity of the plants. The heavy metals, specifically cadmium, bismuth, and lead, were not discovered in any of the samples that were examined in this investigation, as summarized in table 4.3. This finding suggests that there was no contamination of heavy metals, and as a result, the heavy metals can be safely included as an ingredient in a variety of herbal formulations at this time. Out of the four plant parts, the only one that was found to contain heavy metals was the fruit of the *P. emblica* plant (Rao et al., 2011, Kumudhaveni et al., 2020). The other plant parts were only reached for the very first time.

Table 4.3: Determination of heavy metals of PHF-HOPE

	Heavy metals					
	Cadmium (Cd)	**Cadmium (Cd)**	**Bismuth (Bi)**	**Bismuth (Bi)**	**Lead (Pb)**	**Lead (Pb)**
Sample solution	**Sample + NH_4OH**	**Sample + Potassium Ferrocyanide**	**Sample + NH_4OH**	**Sample + H_2S**	**Sample + Dil. HCl**	**Sample + KI**
HOPE	Absent	Absent	Absent	Absent	Absent	Absent

4.7 Preliminary phytochemical screening

The preliminary phytochemical testing of the PHF-HOPE extract showed that it contained a number of different phytoconstituents. The presence of different phytoconstituents in the test samples showed that they were at a range of levels, from modest to high **(**Table 4.4) shows that the biochemicals that were screened had the biggest amounts of flavonoids and reducing sugars. There are a lot of different phytochemicals in plants. These chemicals have many biological uses and can be used in food and drugs. Different phytochemicals, each with its own biological effects, can be found in plant products (Harborne 1998). It is said that phytochemicals play important roles in many biological and medicinal processes in animals when they are eaten. A lot of attention has been paid to the biochemical parts of fruits and vegetables because they can do many chemical and biological things (Halliwell 2007). These acts can be harmful to cells or helpful to cells. One of the main jobs of these phytochemicals is to act as antioxidants (Zhishen et al., 1999). Plant saponins are known to have many medical benefits, including the ability to help with constipation, reduce inflammation, protect blood vessels, boost the immune system, lower cholesterol and blood sugar, kill mollusks, fight fungi and parasites, and lower cholesterol (Sparg et al., 2004). The body uses carbohydrates for a variety of crucial processes, including producing energy and controlling blood sugar. Keeping proteins from being used to break down fatty acids for energy and stopping ketosis (Jequier P, 1994). It is possible for reducing sugar to work as a reducing agent because it has a free aldehyde or ketone group. Some disaccharides, oligosaccharides, and polysaccharides are also reducing sugars, but all monosaccharides are the only ones that are. Reducing sugars like fucoidans has been the subject of a lot of research over the past few decades (Li et al., 2008). This is because they have many biological activities, such as their ability to stop blood clots and viruses, fight tumours and other viruses, boost the immune system, lower blood fats, fight inflammation, protect the stomach, fight complements, and work against hepatopathy, uropathy, and renalpathy. They may also help with surgery and protect the stomach. Alkaloids have been known for a long time to be an important group of chemicals because they have many biological roles, such as pain-relieving properties. One type of secondary product that is found in a lot of plants and is used a lot is flavonoids. They have antibacterial, antifungal, and antiviral effects (Podolak et al., 2010). Anthraquinones have been shown to help with constipation, arthritis, and cancer (Ebbo et al., 2014). It is well known

Table 4.4: Preliminary phytochemical screening of PHF-HOPE extract

Sl. No.	Phytochemical	Aqueous PHF-HOPE
I.	**Detection of alkaloids**	
a.	Dragendroff's test	-
b.	Hager's test	-
c.	Mayer's test	-
d.	Vitali-Morin test	+
II.	**Detection of carbohydrates**	
a.	Molisch's test	+
b.	Test for Starch	-
c.	Test of Pentose	+
III.	**Detection of reducing sugars**	
a.	Benedict's test	+
b.	Fehling's test	+
c.	Aqueous NaOH test	+
d.	Conc. H2SO4 test	+
IV.	**Detection of proteins and amino acids**	
a.	Biuret test	+
b.	Millon's test	-
c.	Ninhydrin test	-
V.	**Detection of flavonoids**	
a.	Alkaline reagent test	-
b.	Lead acetate test	+
c.	Shinoda's test	+
d.	Shibata's reaction	-
e.	Ferric chloride test	+
f.	Pew's test	-
g.	Zinc-hydrochloride	-
h.	Ammonia test	-
VI.	**Detection of phenolic compound**	
a.	Ferric chloride test	+
VII.	**Detection of tannins**	
a.	Braymer's test	+
VIII.	**Detection of saponins**	
a.	Foam test	+
IX.	**Detection of phytosterol**	
a.	Salkowski's test	+
b	Libermann-Burchard's test	+
X	**Detection of lignins**	
a	Labat test	-
XI.	**Detection of quinones**	
a.	Alcoholic KOH test	+
XII.	**Detection of anthraquinones**	
a.	Borntrager's test	-
XIII.	**Detection of coumarins**	
a.	NaOH paper test	+
b.	NaOH test	+

'+'= Present, '-' = Absent

that each phytochemical has a unique relationship with living things. Flavonoids and saponins, which can be found in parts of plants like *Bambusa balcooa* (leaf), *Phyllanthus emblica* (fruit), *Hodgsonia heteroclita* (fruit pulp), and *Punica granatum* (fruit peel), may help fight cancer and diabetes. This could be because they work alone or with other chemicals. In Table 4.4, you can see that the plant has reducing sugars, flavonoids, steroids, proteins and amino acids, phytosterol, lignins, quinones, anthraquinones, and coumarins.

4.8 Determination of biochemical constituents

The biochemical component, including total phenolic and flavonoid contents, of the PHF-HOPE extract showed that there were 241.287±0.31 mg/g GAE and 941.379±0.83 mg (RE/G/ml) flavonoids, respectively. There were several phytoconstituents found in the crude hydro-aqueous extract of PHF-HOPE. A strong phenolic antioxidant is present in PHF-HOPE, suggesting that it may protect living things from oxidative free radicals. Being rich in phenolic antioxidants, PHF-HOPE has the potential to protect living organisms from the harmful effects of oxidative free radicals.

4.9 Assessment of antioxidant activity

4.9.1 Free radical scavenging assay-2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method

The evaluation of PHF-HOPE's antioxidant capability demonstrated the presence of a strong resource of naturally occurring antioxidants. The extracts in the present investigation exhibited significant DPPH radical scavenging action, which increased with higher concentrations of both the extracts and standard ascorbic acid. The maximum DPPH scavenging activity is seen at a concentration of 200 mg/mL. There is notable action shown in comparison to conventional ascorbic acid. The activity exhibited an increase in concentration ranging from 20% to 78%. The scavenging capabilities of DPPH are illustrated in Table 4.5. According to Halliwell (2007), natural antioxidants have been identified as potential sources for medications. However, there is a dearth of understanding regarding their accessibility and appropriateness for intake. Hence, it is crucial to evaluate the efficacy of both in vitro and in vivo systems to establish their suitability for the utilization of conventional drugs (Chan and Cheung 2000). The DPPH test relies on the capacity of antioxidants to cause decolorization, namely at the absorption peak of 517nm. Therefore, DPPH is widely employed to assess the radical-scavenging capability of plants (Jun et al.,

Table 4.5: DPPH scavenging activity of PHF-HOPE extract compared to ascorbic acid as standard

Concentration (µg/mL)	PHF-HOPE (% Inhibition)	Standard (Ascorbic acid)
20	36.164	13.01
40	37.194	17.87
80	51.351	21.23
120	61.261	41.43
160	63.963	59.18
200	75.546	67.76

2004). The generation of the DPPH-H radical induces depolarization proportional to the quantity of accumulated electrons. A higher level of depolarization leads to a more significant reduction in power. The test developed by Wagner et al. in (1996) is the most commonly employed technique for evaluating the ability of a new medication to remove free radicals.

