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Design and Evaluation of Antidiabetic Transdermal Patches Containing Hydroethanolic extract of *Morus alba* Leaves

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Abstract:

This work aimed to develop an anti-diabetic transdermal patch incorporating hydroethanolic extract of *Morus alba* leaves which has well proven hypoglycemic properties. The bioactive components of *Morus alba* leaves are apigenin, quercetin, and rutin which have proven anti diabetic activities. Using the solvent casting approach, herbal transdermal patches were manufactured with Eudragit RL 100 and PVP K 30 polymer. Different polymer ratios serve as a framework for the development of five formulations (F1-F5). Flexibility, thickness, weight variation, flatness, surface pH, folding endurance, percent moisture content, percent moisture uptake, and tensile strength were among the physicochemical properties assessed. The amount of drug was determined using spectrophotometry at wavelength 330 nm. A transdermal patch drug release study was conducted using the paddle over disc method using a 500 mL phosphate buffer pH 7.4. All five batches were seen to be opaque and non-sticky in nature based on the appearance results. The patches' surfaces were smooth. The addition of the hydrophilic polymer PVP K 30 in the right amount increases the medication release in F5. The results of the stability investigation indicated that F5 performed admirably for 180 days in the accelerated conditions. There were no discernible changes in the appear, drug content, or in-vitro release of medications after 24 hours in the previously indicated conditions.

Keywords: *Morus alba*, Transdermal patches, Anti-diabetic, Eudragit RL 100, PVP K 30, Solvent casting method.

Introduction:

Diabetes mellitus is a chronic metabolic disease typified by hyperglycemia, or elevated blood glucose levels, which are brought on by insufficient insulin and frequently coexist with insulin resistance. The majority of people with diabetes have non-insulin dependent diabetes mellitus (NIDDM), a heterogeneous group that includes milder forms of the disease that primarily affect adults (Bhujbal et al., 2011). Since the number of cases is steadily rising and long-term projections are looking less and less promising, diabetes mellitus (DM) has emerged as a major global health issue with a substantial impact. As a result, DM has been designated by international health authorities as one of the diseases that needs to receive priority attention. In 2012, hyperglycemia caused 2.2 million deaths, while in 2019 diabetes mellitus claimed the lives of 1.5 million individuals. Global diabetes prevalence is predicted to reach 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 (WHO, 2021).

Oral hypoglycemic medications such as thiazolidine diones, biguanids, α-glycosidase inhibitors, and sulfonylureas are part of the traditional medication regimen for treating diabetes mellitus, although they come with adverse effects. The noncompliance of patients with medication therapy is a significant issue. According to reports of improved patient compliance, transdermal drug delivery systems (TDDS) provide a better method of delivery (Bhujbal et al., 2011).

The transdermal route is one of the most appropriate, practical, safe, and affordable ways to provide medication, and it has drawn increased interest because of its flexibility and convenience when compared to other routes of delivery (Singhal et al., 2012). Transdermal drug delivery systems (TDDS) are topically applied medications in the form of gels or patches that distribute medications at a predetermined, regulated rate for systemic effects (Gaur et al., 2009). Compared to traditional drug administration methods, transdermal drug delivery systems offer numerous benefits, including a regulated rate of medication release, avoidance of hepatic metabolism, ease of termination, and extended duration of effect. A comparatively large amount of drug is put to the inside of a patch, which is worn on the skin for a long time. The drug penetrates the bloodstream immediately via the skin through a mechanism known as diffusion. The drugs will continue to diffuse into the blood for a considerable amount of time, keeping the constant concentration of drug in the blood flow, because there is a high concentration on the patch and a low concentration in the blood (Fox et al., 2011).

Although insulin and synthetic oral anti-diabetic medications, which are currently used to address diabetic complications, are efficient in lowering blood glucose levels, they have a number of adverse reactions and are unable to control diabetic challenges. (Grover et al., 2002). Across the world, traditional medicinal herbs are utilized to treat a variety of diabetic problems. Older traditional literature describes the use of a variety of herbal medications and minerals to treat diabetes mellitus. Compared to synthetic pharmaceuticals, herbal medications are thought to be safe and have less negative effects (Hayat et al.,2013). In order to give humanity a safer herbal medication substitute, it is crucial to investigate the hypoglycemic potential of medicinal plants.

