Original Research Article

A novel herbal formulation in the management of diabetes

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Abstract

Background and Aim: *Momordica charantia* Linn. is traditionally used as a medicine for diabetes. The present investigation was aimed to formulate and evaluate transdermal patchesof *Momordica charantia* Linn. **Materials and Methods:** The transdermal films containing the herbal drug component fractionated fromethanolicextract of *M. charantia* fruits were prepared by using hydroxy propyl methyl cellulose as a polymer.The films were evaluated for folding endurance, thickness, weight variation, drug contents and *in vitro* diffusion studies and *in vivo* parameterslike acute and sub-acute antihyperglycemic activity in diabetic rats, biochemicalstudies, skin irritation in rats and stability studies. **Result and discussion:**The weightof transdermal patches of *M. charantia* (2 cm^2; 10 mg/patch) and was found to be 0.03 gm.Thickness of patches of *M. charantia* (2 cm^2; 10 mg/patch) was found to be satisfactory. The percentage release of active constituents from transdermal patches of *M.charantia* (2 cm^2; 10 mg/patch) was found to be 47.59% in 10% hydroalcoholic phosphate buffer pH 7.4 at the end of 6 h. The transdermal route exhibited negligible skin irritation and *in vivo* results revealed that the patches successfully decrease the blood glucose level. **Conclusion:** From the results, we concluded that the well-known herbal drug *M. charantia* Linn. have been found to be effective for diabetes through modern pharmaceutical formulation techniques.

Key words: Diabetes mellitus, diethyl ether fraction, *M. charantia* Linn, transdermal patch

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration (hyperglycemia) caused by insulin deficiency and it is often combined with insulin resistance. Non-insulin dependent diabetes mellitus (NIDDM) represents a heterogeneous group comprising milder form of diabetes that occurs predominately in adults and vast majority of diabetic patients have NIDDM.[1]The conventional drug treatment of diabetes mellitus consists of oral hypoglycemic agents like sulfonylureas, biguanid's, α-glycosidase inhibitors and thiazolidine diones which have side effects. An important problem in the drug therapy is the lack of patient compliance. Transdermal drug delivery system (TDDS) thus offers a better route of delivery, reported to have better patient compliance.[1] Transdermal drug administration generally refers to topical application of agents to healthy intact skin either for localized treatment of tissues underlying the skin or for systemic therapy. For transdermal products the goal of dosage design is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin.[2] TDDS can deliver certain medication to systemic circulation in a more convenient and effective way than is possible with conventional dosage form. The potential of skin as a path of drug administration has been amply demonstrated by the acceptability of marketed therapeutic systems.[3] TDDS can minimize first-pass metabolism associated with gastro-intestinal administration of drugs. The TDDS can maintain constant drug level in blood. It is possible to enhance the transdermal permeation of drug using penetration enhancers.[4] The present investigation was aimed to formulate transdermal films incorporating diethyl ether fraction of ethanolic extract of *Momordica charantia* fruits.

Diethyl ether fraction contains mainly charantin, which is a mixture of two compounds, namely, sitosterylglucoside and

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stigmasterylglucoside. Charantin is used to treat diabetes and can potentially replace treatment by injection of insulin, which has not been successful in stimulating the pancreases of the diabetic patients to lower blood sugar to the desired level.[5] Plant derived compounds that show anti-diabetic property such as charantin and others are now being widely accepted as an alternative medicine for treating diabetes mellitus, and they are free from any side effects.

MATERIALS AND METHODS

Materials

Ethanolic extract of *M. charantia* fruit was purchased from Amsar Private Ltd., Indore.

We further carried out the fractionation of extract by using diethyl ether to isolate steroids. Hydroxy propyl methyl cellulose K100M (HPMC), Polyethylene glycol (PEG) were brought from Research Lab Fine Chem. Industries, Mumbai. Alloxan monohydrate was procured from Sigma Aldrich Co, USA. Glipizide, a standard anti-diabetic drug was purchased from Franco-Indian remedies Pvt. Ltd. Glucose; triglyceride and cholesterol kitswere procured from Bio Lab Diagnostics Pvt. Ltd. (Boisar) India. All the chemicals and reagents of analytical grade and were purchased from Hexon Lab. Pvt. Ltd. (Mumbai).

Animals

Wistar albino rats of either sex weighing 160-200 g were maintained under standard environmental laboratory conditions and fed with laboratory diet and water *ad libitum*. All the protocols were performed in accordance with the Institutional Animal Ethics Committee (DYPIPSR/ IAEC/10-11/P-02).