4.9.2 Ferric reducing power assay (FRP)

The comparative reductive capacity of the PHF-HOPE extract and ascorbic acid is illustrated in Figures 4.2a and 4.2b. The lowering capacity of PHF-HOPE extracts was seen to be significant, exhibiting a steady increase with higher concentrations. The conversion of Fe^{3+} to Fe^{2+} in the presence of the extract or the ascorbic acid used as a reference standard serves to measure the reductive capacity (Akinpelu et al.,

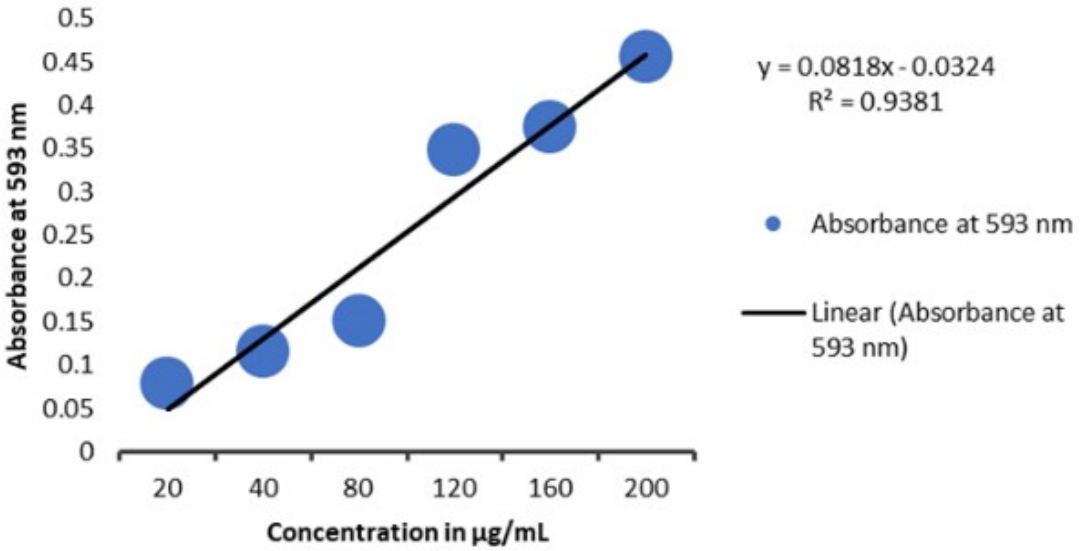


Fig. 4.2a: Ferric reducing power assay showing linear curve of ascorbic acid standard.

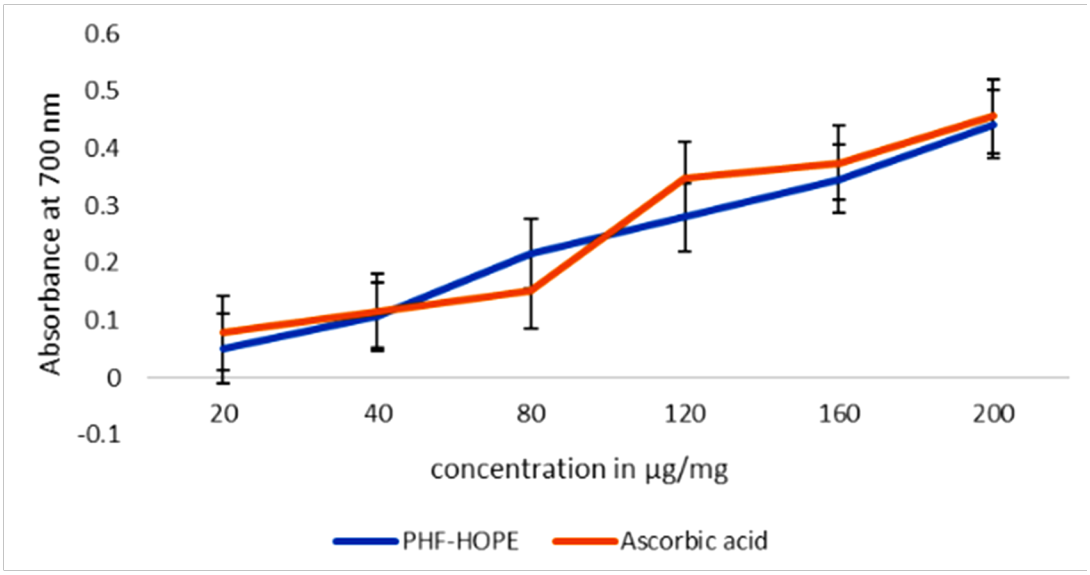


Fig. 4.2b: Ferric reducing power assay of PHF-HOPE extract compared to ascorbic acid as standard.

2010). The extracts' efficacy may be attributed to the presence of flavone hydroxyl, phenolic hydroxyl, or methoxyl groups, free carboxylic groups, keto groups, as well as triterpenes and their derivatives (Najafabad & Jamei 2014). The present investigation revealed that the reducing power had a direct correlation with the concentration of ascorbic acid, and it consistently increased as the concentration increased. The study conducted by Goyal et al., in (2017) examined the absorbance values of reducing ability in *Bambusa balcooa*. When compared to the standard ascorbic acid (0.041) at a concentration of 200 µg/ml, BAQE (0.217), BME (0.079), and BAE (0.027) had the highest absorbance values. In addition, the level of antioxidant activity rises as the inhibitory effect increases (Ajith, 2010).

4.9.3 Hydrogen peroxide scavenging activity

In comparison to normal ascorbic acid, which has an IC_{50} value of 64.6±0.05 mg/ml, the H_2O_2 scavenging activity of the PHF-HOPE extracts demonstrates that they are effective in removing H_2O_2 (IC_{50} = 597.8±0.21 mg/ml by volume). A higher IC_{50} value was found for the extract in comparison to the value found for the standard. Because hydrogen peroxide is a mild oxidizing agent, it can stop some enzymes from working by oxidizing important thiol groups (Middha et al., 2013). This is because hydrogen peroxide produces thiol groups. According to Halliwell and Gutteridge (1993), it also has the capability of causing toxicity by forming hydroxyl radicals, which may occur either through the reaction with Fe^{2+} or Cu^{2+} ions after it is still inside the cells. The formation of hydrogen peroxide (H_2O_2) needs to be limited in

order to prevent such harm. The quantity of hydrogen peroxide that has accumulated in the cells needs to be checked. According to the findings, the PHF-HOPE extract appears to be an effective scavenger of hydrogen peroxide. On the other hand, PHF-HOPE has been shown to be full of natural antioxidants and polyphenols. Both of these substances could be very useful as medicines to stop or slow down the progression of age-related degenerative diseases and oxidative stress. Having said that, it is of the utmost importance to do more research in order to identify the chemicals.
Macwan et al., (2010) made similar conclusions when they evaluated the antioxidant capacity of *Bambusa arundinacea* leaf using three distinct types of solvents: water, methanol, and butanol. They found that the leaf did not exhibit any significant antioxidant properties. In addition to this, they discovered that the methanolic extract had a much larger quantity of antioxidant activity when compared to both water and butanol. As a result, it was found to be an appropriate candidate for the processes involved in extract preparation.

4.10 GC MS analysis

The gas chromatography mass spectrometry (GCMS) approach is an analytical method that combines the capabilities of mass spectrometry (MS) and gas chromatography (GC) to make it possible to perform mass analysis and physical separation simultaneously. The methods of identification, estimation, general detection, and separation of specific mass chemical compounds in the presence of other chemicals are the primary focuses of this methodology. A mass chromatogram is a type of chromatogram that is used to display data obtained from mass spectrometry. The X-axis of a mass chromatogram indicates time, and the Y-axis represents the strength of the signal. Following the GCMS analysis of PHF-HOPE, it was discovered that a great number of bioactive chemicals were present. An investigation using GCMS revealed the presence of 58 bioactive chemicals. The bioactive phytoconstituents that have been found are chemicals that are in high demand for their antioxidative and glucose-lowering properties. In addition to the compounds that were discovered, the chromatograms reflected a great number of other bioactive compounds that were not identified. The phytoconstituents that have been found are put into groups based on their compound name, molecular formula, retention time (RT), area match score, and structure, as shown in Table 4.6 Figure 4.3 displays the chromatograms of the chemicals that have been found during the analysis. The following bioactive compounds were present in the GC-MS analysis carried on aqueous fraction of HOPE: Propanoic acid, 2,2-dichloro-, 1-methylethyl