Morus alba L. is a member of the Moraceae family, which is also known as white mulberry or Tut. Many Asian, South African, and European nations have long employed this plant to treat a variety of illnesses. Native to northern China, the plant is grown all over the world in areas where Bombyx mori are raised and cared for (Dkhil et al., 2015). The fruit, bark, and leaves of the plant have all been used for their many medicinal qualities for a very long time in traditional medicine. There have been reports of hypoglycemic, hypotensive, and diuretic effects from the leaves and shoots of this plant (Phiri and Chagonda, 2012). Traditional medicine has historically utilized mulberry root, stem barks, twigs, and leaves for fever, inflammation, cancer, diabetes, dyspepsia, diarrhea, hypertension, and anthelmintic purposes. It has also been used to improve vision, lower blood pressure, protect the liver, and facilitate the discharge of urine (Emniyet et al., 2014). Triterpenes, coumarins, volatile oils, alkaloids, bioflavonoids (rutin and quercitrin), and several organic acids have been identified in *M. alba* leaves in the form of according to published research. One of the main bioactive components of *M. alba* is 1 deoxynojirimycin, a naturally occurring α-glucosidase inhibitor that prevents type 2 diabetes (Dkhil et al., 2015; Liu et al., 2015). 1. Deoxynojirimycin (DNJ), a new chemical that inhibits intestinal α-glucosidases and decreases blood glucose levels, is the cause of *M. alba*'s antidiabetic effect (Hansawasdi, 2006). Mulberry leaf decoction (20 g/L) has been shown to lower blood glucose levels, prevent histological changes in the pancreas and kidneys of diabetic rats, and block the loss of hepatic glycogen (Khyade, 2018). The primary component of hypoglycemic mulberry leaves is flavones. The primary components of the flavones are kaempferol-3-O-rutinose, quercetin, rutin, and kaempferol. Mulberry leaves include quercetin and rutin, which have antdiabetic and antioxidative activities (Zhen et al., 2013).

So, In the following research work an attempt is made to formulate and evaluate an anti-diabetic herbal transdermal patch of hydroethanolic extract of *Morus alba* leaves.

Materials and methods

Chemicals and reagents

In the present experimental work, all the reagents used were of analytical grade.

Collection and Authentication of Plant Material

The leaves of mulberry (*Morus alba* Linnaeus) were collected from a farm of Indore in the month of September. The Leaves of *Morus alba* were validated and an herbarium specimen was stored for future reference.

Preparation of *Morus alba* Leaves Extracts

The *Morus alba* fresh leaves were cleaned, rinsed, and allowed to air dry at room temperature. After being ground into a fine powder and sieved through a 20-mesh screen, the powder was kept fresh in an airtight glass container. The method outlined in the literature (Garg et al., 2022) was slightly altered in order to create the extracts of *Morus alba* leaves. In order to prepare the extract, 250 g of powdered dried leaves were extracted for one hour at 63 ^{o}C using a hydroethanolic solvent (Ethanol and water in a 60:40 ratio). Whatman No. 1 filter paper was used to filter it. At 30 ^{o}C, the solvent was extracted using a rotatory evaporator. For the further processes, the resultant *Morus alba* hydro ethanolic extract (MAHE) was utilized.

Animals and treatment

150–180 gm male Wistar rats were employed. These were raised in our animal facility, where they were kept in a room with air conditioning (about 22 °C) and a controlled lighting schedule of 12 hours of light and dark. Pellets were used to maintain the animals, and unlimited access to tap water was provided. The Institutional Animal Ethical Committee (IAEC), the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) has approved the study. Before the trial, the rats were given fifteen days to become used to their new surroundings. There were four groups of animals. There are six rats in each group. Fasted animals were deprived of food for at least 16 hrs., but allowed free access to water. Water was given to the first group, which served as the control. A single dosage of STZ (60 mg/kg b.wt) dissolved in citrate buffer was administered to the second group, which was then split into four subgroups following a week of diabetes establishment. The first cohort was maintained as a diabetic control group, and for a period of 12 weeks, the second and third subgroups had daily gastric intubation to receive the standard medicine (glibenclamide, 10 mg/kg) and MAHE (200 mg/kg b.wt), respectively(Swathi et al., 2017).