Preparation of transdermal patches of *Momordica charantia*

Transdermal patches were prepared by solvent evaporation technique. The polymer (HPMC) and diethyl ether fraction of *M. charantia* were weighed. PEG, which acts as a plasticizer and permeation enhancer, was used in the concentration of 30 % v/v. Ethanol was used as a solvent. PEG 400 (30% weight of polymer) was dissolved in ethanol with stirring, which serve the purpose of plasticizer as well as penetration enhancer and then calculated amount of HPMC (500 mg) was dispersed in solvent ethanol. Diethyl ether fraction of *M. charantia* (290 mg) was dissolved in ethanol; this solution was then added to polymer base and stirred continuously to get uniform solution. The final volume was made up using water. The above solution was then poured into Petri plate coated with liquid paraffin and then dried at room temperature. After drying, patches were removed and cut into 2 cm^2 area and wrapped with aluminum foil and kept in desiccators until they were used for further study.[6]

Evaluation parameters of transdermal patches of *M. charantia*

Weight variation test

The weight of each patch was taken using analytical weighing balance (Shimadzu). The mean weight of the film as well as deviation from the mean was obtained and the data was recorded.

Folding endurance

The folding endurance was measured manually for the prepared patches. The patches were repeatedly folded at the same place till it broke. The number of times the patches could be folded at the same place without breaking gives the accurate value of folding endurance.

Thickness

The thickness of patches was determined using digital vernier caliper (Radical Co Mitu-toyo). The mean thickness was measured at five different points of film.

Drug content study

Transdermal patches were taken (2 cm^2area) individually, crushed, and taken in a 100-ml volumetric flask (pH 7.4 phosphate buffer). The medium was stirred with a teflon-coated magnetic bed for 2 h. The contents were filtered using Whatmann filter paper and the suitable dilutions of the filtrate were prepared by using phosphate buffer pH 7.4. Absorbance of dilutions were was measured by using UV- Vis spectrophotometer (Shimadzu) 1701, at 276.5 nm against phosphate buffer pH 7.4 as a blank.

Drug polymer interaction studies

Drug-polymer interaction was investigated using Fourier Transform Infrared (FTIR). FTIR of diethyl ether fraction of *M. charantia,* polymer (HPMC) and mixture of diethyl ether fraction and polymer were taken using Shimadzu 8400S FTIR spectrophotometer with KBr pellets.[7,8]

In vitro evaluation of transdermal patches

The *in vitro* skin permeation experiments were conducted using Franz diffusion cell (receptor compartment capacity: 20 ml). Full thickness skin from dorsal region of Wistar rat, whose hair had been removed on the previous day with hair removal cream, was used as membrane. The rats were sacrificed by cervical dislocation and the fatty material was removed from dissected skin and skin was washed with phosphate buffer used immediately. The receiver compartment was filled with 20 ml of 10 % hydroalcoholic phosphate buffer, pH 7.4. The transdermal patch was firmly pressed onto the center of the rat skin and then the skin was mounted on the donor compartment. The donor compartment was then placed in position such that the surface of dermis side skin just touches the receptor fluid surface. The whole assembly was kept on a thermostatically controlled magnetic stirrer set at 37 ± 2^0C and the content in the receiver compartment was continuously stirred at a constant speed (100 rpm) using a magnetic bead. The samples (2 ml) were withdrawn at the intervals of half hour up to 6 hr. and replaced with same amount (2 ml) of 10% hydroalcoholic phosphate buffer to maintain the sink condition. The samples were analyzed for drug content using UV- Vis spectrophotometer(Shimadzu 1701) at 276.5 nm. The cumulative % drug release from the transdermal patch of *M. charantia* was then calculated by using PCP DISSO software.[1,9]

In vivo evaluation of transdermal patches

Skin irritation study

The rats whose hair were removed on previous day, were divided into three groups (n = 6) and treated as follows.Group I – Normal control group, animals in group II were treated with formalin solution (a standard irritant; 0.8% v/v) upto 7 days, animals in group III were treated with transdermal patches of *M. charantia* (2 cm^2, 10 mg/patch) by using USP adhesive tape for 7 days.

The animals were treated daily with formalin solution and new transdermal patches of *M. charantia* upto 7 days by using USP adhesive tape. After 7 days animals were sacrificed and treated skin samples were processed for histological examination.