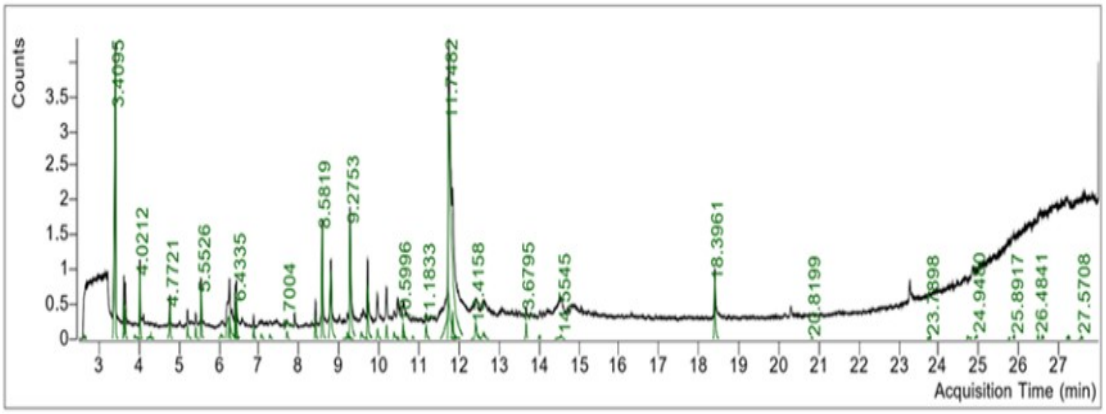
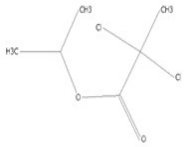
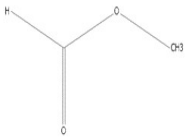
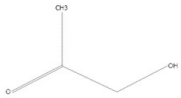
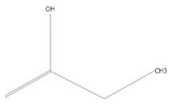
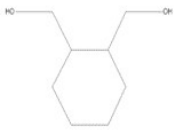
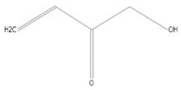
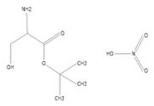
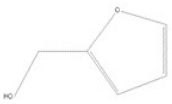
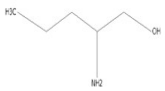
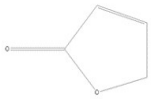
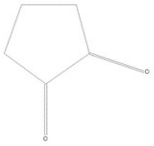
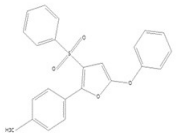
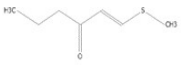
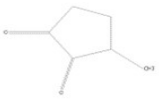
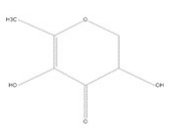


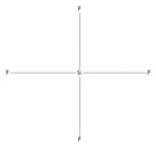
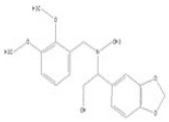
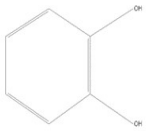
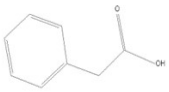
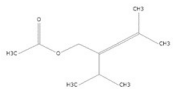
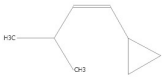
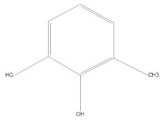
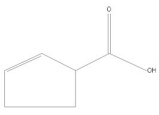
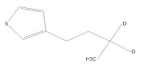
Figure 4.3: GC-MS chromatogram of aqueous fraction of PHF-HOPE.

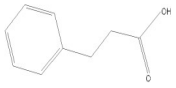
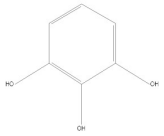
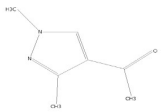
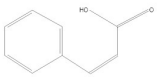
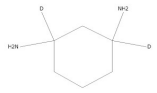
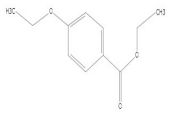
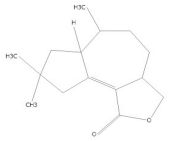
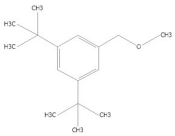
easter; Methyl formate; 2-Propanone, 1-hydroxy Propanoic acid; [(1R,2R)-2-1-Hydroxybut-3-en-2-one; L-Serine 1,1-dimethylethyl ester nitrate; 2-Furanmethanol; dl-2-Amino-1-pentanol; 2(5H)-Furanone; 1,2-Cyclopentanedione; 2-Phenoxy-4 (phenylsulfonyl)-5-(p-tolyl)furan; Methoxypropionaldehyde; 1-Methylsulfanyl-1-hexen-3-one; Propane, 2-methyl-; 2H-Pyran-2,6(3H)-dione; 1,2-Cyclopentanedione, 3-methyl-; 2,4-Imidazolidinedione,1-methyl-; (1)-Ethoxyhex-1-ene; 2-(5-Methylfuran-2-yl)ethanol; 2-acetyl-2-hydroxy-.gamma.-butyrolactone; 4H-Pyran-4-one; 4H-Pyran-4-one; 2,3-dihydro-3,5-dihydroxy-6-; Benzoic acid; Silicon tetrafluoride; (2S)-2-(1,3-benzodioxol-5-yl)-2-[(2,3-dimethoxyphenyl)methyl-methyl -amino]ethanol;Catechol; 2(3H)-Furanone, dihydro-5-(2-methylpropyl)-; 5-Hydroxymethyl-2-furaldehyde; hyltriaconta-10,12,14,16,18,20-hexaene-dioate; Benzeneacetic acid; 1-acetoxy-2-isopropyl-4-methyl-2,3-pentadiene; cis-1-Cyclopropyl-3-methyl-1-butene; 1,2-Benzenediol, 3-methyl-; 2-Cyclopentene-1-carboxylic acid; 3-(3,3-dideutero-n-butyl)thiophene; Hydrocinnamic acid; 2-(N-Methylamino)-4,5-dimethoxyanilinemonohydrochloride; 1,2,3-Benzenetriol; 1,2-Ethanediol; 1-(1,3-Dimethyl-1H-pyrazol-4-yl)-ethanone; (Z)-3-Phenyl-2-propenoic acid; 1,3-Cyclohexane-1,3-d2-diamine, cis-; Benzoic acid, 4-ethoxy-, ethyl ester; (3aS,6S,6aS)-6,8,8-trimethyl-3,3a,4,5,6,6a,7,9-octahydroazuleno[4,5-c]furan-1-one; 1,3-ditert-butyl-5-(methoxymethyl)benzene; 1-Butanamine; n-Hexadecanoic acid; 4,9-Dimethyldiamantane; N-[1-[(6-chloranylpyridin-3-yl)methyl]methyl]imidazole-2 -yl]nitramide; (Z)-N-Benzyl 3,5,5-trimethyl-4-(3'-phenylbuta-1',2'-dienyl)furan-2 (5H)-imin; (1SR,5RS,9SR,13RS,18RS)-18 allylhexacyclo [16.3.0.0(15).0(5,9).0 (9,13).0(13,17]henicos-16-ene17]henicos-16-ene; 4-(2-Thienyl)-3-methylsulfonylthiomethy-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrolidin-1-yloxyl radical; 2-hydroxy-2-[6-methoxy-2-(4-methylphenyl)-3-imidazo[1,2-b]pyridazinyl] acetic acid ethyl ester; Nonamethyl(methoxymethyl)ferrocene; Dimethyl 4-methyl-6-

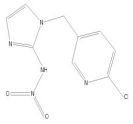
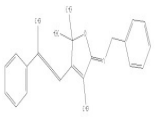
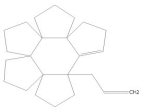
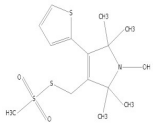
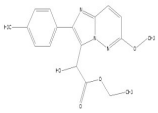
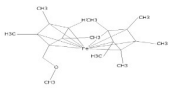
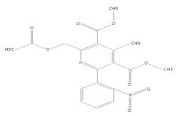
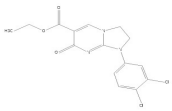
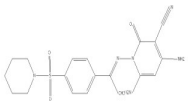
Table 4.6: Phytoconstituents of aqueous fraction of PHF-HOPE