Experimental procedure

A blood sample was taken from the tail vein 24 hours post-injection, and the Accu-Chek Glucometer was used to determine the blood glucose level using the glucose oxidase method both before and 72 hours post-STZ injection. Animals with blood glucose levels greater than 250 mg/dl were chosen for additional anti-diabetic treatment.

On the first, seventh, fourteen, and twenty-first days of the treatment, blood samples were drawn by pricking the rats' tail veins. The blood samples were then used right away to measure blood glucose using the Accu-Chek glucometer. (Gajdosik et al., 1999).

Formulation of Transdermal Patch

Utilizing the Solvent casting approach, transdermal patches of *Morus alba* leaves extract were formulated (Figure no.1). The solvent for patch preparation was methanol and water in a 70:30 ratio; the plasticizer was PEG 400; the polymers were PVP K 30 and Eudragit RL 100. Menthol was applied as an enhancer of penetration. The polymer, which was precisely weighed in various ratios in methanol: water (70:30), was dissolved to create the patch. PEG and menthol were added to this polymeric solution, and for two hours, they were thoroughly mixed with the aid of a magnetic stirrer. To this solution, the extract was incorporated in weighed doses while being constantly stirred. After that, the solution was put into a petridish that had been greased with glycerin, and it was left to dry for 24 hours at room temperature. Based on various polymer ratios, five formulations: F1, F2, F3, F4, and F5 are prepared (Table no.1). After that, the patches were divided into 1 cm by 1 cm pieces and preserved in polyethylene bags and aluminum foil, then placed in desiccators for additional research. (Trivedi, 2011; Sharma & Verma,2023).

Table no.1. Formulation of Transdermal Patch

Formulation Code	Extract (mg)	PVP K 30 (mg)	Eudragit RL 100 (mg)	Polyethylene glycol 400 (ml)	Menthol (ml)	Methanol: Water(ml) (70:30)
F1	200	350	100	30	0.5	10