Antihyperglycemic activity in diabetic rats

Induction of diabetes

Diabetes was induced by of alloxan monohydrate (120 mg/kg, i.p) in saline solution. The diabetic state was confirmed 48 hr after alloxan injection by hyperglycemia. Surviving rats with fasting blood glucose level higher than 250 mg/dl were included in the study.

Acute study

The hairs on the backside of the rat were removed with a hair removal cream on the previous day of the experiment. Following an overnight fast, rats were divided into four groups(n = 6). The rats were treated as following:

Group I (NC) received 0.9% w/v saline (1 ml/kg, p.o.), group II (DC) received 0.9% w/v saline (1ml/kg, p.o.), group III (STD) received Glipizide(5 mg/kg, p.o), group IV (HT) received herbal tablet (89 mg/kg, p.o). Animals in group IV (TEST) were treated with new transdermal patch of *M. charantia* (2 cm^2; 10 mg/kg) by using USP adhesive tape [Figure 1].

At 0 min, 30 min, 1 hr, 2hr, 6 hr, and 24 hr after drug administration and patch application, blood was collected by puncturing tail vein under light ether anesthesia by using fine syringe in epindroff. Plasma glucose levels were estimated by the GOD/POD method

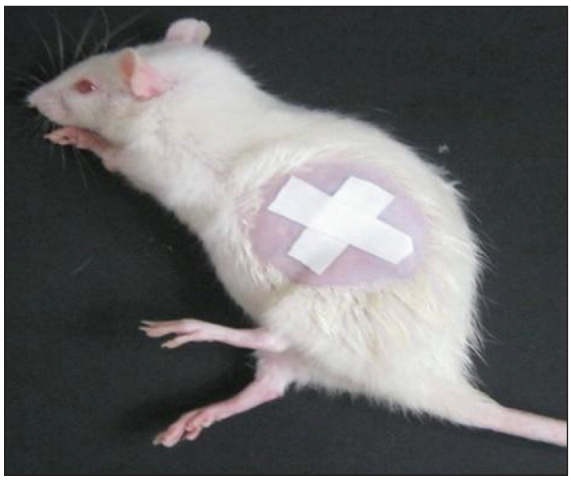

Figure 1: Application of patch to rat skin

Sub-acute study

The hairs on the backside of the rat were removed with a hair removal cream on the previous day of the experiment. Following an overnight fast, rats were divided into four groups (n = 6). The rats were treated as following:

Group I (NC) received 0.9% w/v saline (1 ml/kg/day, p.o.) for 14 days, group II (DC) received 0.9% w/v saline (1ml/kg/day, p.o.) for 14 days, group III (STD) received Glipizide (5 mg/kg/day, p.o) for 14 days. Group IV (HT) received herbal tablet (89 mg/kg/day, p.o) for 14 days. Animals in group IV (TEST) were treated daily with new transdermal patch of *M. charantia* (2 cm^2; 10 mg/kg) upto 14 days by using USP adhesive tape.

At day 1, day 8, and day 14 after drug administration and patch application, blood was collected by puncturing retro-orbital plexus under light ether anesthesia by using fine glass capillary in epindroff. Plasma glucose levels were estimated by the GOD/POD method. At day 14, blood was collected from animals of all groups and serum was separated off. TC, TG, HDL-C measured by using enzymatic kit of Bio Lab Diagnostics Pvt. Ltd. Company. According to that result LDL-C, VLDL-C andatherogenic index were calculated.

Biochemical evaluation

At the end of each week of treatment, all the animals were anesthetized with anesthetic ether and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary and collected in epindroff tubes and used for estimation of plasma glucose (GOD/POD method). Likewise at the end of $7^{th}$and 14^{th} day of treatment blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary and collected in epindroff tube. The serum wasused for estimation of total cholesterol (COD/POD method), triglyceride (GPO/POD method), HDL-C, LDL-C, and VLDL-C.

Statistical analysis

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

RESULTS

Physicochemical parameters of transdermal patches of *M. charantia*

In the present study, transdermal patches of *M. charantia* were prepared using HPMC K100M. PEG was used as plasticizer as well as penetration enhancer.The films were evaluated for their physical characteristics, such as weight variation, folding endurance, thickness, drug content study, and release characteristics. The physicochemical properties *viz.* weight variation test, folding endurance, thickness, and drug content of transdermal patches were within the limits [Table 1].