Sl. No	Compound Name Chromatogram	Molecular Formula	2D Structure	RT	Area
1	Propanoic acid, 2,2-dichloro-, 1-methylethyl ester (CAS: 54587-48-3)	$C_6H_{10}C_{l2}O_2$		2.6328	835967
2	Methyl formate (CAS: 107-31-3)	$C_2H_4O_2$		3.4095	78726149
3	2-Propanone, 1-hydroxy- (CAS: 116-09-6)	$C_3H_6O_2$		3.6184	7775615
4	Propanoic acid (CAS: 79-09-4)	$C_3H_6O_2$		3.6544	7277315
5	[(1R,2R)-2-(hydroxymethyl) cyclohexyl]methanol (CAS: 997061-82-4)	$C_8H_{16}O_2$		3.8949	1741525
6	1-Hydroxybut-3-en-2-one (CAS: 997005-05-6)	$C_4H_6O_2$		4.0212	12182225
7	L-Serine 1,1-dimethylethyl ester nitrate (CAS: 997287-70-0)	$C_7H_{16}N_2O_6$		4.2942	1757214
8	2-Furanmethanol (CAS: 98-00-0)	$C_5H_6O_2$		4.7721	7953172
9	dl-2-Amino-1-pentanol (CAS: 4146-04-7)	$C_5H_{13}NO$		5.2085	3349946
10	2(5H)-Furanone (CAS: 497-23-4)	$C_4H_4O_2$		5.4136	2514379

11	1,2-Cyclopentanedione (CAS: 3008-40-0)	$C_5H_6O_2$		5.5526	9767977
12	2-Phenoxy-4-(phenylsulfonyl)-5-(p-tolyl) furan (CAS: 997813-08-2)	$C_{23}H_{18}O_4S$		6.0482	2450647
13	Methoxypropionaldehyde (CAS: 0-00-0)	$C_4H_8O_2$	NA	6.2366	21929493
14	1-Methylsulfanyl-1-hexen-3-one (CAS: 997060-77-9)	$C_7H_{12}OS$		6.2613	4529353
15	Propane, 2-methyl- (CAS: 75-28-5)	C_4H_{10}		6.4069	8221483
16	2H-Pyran-2,6(3H)-dione (CAS: 5926-95-4)	$C_5H_4O_3$		6.4335	9857355
17	1,2-Cyclopentanedione, 3-methyl- (CAS: 765-70-8)	$C_6H_8O_2$		6.8648	2665067
18	2,4-Imidazolidinedione, 1-methyl- (CAS: 616-04-6)	$C_4H_6N_2O_2$		7.0590	1459679
19	(E)-1-Ethoxyhex-1-ene (CAS: 997036-66-8)	$C_8H_{16}O$		7.2731	1392838
20	2-(5-Methylfuran-2-yl) ethanol (CAS: 997032-74-9)	$C_7H_{10}O_2$		7.7004	1948012
21	2-acetyl-2-hydroxy-.gamma.-butyrolactone (CAS: 135366-64-2)	$C_6H_8O_4$		8.4119	4955458
22	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl (CAS: 28564-83-2)	$C_6H_8O_4$		8.5819	25899854

23	Benzoic acid (CAS: 65-85-0)	$C_7H_6O_2$		8.8006	26810440
24	Silicon tetrafluoride (CAS: 7783-61-1)	F4Si		9.2092	2138036
25	(2S)-2-(1,3-benzodioxol-5-yl)-2-[(2,3- dimethoxy-phenyl)methyl-methyl-amino]ethanol (CAS: 997704-32-2)	$C_{19}H_{23}NO_5$		9.2568	2228348
26	Catechol (CAS: 120-80-9)	$C_6H_6O_2$		9.2753	37640573
27	2(3H)-Furanone, dihydro-5-(2-methylpropyl)- (CAS: 18432-37-6)	$C_8H_{14}O_2$		9.5593	3312389
28	5-Hydroxymethyl-2-furaldehyde (CAS: 67-47-0)	$C_6H_6O_3$		9.7153	15087763
29	Dimethyl triaconta-10,12,14,16,18,20-hexaene (CAS: 997947-85-9)	$C_32H_50O_4$	NA	9.9018	25031
30	Benzeneacetic acid (CAS:103-82-2)	$C_8H_8O_2$		9.9656	3059388
31	1-acetoxy-2-isopropyl-4-methyl-2,3-pentadiene (CAS: 133795-61-6)	$C_{11}H_{18}O_2$		10.1890	3210163
32	cis-1-Cyclopropyl-3-methyl-1-butene (CAS: 997017-34-9)	C_8H_{14}		10.3797	2682508
33	1,2-Benzenediol, 3-methyl- (CAS: 488-17-5)	C_7H_8O		10.5996	4570164
34	2-Cyclopentene-1-carboxylic acid (CAS: 2348-89-2)	$C_6H_8O_2$		10.6279	1786470
35	3-(3,3-dideutero-n-butyl) thiophene (CAS: 997054-27-6)	$C_8H_{10}D_2S$		10.8444	668227

36	Hydrocinnamic acid (CAS: 501-52-0)	$C_9H_{10}O_2$		11.1833	4460913
37	2-(N-Methylamino)-4,5- di-methoxyanilinemonohydrochloride (CAS: 0-00-0)	$C_9H_{14}N_2O_2$	NA	11.7398	45788270
38	1,2,3-Benzenetriol (CAS: 87-66-1)	$C_6H_6O_3$		11.7482	201228029
39	1,2-Ethanediol (CAS: 107-21-1)	$C_2H_6O_2$		11.8199	563929
40	1-(1,3-Dimethyl-1H-pyrazol-4-yl)-ethanone (CAS: 52773-23-6)	$C_7H_{10}N_2O$		11.8450	8343314
41	(Z)-3-Phenyl-2-propenoic acid (CAS: 102-94-3)	$C_9H_8O_2$		12.4158	7067787
42	1,3-Cyclohexane-1,3-d2-diamine, cis- (CAS: 70795-41-4)	$C_6H_{12}D_2N_2$		12.6155	3625234
43	Benzoic acid, 4-ethoxy-, ethyl ester (CAS: 23676-09-7)	$C_{11}H_{14}O_3$		13.6795	3733167
44	(3aS,6S,6aS)-6,8,8-trimethyl-3,3a,4,5,6,6a,7,9- octahydro-azuleno[4,5-c]furan-1-one (CAS: 23676-09-7)	$C_{15}H_{22}O_2$		14.0155	632775
45	1,3-ditert-butyl-5-(methoxymethyl)benzene (CAS: 997326-12-0)	$C_{16}H_26O$		14.0160	843393
46	1-Butanamine (CAS: 109-73-9)	$C_4H_{11}N$		14.5545	2238352
47	n-Hexadecanoic acid (CAS: 57-10-3)	$C_{16}H_{32}O_2$		18.3961	16210127
48	4,9-Dimethyldiamantane (CAS: 0-00-0)	$C_{19}H_{24}$	NA	20.8199	549067

49	N-[1-[(6-chloranylpyridin-3-yl) methyl]imidazol2-yl]nitramide (CAS: 997391-56-0)	$C_9H_8ClN_5O_2$		23.7898	785603
50	(Z)-N-Benzyl 3,5,5-trimethyl-4-(3'-phenylbuta1',2'-dienyl)furan-2(5H)-imine (CAS: 997699-39-4)	$C_{24}H_{25}NO$		24.7184	1133912
51	(1SR,5RS,9SR,13RS,18RS)-18- allylhexacyclo[16.3.0.0(15).0(5,9).0 (9,13).0(13, 17]henicos-16-ene (CAS: 106115-36-0)	$C_{24}H_{34}$		24.9480	1155871
52	4-(2-Thienyl)-3-methylsulfonylthiomethyl2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrolidin1-yloxyl radical (CAS: 997705-58-6)	$C_{14}H_{20}NO_3S3$		25.7751	262318
53	2-hydroxy-2-[6-methoxy-2-(4-methylphenyl)-3- imidazo[1,2-b] pyridazinyl]acetic acid ethyl ester (CAS: 997692-10-9)	$C_{18}H_{19}N_3O_4$		25.8917	668197
54	Nonamethyl(methoxymethyl) ferrocene (CAS: 997734-47-9)	$C_{21}H_{32}FeO$		26.4841	654496
55	Dimethyl 4-methyl-6-(acetoxymethyl)-2-(2'- nitrophenyl)-3,5-dicarboxylate (CAS: 997835-26-8)	C19$H_{18}N_2O_8$		26.4841	839175
56	1-(3,4-dichlorophenyl)-7-keto-2,3-dihydroimidazo[1,2-a]pyrimidine-6-carboxylic acid ethyl ester (CAS: 997725-04-2)	$C_{15}H_{13}C_{l2}N_3O_3$		27.2300	549985
57	4,6-Diamino-2-oxo-1-[1-(1-(4-(piperidin-1-ylsulfonyl)phenyl) ethylidene-amino)-1,2- dihydro-pyridine-3-carbonitrile (CAS: 997855-77-5)	$C_{19}H_{22}N_6O_3S$		27.2570	468658
58	1-methyl-3,4-bis(trifluoromethylthio) pyrazole (CAS: 114861-78-8)	$C_6H_4F_6N_2S_2$		27.5708	506173

(acetoxymethyl)-2-(2'-nitrophenyl)-3,5-dicarboxylate; 1-(3,4-dichlorophenyl)-7-keto-2,3-acid ethyl ester; 4,6-Diamino-2-oxo-1-[1-(1-(4-(piperidin-1-dihydropyridine-3-carbonitrile; 1-methyl-3,4-bis(trifluoromethylthio)pyrazole.

n-Hexadecanoic acid; It has an Anti-oxidant, Hypocholesterolemic, Nematicide, Anti -androgenic, Hemolytic, Pesticide, Lubricant, 5-Alpha reductase inhibitor, antipsychotic. (Tyagi and Agarwal 2017). Hydrocinnamic acid; it acts as an antioxidant property and used for flavouring, food additives, spices, fragrance (Grover and Patni 2013).