F2	200	300	150	30	0.5	10
F3	200	250	200	30	0.5	10
F4	200	200	250	30	0.5	10
F5	200	100	350	30	0.5	10

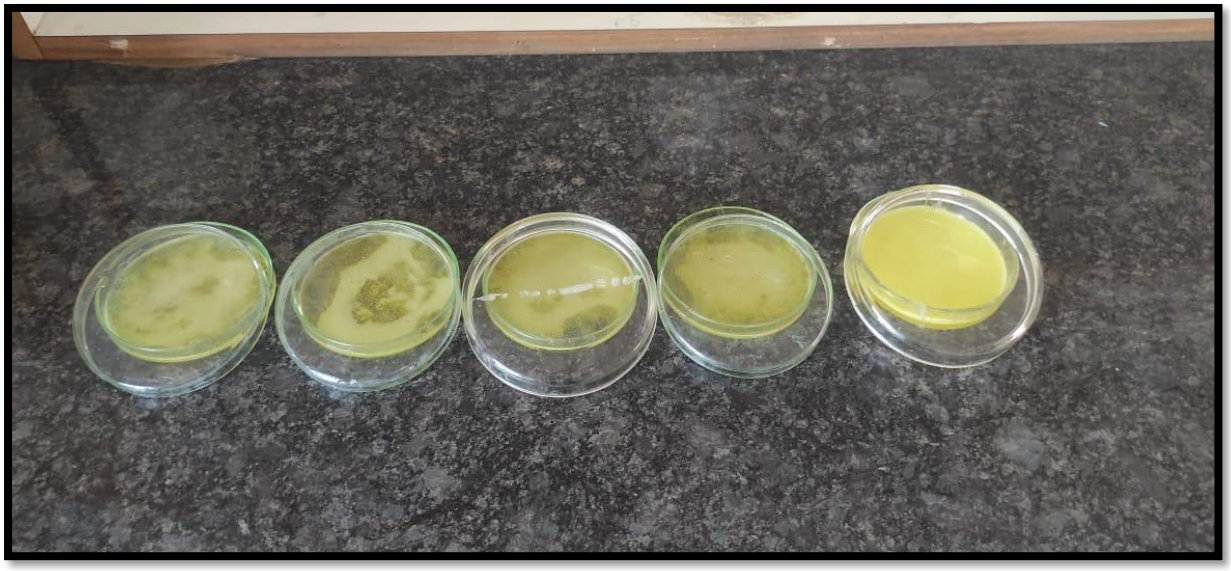

Figure 1. Prepared transdermal patch of hydroethanolic extract of *Morus alba leaves.*

Evaluation of transdermal patch

- ***Skin Irritation studies***

The albino Wistar rats were housed in cages with free access to water and standard lab diet. The dorsal abdomen skin of the rats was meticulously removed while minimizing peripheral damage prior to the 24-hour research. The formalin 0.8% v/v aqueous solution was utilized as a typical skin irritant. Rats were exposed to either a conventional irritant or a transdermal patch carrying a medicine on the first day of the test period by evenly applying the respective substances to the shaved area. The rats were then returned to their cages. After 72 hours of exposure, the patches were removed, and the test area was rinsed with double-distilled water. (Singh and Bali, 2016). The erythema score was read and recorded using the Draize scoring system (Draize et al. 1944). Scores of 0 indicated that there was no erythema, 1 indicated a very faint erythema (light pink), 2 indicated an established erythema (dark pink), 3 indicated an intermediate to serious erythema (light red), and 4 indicated a severe erythema (dark red).

- ***Physical Appearance***

The visual examination was done for each transdermal patch to check the smoothness, clarity, and stickiness (Kharia et al, 2020).

- ***Thickness***

The digital Vernier caliper was used to measure the patch thickness. The average thickness of the film was determined at five distinct locations (Bhujbal et al. 2011).

- ***Weight Variation***

An analytical weighing balance (Shimadzu) was used to determine the weight of each patch. The weight of the film was measured, and the data was documented along with the mean and deviation from the mean. (Bhujbal et al. 2011).

- ***Flatness***

Once the patches were chosen, two strips were removed from both sides and one from the center. By evaluating the length of each strip and the variations in length resulting from non-uniform flatness, one may approximate the percentage of constriction, where 0% constriction corresponds to 100% flatness. By calculating the constriction of the strips and using the formula, flatness was determined. (Bharkatiya, et al. 2010).

% Constriction =L1 – L2/L1 x 100

Where, L1 = Initial length,

L2 = Cut film length of Strip

- ***Surface pH***

The transdermal patch's surface pH was measured with a pH meter. The patch was moistened with water. The pH was measured by pressing the electrode up against the patch's surface. Three runs of the procedure were conducted, with an average and standard deviation. (Patel, et al. 2021.)

- ***Folding Endurance***

For the prepared patches, folding endurance was measured by hand. The patches were folded in the same spot over and over until they snapped. The precise measurement of folding durability is determined by counting how many times the patches could be folded in the same direction without breaking. (Bhujbal et al. 2011).

- ***Percent Moisture Content***

Patches were individually weighed, then kept in desiccators containing activated silica for a 24 hrs. at room temperature. Up until it stabilized, the weight of the films was measured several times. The moisture content percentage was calculated using the formula below. (Rao, et al. 2019).

Percentage Moisture Content = Initial weight - Final weight / Final Weight x 100

- ***Percent Moisture Uptake***

The generated patches were placed in desiccators containing saturated potassium chloride solutions and exposed to 84% relative humidity at room temperature in order to measure the amount of moisture absorption. Following the measurement of the films, the following formula was used to calculate the percentage of moisture uptake. (Rao, et al. 2019)

% Moisture Uptake = Final weight - Initial weight / Initial weight x 100

- ***Tensile Strength***

To determine the prepared patch's elongation (a measure for tensile strength)nit was tugged using a pulley. To strengthen the pulling power, weights were gradually added to the pan until the patch broke. The elongation, or the distance the pointer traveled before rupturing the

patch, was estimated using a magnifying glass and graph paper. It was determined that the tensile strength was kg/cm2. (Singhal, et al. 2012).

- ***Drug Content***

Following careful weighing and dissolution in 100 ml of pH 7.4 phosphate buffer, 100 mg of patch was added, and the mixture was vigorously agitated for 24 hours in a shaker incubator. The solution was filtered before being spectrophotometrically analyzed at 330 nm. For each formulation, the measurement was carried out three times, and the average and standard deviation were recorded (Kanabar, et al. 2015).

- ***In vitro Dissolution Study***

Using a 500 mL phosphate buffer pH 7.4 and the paddle over disc method, a drug release study of transdermal patches was performed. 500 cc of dissolution medium were utilized, and the paddle over disc apparatus was employed and run at a speed of 50 rpm with dissolution media at 32°C ± 0.5°C. Every time, a UV-visible spectrophotometer was used to extract 5 ml aliquots for up to 24 hours, and they were then evaluated spectroscopically (Cherukuri, et al. 2017). The experiment was carried out three times, and the results were averaged.

- ***Release Kinetics***

The drug release kinetics were studied using a variety of mathematical models, such as the Higuchi matrix, the Korsmeyer-Peppas models, the Hixson Crowell model, and the zero-order and first-order kinetic models. To explore the release mechanism and release rate kinetics of the dosage form, the collected data on drug dissolving in vitro was incorporated into these graphical models (Gangurde, 2011). By comparing the acquired r-values, the best-fit model in this instance was determined.

- ***Stability Study***

A stability investigation of the optimized and selected patch was performed using a stability chamber at a temperature of 40 ±2 °C and a relative humidity (RH) of 75±5% in compliance with ICH criteria. During the six months, the patch was kept under observation, its physicochemical stability was assessed by looking at its physical characteristics, drug content, and dissolving tests. (Parhi & Padilam, 2018).

Statistical analysis

Data are expressed as mean ± SEM and statistically analyzed by ANOVA followed by Dunnett test.

Result and Discussion

- ***Antihyperglycemic activity***

In the present study, diabetes mellitus was induced in rats through a STZ injection that causes the destruction of cells of islets of Langerhans (Proctor et al., 2006) This effect was represented in the current study through the elevation of blood glucose in STZ induced diabetic control rats. The administration of MAHE (200 mg/kg b.wt) showed significant ($p<0.01$) reduction of blood glucose levels (Table 2)

Table 2. Antihyperglycemic activity of Hydroethanolic extract of *Morus alba* leaves on STZ-induced diabetic rat

Groups	Zero Day (Fasting Blood Glucose Level)	1st day (After STZ induction of Diabetes Blood Glucose Level)	7th day (After induction of diabetes with Treatment Blood Glucose Level)	14th day (After induction of diabetes with Treatment Blood Glucose Level)	21st day (After induction of diabetes with Treatment Blood Glucose Level)
NC	97.72±1.37	97.72±1.51	95.70±1.20	98.63±1.03	96.23±1.16
DC	96.53±1.13	292.34±2.07	309.72±1.43	318.15±2.562	312.26±1.42
Standard	98.56±1.29	295.77±1.52	253.50±2.01**	164.02±1.38**	128.52±1.27***
MAHE	97.42±1.61	290.47±2.08	271.24±1.23*	141.32±1.60**	156.43±1.29**

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; ****P*< 0.05, *****P*< 0.01, * *P*< 0.001 as compared with diabetic control**

Skin Irritation Study

Rat skin irritation study was done to see if the Transdermal patch may irritate or sensitize skin. For 72 hours, the animals' erythema (skin redness and flushing) and edema (papules and wheals) progress was monitored. The erythema score was read and recorded using the Draize scoring method.