4.11 In vivo anti-hyperglycemic activity

The term "rich in antioxidants" has become widely used, and there are countless in vitro reports on plants being used as natural antioxidants in diets (Halliwell, 2007; Gutteridge and Halliwell, 2010), but it is still unclear whether animals and humans can biologically absorb them. According to Halliwell (2012), it is crucial to determine if a food or drink has antioxidant properties before recommending it for consumption. This involves testing the product in both laboratory and living-organism settings to ensure consistency in the results. While there are instances where the activity in both the in vitro and in vivo systems is identical, this is not always the case (Halliwell et al., 2005; Halliwell, 2009; Boom gaarden et al., 2010). This study set out to look into the potential hypoglycemic effects of an aqueous PHF-HOPE extract on rats with alloxan-induced diabetes. Previous research on the treatment of diabetes (Goyal et al., 2017; Usha et al., 2017, 2021; Saini et al., 2022). The plant species and parts chosen for this study included *Bambusa balcooa* (leaf), *Phyllanthus emblica* (fruit), *Hodgsonia heteroclita* (fruit pulp), and *Punica granatum* (fruit peel).

4.12 Acute toxicity test

The oral administration of the PHF-HOPE extract resulted in no mortality being seen in the animals that were used for the experiment at a dosage of 2000 mg/kg given. Therefore, for the in vivo tests, it was determined that a dose of one tenth (400 mg/ kg) of the maximum dose and one twentieth (200 mg/kg) of the maximum dose were safe. After 42 days, there was no evidence of clinical or toxicological signs, nor was there any delay in death found in the animals that were used in the trial.

4.13 Effect of interventions on biochemical parameters

4.13.1 Induction of hyperglycemia

Before the trial began, the rats were separated into six different groups. With the exception of group one, all of the other groups were given alloxan for administration. Before beginning the experiment, the rats' blood glucose levels were measured, and the results showed that there was no discernible difference between the groups. Following the injection of alloxan for three days, a considerable rise in glucose levels was seen, and this increased glucose level was deemed to be in the diabetic group.

4.13.2 Blood glucose level

The experimental rats' blood glucose levels at various time periods are displayed in

Table 4.7: The blood glucose levels (measured in mg/dL) at different time points for various experimental groups.

Exp. groups	0th day	3rd Day	7th	14th	21st	28th	35th	42nd
Normal	99.12±11.89	102.34±10.234	106.68±6.4	100.11±5.00	101.4±11.154	102.36±4.50	108.08±6.48	102.15±6.129
DC	98.81±4.94	240.12±7.20	288.28±31.71	301.05±18.96	318.03±12.72	328.08±17.06	321.26±21.84	341.9±27.352
PHF-HOPE 200mg/kg	99.31±5.95	240.24±12.01	201.44±11.68	192.85±11.37	186.14±9.67	170.05±13.26	168.62±12.14	152.72±9.16
PHF-HOPE 400mg/kg	99.45±4.97	240.02±16.80	199.06±13.53	156.29±28.13	152.54±10.52	152.21±9.89	141.28±7.48	136.1±8.98
DG -0.25 mg/kg	99.85±5.99	240.36±19.22	170.22±8.34	142.08±11.79	141.2±7.90	128.2±10.51	130.12±11.71	130.41±6.52

DC= Diabetic control, DG= Diabetic glibenclamide

Table 4.7. Blood glucose levels increased in the alloxan group compared to the control group after three days of injections. Nevertheless, as the investigation progressed, blood glucose levels decreased in the PHF-HOPE treatment groups. Similar to the effects of the conventional medication (DG), the group treated with 400 mg/kg body weight with PHF-HOPE demonstrated a notable reduction in blood glucose levels. According to Tanko et al., (2008), the insulin-like properties of PHF-HOPE extract may either increase glucose uptake or decrease hepatic gluconeogenesis, which would explain its hypogycemic action. It might also work by either getting the β-cells in the islets of Langerhans to make insulin or freeing insulin from its bound state (Parmar et al., 2007; Middha et al., 2012). In order to provide a better view of the average blood sugar levels throughout the weeks or months, HbA1c values were also assessed. The results showed that the HbA1c levels of untreated diabetic rats were much higher than those of normal control rats, showing that a dosage of 150 mg/kg body weight was effective in inducing diabetes mellitus (DM) in Wistar rats. The PHF-HOPE group's HbA1c readings were considerably lower

Table 4.8: Blood tests conducted to measure specific parameters related to lipid levels and glycemic control.

Exp. groups	Triglyceride (mg/dL)	TC (mg/dL)	HDL (mg/ dL)	LDL (mg/ dL)	VLDL (mg/ dL)	HbA1c (mg/ g of Hb%)
Normal control	68.02±4.08	86.63±2.59	42.41±1.35	28.59±1.40	11.88±4.39	0.41±0.01
DC	132.21±9.51[a]	152.62±6.41[a]	23.85±1.24[a]	81.78±4.25[a]	39.53±1.18[a]	2.02±0.04[a]
PHF-HOPE 200mg/kg	76.05±1.14[b]	98.06±4.90[b]	37.36±0.89[b]	43.47±2.82[b]	16.28±0.55[b]	0.74±0.02[b]
PHF-HOPE 400mg/kg	88.18±2.46[b]	90.28±4.15[b]	39.77±1.82[b]	39.64±0.75[b]	14.75±0.30[b]	0.63±0.3[b]
DG-0.25 mg/ kg	66.23±4.10[c]	87.39±3.40[c]	40.58±3.08[b]	31.69±0.91[c]	12.86±0.56[c]	0.44±0.02[b]

DC= Diabetic control, DG= Diabetic glibenclamide, TC=Total Cholesterol, HDL= High-Density Lipoprotein, LDL= Low-Density Lipoprotein, VLDL= Very Low-Density Lipoprotein, HbA1c= Haemoglobin A1c. Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p < 0.05$. Those which are not sharing the same letters are significantly different.

than those of the diabetic control group, but they were not significantly lower than those of the standard medication group diabetic glibenclamide (DG). This data demonstrates that diabetic rats treated with HOPE had superior long-term management of their blood glucose levels, perhaps due to a favourable effect on lowering HbA1c levels. There may have been a therapeutic impact on diabetes management; although the decrease in HbA1c levels was not statistically significant when compared to the standard treatment group, it was considerably lower than the diabetic control group.

4.13.3 Blood lipid and HBA1C profiling

The levels of lipids and HbA1c in the various experimental groups are presented in Table 4.8. In comparison to the normal control group, the diabetic control group (DC) had higher levels of triglycerides, total cholesterol, LDL, VLDL, and HbA1c. Nevertheless, treatment with PHF-HOPE at dosages of 200 mg/kg and 400 mg/kg each reduced these lipid levels. Results indicated that, compared to the diabetic control group, the PHF-HOPE-treated groups had lower levels of triglycerides, total cholesterol, LDL, VLDL, and HbA1c. Results showing a decrease in triglyceride and LDL levels indicate that PHF-HOPE may have a lipid-lowering impact, even though levels of TC, LDL, and VLDL were almost the same in the PHF-HOPE-treated groups as in the control and standard medication groups. The fact that diabetic rats treated with PHF-HOPE had lower HbA1c levels than those treated with diabetic control (DC) suggests that the former group had better long-term blood glucose management.

4.13.4 Liver glycogen total protein serum urea, creatinine, SGOT, and SGPT

Table 4.9: The table presents the results of biochemical analyses conducted to measure specific parameters related to liver glycogen levels, total protein content, and markers of kidney and liver function.