Compared to their negative control counterparts, transdermal patch-treated rats showed no signs of cutaneous reactions like erythema or edema. In contrast, 100% of the rats in the 0.8% w/v aqueous formalin treatment group displayed significant erythema after 48 hours, indicating serious irritation. This predicted that the produced transdermal films would have a non-allergenic and non-irritant profile (Table no.3)

Table no.3. Acute Dermal Irritation Study of Optimized Transdermal Patch F5

Skin Reaction	**Erythema**			**Edema**		
Observation time, hours	24	48	72	24	48	72
Total score	0	0	0	0	0	0
Mean Score	0	0	0	0	0	0
Total of mean score	0					
PII	0					
Remarks	**Non-irritating**					

All five batches were discovered to be opaque and non-sticky based on the appearance results. The patches' surface was smooth. As a result, it was determined that the preparation had no undissolved particles and that all of the excipients had been correctly dissolved.

Table no.4. Evaluation Data of Thickness, Weight Variation, Flatness, Surface pH and Folding Endurance of Transdermal Patches

Formulation Code	Thickness (mm) ± SD	Weight Variation (mg) ± SD	Flatness (%)	Surface pH	Folding Endurance
F1	0.43 ± 0.07	35.26±0.05	99.02± 0.11	6.3± 0.2	157±1.2
F2	0.41 ± 0.09	34.15±0.06	98.24 ± 0.27	6.4 ± 0.1	164±0.7
F3	0.42 ± 0.02	36.25±0.02	99.27± 0.04	6.3 ± 0.1	159±1.1
F4	0.43 ± 0.03	35.67±0.05	98.26± 0.32	6.0 ± 0.7	162±1.2
F5	0.42 ± 0.06	35.11±0.04	100	6.2 ± 0.2	189±1.3

Values are shown as mean ± SD, n= 3

Using a Vernier caliper, the thickness was measured and found to be uniform, ranging from 0.41±0.09 to 0.43 ± 0.07 mm. All of the patches from F1- F5 had the optimal thickness overall, but batch F2, F3, and F5 showed the best results as reported in Table no.4. The weight variation of the transdermal patches was found to be between 34.15± 0.06 mg and 36.25±0.02 mg. The patches' standard deviation values were low, indicating the lower weight variation among the prepared transdermal patches. The level of immediate constriction in the patch is indicated by flatness, and all of the formulations had similar strip lengths before and after cutting or separating the patch, with flatness ranging from 98.24 ± 0.27 to 100%, indicating complete flatness. The results are displayed in Table No. 4. The analysis revealed that every formulation had a pH that was closer to skin pH. Five batches' pH values were determined to be within the skin-friendly range of 6.0 ± 0.7 to 6.4 ± 0.1. The pH of batch F5 was found to be adequate at 6.2 ± 0.2. The investigation verified that every formulation had folding endurance values more than 150, with F5 demonstrating a higher endurance of 189±1.3. It implied that the patches were maintaining their integrity when applied to the skin and had high strength and flexibility. The findings were displayed in Table No. 4.

It was discovered that the transdermal patch batches' average moisture content ranged from 1.26 ± 0.11% to 1.56 ± 0.12%. The patches are more stable when there is less moisture present. The outcomes are shown in Table No. 5. It was shown that the average moisture uptake ranged between 2.012 ± 0.14% and 3.062 ± 0.21%. The patch's ability to absorb moisture did not significantly change when exposed to 84% relative humidity; nevertheless, a little increase in moisture acquisition was noted, necessitating the use of foil for moisture barrier storage. The findings are displayed in Table No. 5. High tensile strength is a need for an appropriate transdermal patch. The tensile strength of each of the five patches was determined to be satisfactory, ranging from 0.74±0.22 to 0.95±0.08 kg/mm2, indicating sufficient strength under stress. The drug content of the produced patches ranged from 82.6 ± 0.2 to 92.2 ± 0.3%, indicating a proper and even distribution of the drug. In the drug content analysis, formulation F5 performed the best out of all of them. Table No. 5 shows all of the results.

Table no.5. Evaluation Data of Folding Endurance, % Moisture Content, % Moisture Uptake & Tensile Strength of Transdermal Patches

Formulation Code	% **Moisture Content**	% **Moisture Uptake**	**Tensile Strength (kg/mm^2)**	**Drug Content (%)**
F1	1.42 ± 0.21	3.011 ± 0.22	0.74± 0.22	82.6 ± 0.2
F2	1.56 ± 0.12	2.048 ± 0.68	0.78± 0.02	85.2 ± 0.1
F3	1.51± 0.17	3.062 ± 0.21	0.84± 0.21	87.1 ± 0.6
F4	1.49 ± 0.27	2.072 ± 0.19	0.81± 0.03	88.6 ± 0.2
F5	1.26 ± 0.11	2.012 ± 0.14	0.95± 0.08	92.2 ± 0.3

Values are shown as mean ± SD, n= 3

- ***In-Vitro* Drug Release Study**

Morus alba transdermal patches were created using varying ratios of Eudragit RL100 and PVPK 30 to create a film with the required physicochemical characteristics. Here, the addition of PVP K 30, a hydrophilic polymer that gives the film sufficient resilience,

smoothness, and flexibility, decreased the rigidity of Eudragit. The lowest drug release was seen in the films F1 and F2, which may have been caused by Eudragit RL 100's hydrophobicity and limited moisture absorption capacity, which inhibit drug release. On the other hand, because the Film F5 (Eudragit RL 100: PVP K 30; 350:100) contains the hydrophilic material PVP K 30 in appropriate ratio, it has been discovered to enhance drug release. PVP K 30 increases the solubility of medicinal compounds by inhibiting crystallization (Vakadapudi et al., 2014). A slow steady release has been noted. Up to 24hrs, the steady state was sustained. The Eudragit polymeric network, which limits the diffusion of the extracted molecules out of the film, may be the cause of the delayed release. Table No. 6 contains the in vitro drug release information for each developed batch of transdermal patches. The information unequivocally demonstrates that formulation F5 delivered a higher proportion of drug than the other formulations. The addition of hydrophilic polymer PVP K 30 in the right amount improved the medication release. The drug release appears to increase in this proportion of polymers, according to the cumulative percentage drug release vs. time graph (Figure no. 2).

Table no.6. Results of *In-vitro* Drug Release Study

Time (hrs)	F1	F2	F3	F4	F5
1	3.23±0.42	4.58±0.16	6.26±0.45	7.21±0.15	9.45±0.25
2	6.62±0.52	7.21±0.32	8.27±0.12	13.16±1.4	15.12±0.23
4	10.27±0.12	11.12±0.23	10.18±0.11	20.12±1.1	22.11±.0.26
6	14.38±1.1	15.21±1.3	16.66±0.21	24.3±1.05	30.01±1.41
8	20.11±1.6	21.02±0.13	23.22±0.17	36.21±1.23	42.22±1.42
10	26.15±0.16	30.24±1.6	41.10±0.24	48.27±1.07	54.26±1.6
12	35.18±1.02	41.27±1.1	53.24±0.72	60.09±1.7	62.17±1.4
24	52.23±1.52	57.11±1.3	74.28±0.26	89.17±1.04	96.15±1.3