Exp. groups	Liver glycogen (g/100mg wet)	Total protein (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	SGOT(IU/L)	SGPT(IU/L)
Normal	6.01±0.30	10.13±0.60	20.81±1.24	0.32±0.01	99.12±0.50	82.14±4.92
DC	2.83±0.16[a]	5.16±0.32[a]	49±2.45[a]	1.06±0.05[a]	191.31±11.47[a]	156.02±8.58[a]
PHF-HOPE 200mg/kg	4.89±0.25[b]	8.01±0.64[b]	23.12±1.29[b]	0.49±0.02[b]	123.19±6.15[b]	96.32±5.97[b]
PHF-HOPE 400mg/kg	5.11±0.30[b]	9.21±0.50[b]	21.62±1.29[b]	0.41±0.028[b]	111.63±5.46[c]	86.31±5.17[c]
DG -0.25 mg/kg	5.92±0.41[b]	10.67±0.53[c]	24.32±1.7[b]	0.37±0.02[c]	102.01±5.30[d]	83.62±4.18[d]

DC= Diabetic control, DG= Diabetic glibenclamide, SGOT= Serum Glutamic-Oxaloacetic Transaminase, SGPT= Serum Glutamic-Pyruvic Transaminase . Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p < 0.05$. Those which are not sharing the same letters are significantly different.

Diabetic rats exhibited elevated levels of blood urea, and creatinine, which serve as crucial indicators of renal impairment (Table 4.9). Glycogen serves as the main storage form of glucose inside cells and tissues. Insulin affects its regulation by stimulating glycogen synthase and inhibiting glycogen phosphorylase, which results in increased glycogen production (Alam et al., 2018). The diabetic rats saw a significant reduction in liver glycogen and total protein levels, which can be attributed to the absence of insulin. The administration of PHF-HOPE to diabetic rats resulted in the restoration of liver glycogen levels to a normal state, perhaps due to an increase in insulin secretion. Serum levels of SGPT and SGOT were higher in rats that had alloxan-induced diabetes. The potential cause of this phenomenon might be the release of enzymes from the tissues, which then enter the bloodstream as a result of the harmful impact of alloxan (Alagammal et al., 2012). SGPT and SGOT were used to measure how badly the liver was damaged in diabetic rats that were given streptozotocin (Kasetti et al., 2019). The study found that the PHF-HOPE extract cut down on the amounts of SGPT and SGOT enzymes in the livers of rats that had been given the poison alloxan. The normalization of SGPT and SGOT levels following treatment with both glibenclamide and PHF-HOPE enhances the antidiabetic effectiveness of the extract. In addition, SGPT and SGOT levels serve as indicators of liver function, and the return to normal levels of these measurements signifies the restoration of normal liver function.

4.14 Enzymatic antioxidant assays

4.14.1 Super-oxide dismutase (SOD, EC 1.15.1.1)

Table 4.10: SOD enzyme activities (U/mg/ml) of various organ tissues (Pancreas, Spleen, Liver, Brain, and Kidney) in different experimental groups.

Exp. groups	Pancreas (U/mg/ml)	Spleen (U/mg/ml)	Liver (U/mg/ml)	Brain (U/mg/ml)	Kidney (U/mg/ml)
Normal control	114.16±6.84	44.85±1.75	199.01±9.90	12.82±0.65	201.14±9.25
DC	69.83±3.96^{a}	32.49±3.26^{a}	106.83±7.26^{a}	6.89±0.32^{a}	99.82±7.12^{a}
PHF-HOPE 200mg/kg	76.92±6.25^{b}	37.61±1.88^{b}	109.85±4.13^{a}	7.82±0.26^{b}	103.82±5.25^{b}
PHF-HOPE 400mg/kg	81.13±4.93^{b}	38.48±1.27^{b}	125.11±6.22^{b}	7.97±0.48^{b}	158.61±4.28^{c}
DG -0.25 mg/ kg	104.31±5.17^{c}	39.09±2.32^{b}	142.56±8.66^{c}	8.14±0.74^{b}	196.83±8.34^{d}

DC= Diabetic control, DG= Diabetic glibenclamide Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p < 0.05$. Those which are not sharing the same letters are significantly different.

According to Arivazhagan et al., (2000) and Chis et al., (2009), superoxide dismutase (SOD) is an essential antioxidant defence enzyme that assists in the protection of tissue against oxygen-free radicals. It does this by catalyzing the dismutation of superoxide radicals, therefore turning them into hydrogen peroxide and molecular oxygen. When GPx works in conjunction with the activity of SOD, the reactive oxygen species that it produces are more effective. It does this by helping to clean up hydrogen peroxide (H_2O_2) and other organic hydroperoxides (ROOH) that are made by superoxide dismutase (SOD) (Halliwell, 2001). This keeps the membrane from losing its lipids. (Table 4.10) displays the findings of the levels of superoxide dismutase (SOD) in a variety of organ tissues and groups. When compared to the normal group, the level of superoxide dismutase (SOD) in the diabetic control group (DC) was considerably reduced. On the other hand, the levels of superoxide dismutase (SOD) in the treatment groups rose after they were administered HOPE at dosages of 200 mg/kg and 400 mg/kg. While the levels of superoxide dismutase (SOD) in the treatment groups were greater than those in the diabetic control group, the SOD levels in the treatment groups were not substantially higher than the SOD levels that were seen in the normal and standard medication (DG = 0.25 mg/kg) groups. With regard to the pancreas, it was observed that the normal group exhibited a level of superoxide dismutase (SOD) that was 114.16±6.84 U/mg/ml, but the DC group had a lower level of 69.83±3.96 U/mg/ml. When administered at dosages of 200 mg/kg and 400 mg/kg, the PHF-HOPE treatment led to the production of superoxide dismutase (SOD) levels of 76.92±6.25 U/mg/ml and 81.13±4.93 U/mg/

Table 4.11: CAT enzyme activities (Units per litre - U/l) in various organ tissues (Pancreas, Spleen, Liver, Brain, and Kidney) for different experimental groups.

Exp. groups	Pancreas (U/L)	Spleen (U/L)	Liver (U/L)	Brain (U/L)	Kidney (U/L)
Normal control	1.5±0.09	1.024±0.03	2.6±.0.14	2.22±0.12	1.8±0.06
DC	0.76±0.04[a]	0.29±0.01[a]	1.08±0.06[a]	0.99±0.06[a]	0.52±0.04[a]
PHF-HOPE 200mg/ kg	0.79±0.07[a]	0.44±0.01[b]	1.23±0.09[a]	1.24±0.05[b]	0.63±0.04[b]
PHF-HOPE 400mg/ kg	0.81±0.05[b]	0.46±0.02[b]	1.83±0.09[b]	1.64±0.04[b]	0.73±0.04[b]
DG -0.25 mg/kg	1.2±0.06[c]	0.69±0.01[c]	2.09±0.11[c]	2.09±0.15[c]	1.04±0.06[c]

DC= Diabetic control, DG= Diabetic glibenclamide Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p < 0.05$. Those which are not sharing the same letters are significantly different.

ml, respectively. The standard medication group, administered at a dosage of DG-0.25 mg/kg, had a SOD level of 104.31±5.17 U/mg/ml. Other organ tissues exhibited patterns that were comparable. It was shown that the diabetic control group had reduced levels of superoxide dismutase (SOD) in the spleen, liver, brain, and kidney when compared to the normal group. The administration of PHF-HOPE at dosages of 200 mg/kg and 400 mg/kg led to increased levels of superoxide dismutase (SOD), but these levels were not substantially different from those of the normal and standard medication groups.

4.14.2 Catalase assay (CAT)

To protect against free radical damage, the body uses endogenous antioxidant enzymes like CAT. According to Ahmad et al., (2011), catalase plays a crucial function in protecting cells from oxidative stress by efficiently breaking down hydrogen peroxide (H_2O_2) into oxygen and water. The diabetic control group (DC) had substantially reduced CAT levels in the pancreas, spleen, liver, brain, and kidney when compared to the normal group. In contrast to the control group, those treated with PHF-HOPE at dosages of 200 and 400 mg/kg showed no statistically significant increase in CAT levels. However, compared to the diabetic group, the PHF-HOPE treated groups did have somewhat greater CAT levels. The CAT level in the pancreas was 1.5±0.09 U/l in the normal group and 0.76±0.04 U/l in the diabetes group. The levels of CAT were 0.79±0.07 U/l and 0.81±0.05 U/l in the 200 mg/kg and 400 mg/kg HOPE groups, respectively. A CAT level of 1.2±0.06 U/l was observed in the conventional medication group (DG = 0.25 mg/kg). Other organ tissues showed comparable trends. The CAT levels in the spleen, liver, brain, and kidney were lower in the diabetes group compared to the normal group. (Table 4.11)

Table 4.12: MDA enzyme activities (Units per litre - U/l) in various organ tissues (Pancreas, Spleen, Liver, Brain, and Kidney) for different experimental groups.