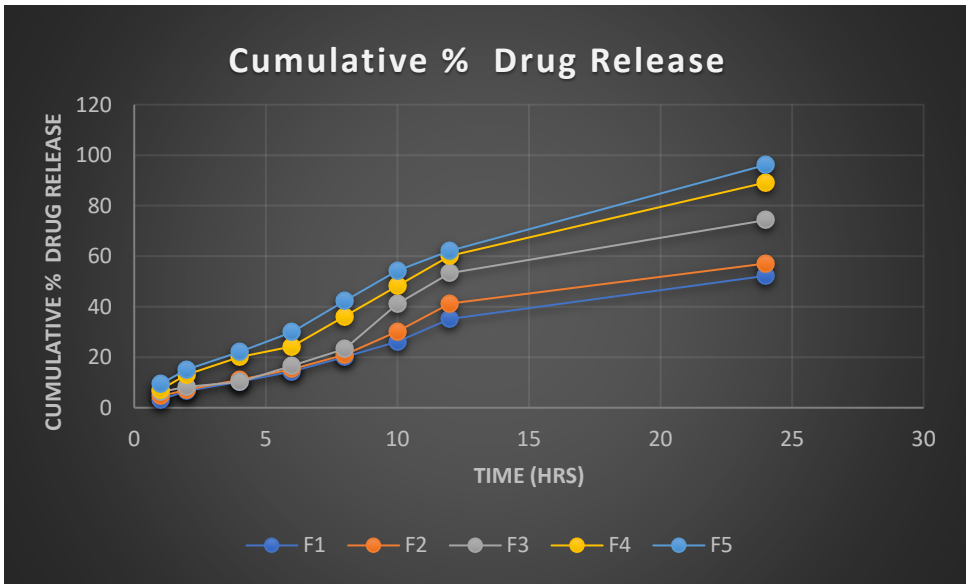

Figure no.2. *In-vitro* Drug Release Profile of Formulations F1-F5

- **Release Kinetics**

The data was fitted into various kinetic models, including Zero-order, First-order, Higuchi equation, Korsemeyer-Peppas, and Hixson-Crowell equation, after the in-vitro drug release of the optimized formulation F5. Results drawn are illustrated in table no.7

Table no.7. *In-vitro* Release Kinetics of Optimized Formulation F5

Formulation Code	Correlation coefficient (r^2)					Diffusion Exponent 'n'
	Zero order	First order	Higuchi	Hixson	Korsmeyer Peppas	
F5	0.975	0.9207	0.933	0.9013	0.9357	0.47

From the above data, it was concluded that prepared patch follows zero order drug release kinetics with the higher regression coefficient value of r^2= 0.975. The exact mechanism of skin permeation was confirmed by fitting the data in Korsmeyer- Peppas model, where the regression coefficient was found to be 0.9357 with release exponent n= 0 .47. Hence, it was ascertained that the drug is releasing through anomalous non- Fickian diffusion.

- **Stability Study:**

Based on the drug release results, F5 was determined to be the optimal formulation and was used in further studies. For six months, the stability investigation was conducted. The in-vitro drug release, drug content, and physical properties of the patch were examined. The results of this investigation demonstrated that formulation remained stable for 180 days under expedited conditions. There were no discernible changes in the look, drug content, or in-vitro release of medications after 24 hours in the previously indicated conditions. Statistical test of data obtained with drug content and drug release study also confirmed the absence of significant difference ($P < 0.05$) among stored and fresh patches, indicating the sufficient stability. Results of stability study are shown in Table no.8.

Table no.8. Stability Study of Patch F5 Stored at 40ºC/75% RH

Tested After Days	Physical Appearance	Drug Content (%)	*In-vitro* Drug Release
0	Smooth, opaque, Non-sticky film	92.2 ± 0.3	96.15±1.3
30	Smooth, opaque, Non-sticky film	92.2± 0.3	96.15±1.1
60	Smooth, opaque, Non-sticky film	92. 2± 0.1	96.14±1.8
90	Smooth, opaque, Non-sticky film	92. 1± 0.2	96.14±1.2
180	Smooth, opaque, Non-sticky film	91.8± 0.1	96.14±1.2

Conclusion

In the present study an Anti-Diabetic transdermal patch of hydroethanolic extract of *Morus alba* leaves are prepared by solvent casting method using a combination of PVP K 30 and Eudragit RL 100 polymer, using PEG as plasticizers and Menthol as a permeation enhancer. *Morus alba* leaves contains quercetin and rutin, which have antidiabetic and antioxidative activities. The optimized formulation F5 showed good physicochemical properties like thickness, weight variation, drug content, folding endurance, moisture content. The drug release in F5 is increased because of adding hydrophilic polymer PVP K 30 in appropriate quantity. A slow and steady release has been observed. The steady state was maintained up to 24 h. The slow release might be due to the Eudragit polymeric network that restricts the diffusion of the dug molecules out of the film.

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