Exp. groups	Pancreas (U/L)	Spleen (U/L)	Liver (U/L)	Brain (U/L)	Kidney (U/L)
Normal control	5±0.36	2.31±0.19	8.3±0.43	1.21±0.06	1.09±.05
DC	7.6±0.31[a]	9.13±0.52[a]	19.4±0.95[a]	2.98±0.19[a]	7.32±0.47[a]
PHF-HOPE 200mg/kg	7.1±0.42[a]	8.34±0.49[a]	15.8±0.81[b]	2.51±0.16[a]	6.13±0.44[a]
PHF-HOPE 400mg/kg	6.8±0.41[c]	7.09±0.46[b]	14.2±0.85[b]	2.03±0.14[b]	5.82±0.36[b]
DG -0.25 mg/kg	6.2±0.36[c]	4.88±0.31[c]	11.9±0.77[c]	1.98±0.11[b]	3.41±0.25[c]

DC= Diabetic control, DG= Diabetic glibenclamide. Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p < 0.05$. Those which are not sharing the same letters are significantly different.

shows that CAT levels in these tissues were marginally higher after PHF-HOPE therapy compared to the diabetes group.

4.14.3 Malondialdehyde (MDA)

As cellular free radical levels rise, MDA synthesis rises as one of the end products of polyunsaturated fatty acid peroxidation. One well-known indicator of oxidative stress is the amount of malondialdehyde (MDA) (Goyal et al., 2017). Diabetic control subjects had considerably greater amounts of malondialdehyde (MDA) in several organs and tissues than the control group, including the pancreas, spleen, liver, brain, and kidney. Although the differences were not statistically significant, treatment with PHF-HOPE at 200 mg/kg and 400 mg/kg dosages resulted in somewhat decreased levels of MDA in these tissues. The normal group showed an MDA level of 5±0.36 U/l in the pancreas, but the diabetes group showed a higher level of 7.6±0.31 U/l. The muscle damage assessment (MDA) values in the 200 mg/kg and 400 mg/kg PHF -HOPE groups were 7.1±0.42 U/l and 6.8±0.41 U/l, respectively. Referring to Table 4.12, the MDA level for the standard medication group (DG = 0.25 mg/kg) was 6.2±0.36 U/l. Comparatively, the diabetic group had noticeably elevated MDA levels in the spleen, liver, brain, and kidney when contrasted with the control group. Although the diabetic group showed statistically significant increases in MDA levels, the PHF-HOPE group showed just a small decrease in the same tissues.

4.15 Histopathological studies

In the normal control rat pancreas, histopathological examination revealed well-preserved islets of Langerhans. These islets contain pale, rounded, and ovoid β-cells located centrally, as indicated by the arrows (Figure 4.4). The islets are appropriately embedded within the exocrine portion of the pancreas, highlighting the typical and

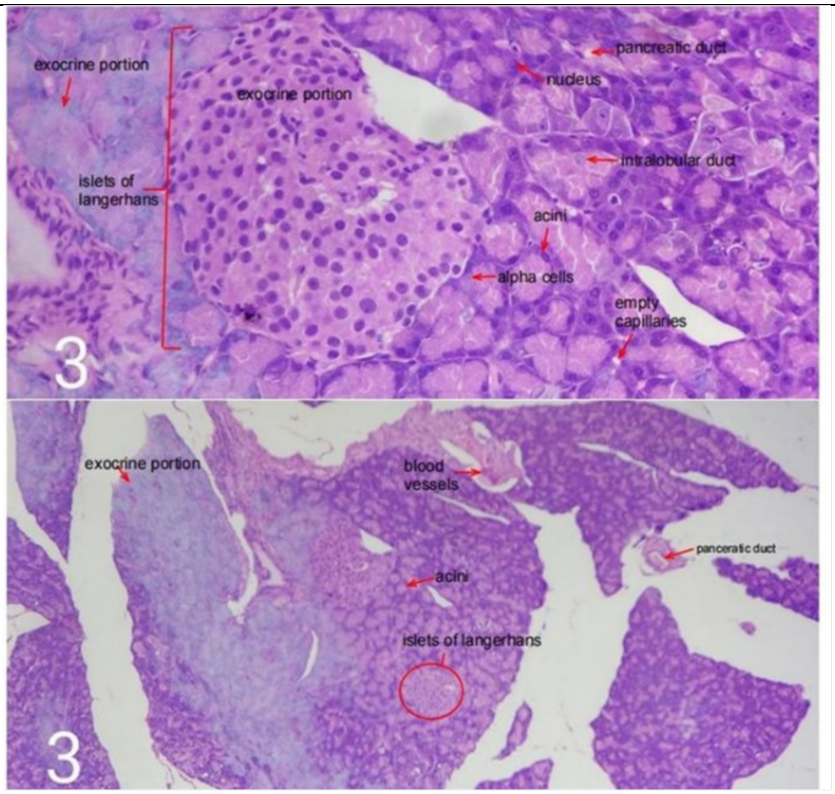


Fig. 4.4: Normal control rat pancreas showing normal islets of Langerhans with pale rounded and ovoid β-cells in the center (arrow), embedded in exocrine portion of pancreas (H&E).

healthy architecture of pancreatic tissue in the control group.

In the diabetic control rat pancreas, distinct histopathological alterations were evident. Notably, there was a noticeable shrinkage of the islets of Langerhans, accompanied by degeneration and necrosis of constituent cells. The nuclei within these cells appeared densely basophilic, indicative of pathological changes, and karyolysis is clearly evident (Figure 4.5). These observations collectively suggest structural and cellular abnormalities within the pancreatic tissue, reflective of the impact of diabetes on the morphology and integrity of the islets of Langerhans. In the pancreas of diabetic rats treated with glibenclamide, histopathological analysis revealed a positive impact on the islets of Langerhans. The islets exhibit a restoration to normal, characterized by the presence of pale, large, round to ovoid-shaped cells, as highlighted by the arrow. These cells were embedded within the exocrine portion of the pancreas (Figure 4.6). The observed preservation of the islet structure and cellular morphology suggests a beneficial effect of glibenclamide treatment on the pancreatic tissue in diabetic conditions.

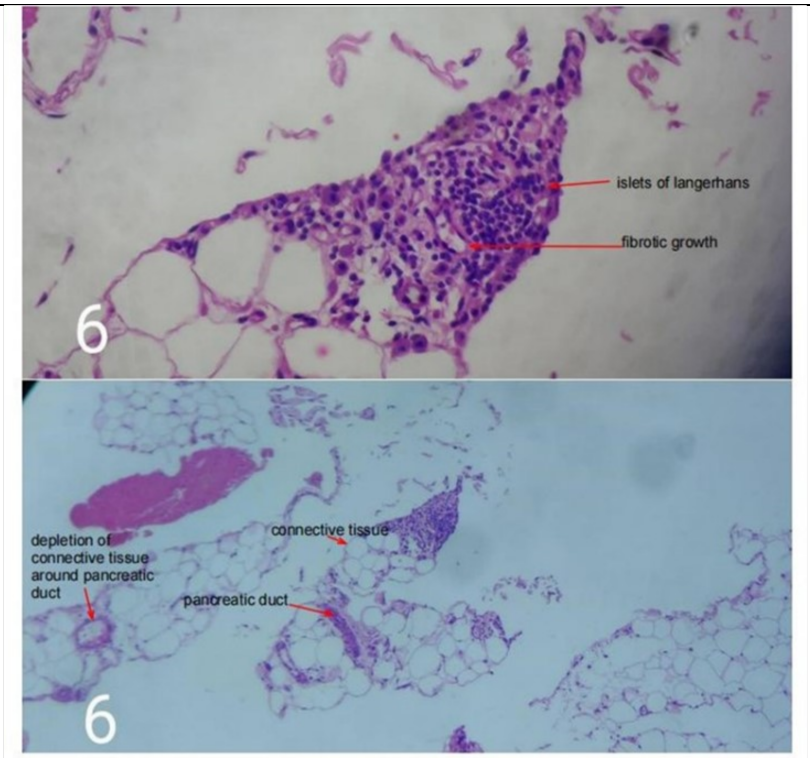


Fig. 4.5: Diabetic control rat pancreas showing shrinkage of islets of Langerhans with degeneration and necrosis of components cells where its nucleus appeared densely basophilic and karyolysis is evident (arrow) (H&E).

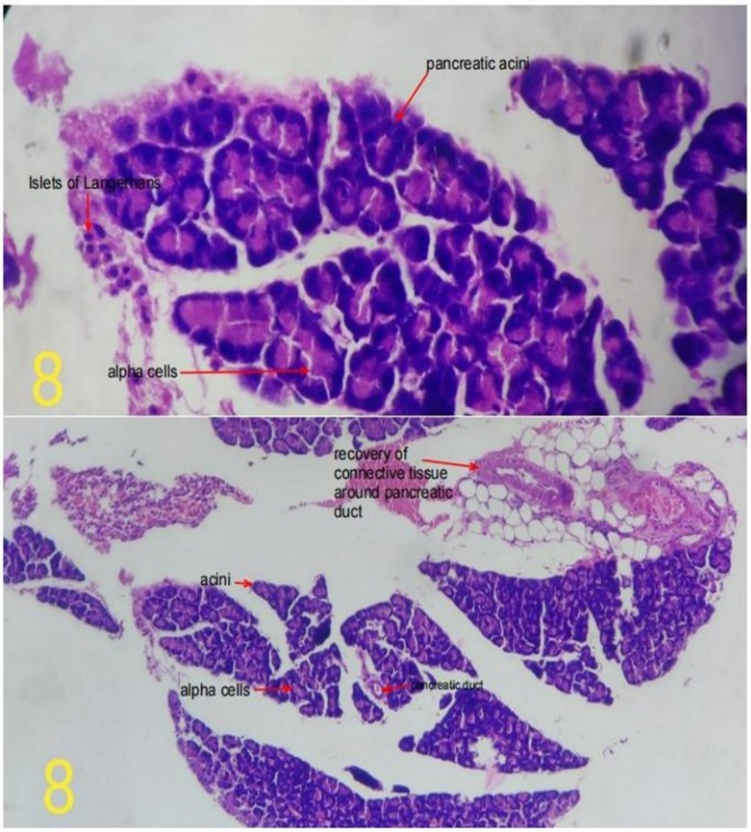


Fig. 4.6: Pancreas of diabetic rat treated with Glibenclamide showing normal islets of Langerhans with its normal pale large round to ovoid shaped containing cells (arrow) that embedded in exocrine portion of pancreas (H&E).

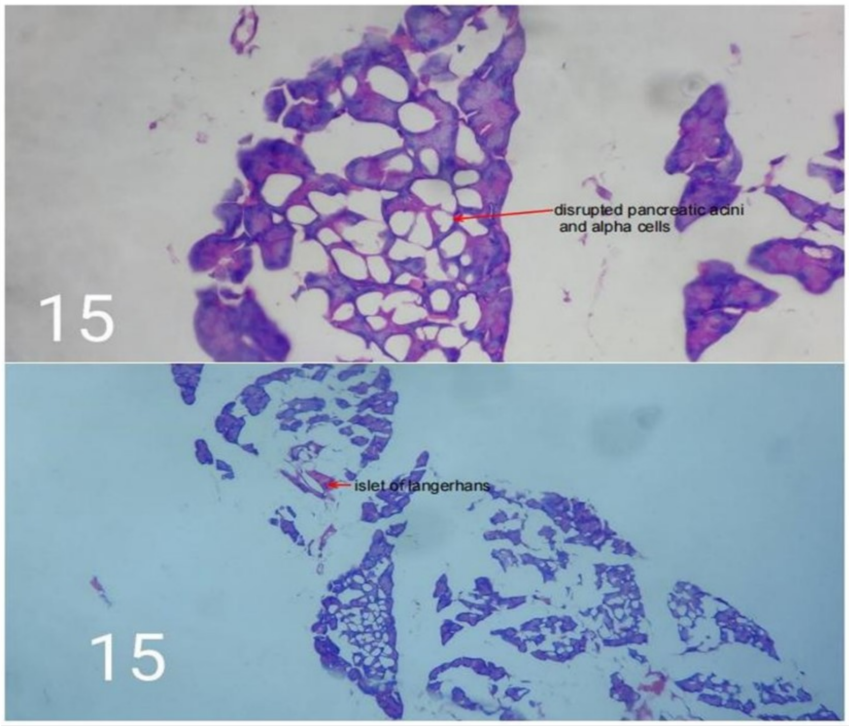


Fig. 4.7: Pancreas of diabetic rat treated with Low dose of HOPE showing normal sized islets of Langerhans but some degeneration of the β cell in the center were noticed (arrow) (H&E).

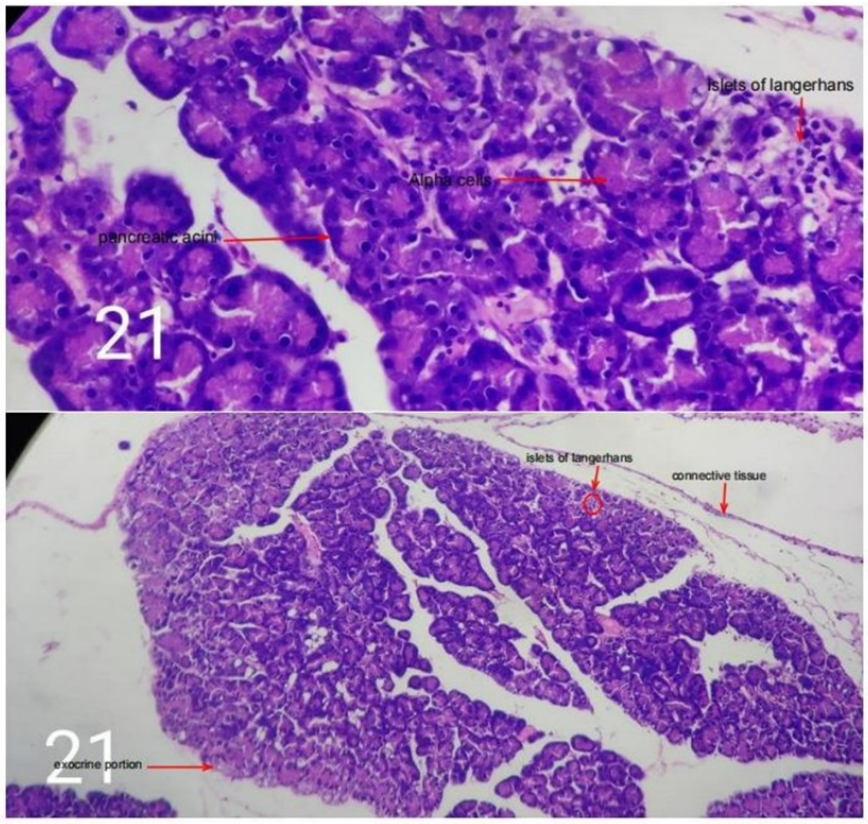


Fig. 4.8: Pancreas of diabetic rat treated with high dose of HOPE showing normal sized islets of Langerhans but better regeneration of the β cell in the centre were noticed (arrow) (H&E).

In the pancreas of diabetic rats treated with a low dose (200mg/kg B.W.) of PHF-HOPE, histopathological examination revealed islets of Langerhans of normal size. However, some degeneration of the β cells in the central region of the islets was observed. This suggests that while the treatment with a low dose of PHF-HOPE had a positive effect in maintaining overall islet size, there was still evidence of β cell degeneration within the islets (Figure 4.7). Further investigation may be warranted to understand the specific impact and potential mechanisms of this observed degeneration in the context of PHF-HOPE treatment for diabetic rats. In the pancreas of diabetic rats treated with a high dose (400mg/kg B.W.) of PHF-HOPE, histopathological examination revealed islets of Langerhans of normal size. Notably, there was a significant improvement in the regeneration of β cells in the central region of the islets. This suggests that the high dose of PHF-HOPE treatment had a positive impact on both the maintenance of overall islet size and the regeneration of β cells within the islets (Figure 4.8). The observed improvement in β cell regeneration may contribute to the therapeutic efficacy of PHF-HOPE in mitigating the effects of diabetes in the pancreatic tissue.