From the FTIR spectra, it was clearly evident that there were no interactions of the drug with the polymer. The main peaks in the spectrum of diethyl ether fraction of *M. charantia* do not show any

substantial difference when fraction was combined with polymer

Transdermal patches of *M. charantia* (2 cm^2area; 10 mg/patch) showed good release characteristics in hydroalcoholic phosphate buffer medium than in phosphate buffer solution. *In vitro* release profile of transdermal patches of *M. charantia* (2 cm^2; 10 mg/patch) showed 47.59% drug release in six hours [Figure 2].

Skin irritation test

The histopathological examination of the skin indicates that prepared systems produced mild inflammation. Formalin produced high grade of irritation, indicated by severe inflammation.

Anti-hyperglycemic activity in diabetic rats

Acute study

Group treated with Glipizide showed significant decrease in plasma glucose level at 1 hr, 2 hr, and 6 hr when compared with diabetic control group.Group treated with HT, TEST group showed significant decrease ($P < 0.01$) in plasma glucose level at 2 hr and 6 hr when compared with diabetic control group [Table 2].

Sub-acute study

The transdermal patches produced significant decrease in blood glucose levels upto 14 days indicating that these devices provide optimum Anti-hyperglycemic effects upon long-term application also.Groups treated with Glipizide showed significant decrease ($P < 0.01$) in plasma glucose level on day 8 and onwards, this significant anti-diabetic activity remains also at the end of treatment schedule (on day 14). Also groups treated with HT, TEST showed significant decrease ($P < 0.01$) in plasma glucose level at the end of treatment schedule (on day 14) [Table 3].

Biochemical parameters

The elevated lipid profile levels (total cholesterol, triglycerides, low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol) were significantly decreased with transdermal patches at the end of day 14 treatment compared to diabetic control group. Whereas high density lipoprotein-cholesterol level was significantly increased with transdermal patches at day 14 of treatement [Table 4].

Table 1: Physiochemical properties of Transdermal patches of *M. charantia*

Parameters	Transdermal patches of *M.charantia* (2 cm^2, 10 mg/patch)
Weight variation test	00.03 ± 0.00 gm
Folding endurance	360 ± 07.90
Thickness	00.05 ± 0.00 mm
Drug content	97%

Value represent mean ± SD (n = 5)

DISCUSSION

In this study, transdermal patches of *M. charantia* (2 cm^2; 10 mg/kg), produced a significant fall in blood glucose of diabetic rats, hence the transdermal patches of *M. charantia* have anti-hyperglycemic activity. The blood glucose lowering effects of

Table 2: Effect of transdermal patches on plasma glucose level in diabetic rats (acute study)

Gr.	Plasma glucose level (mg/dl)					
	0 min	30 min	1 hr	2 hr	6 hr	24 hr
NC	79.79±02.50	78.46±01.77	79.20±01.90	78.43±02.00	78.23±01.77	78.32±01.78
DC	290.00±02.05	291.54±02.07	291.78±01.43##	293.24±02.562##	292.12±01.42##	290.19±01.86##
STD	290.93±02.02	289.77±01.52	240.70±02.01**	180.62±02.183**	140.54±02.03**	284.88±01.86
HT	290.24±02.02	290.00±02.02	286.61±01.25	277.01±02.686**	259.71±01.71**	287.51±01.36
TEST	289.69±01.89	289.79±01.64	288.54±02.03	276.22±02.36**	260.42±01.72**	286.63±01.85

Results are presented as mean ± SEM. (n = 6), ANOVA followed by Dunnett's test.* ($P < 0.05$), **($P<0.01$) when compared with diabetic control group. Where, NC: Normal control group received 0.9% w/v saline (1ml/ kg, p.o.), STD: Glipizide (5 mg/kg, p.o), HT: Herbal tablet (89 mg/kg, p.o.), TEST: Transdermal patch of *M. charantia* (2 cm2; 10 mg/patch). ## - ($P<0.01$) when compared with normal control group

Table 3: Effect of transdermal patches on plasma glucose level in diabetic rats (subacute study)

Groups	Plasma glucose level(mg/dl)		
	Day 1	Day 8	Day 14
NC	79.58 ± 02.10	80.28 ± 01.95	78.46 ± 01.97
DC	290.13 ± 01.96	295.15 ± 01.33##	283.56 ± 01.70##
STD	290.82 ± 02.05	223.78 ± 04.85**	160.96 ± 02.75**
HT	290.02 ± 02.08	241.79 ± 03.97**	210.94 ± 01.90**
TEST	289.89 ± 01.47	278.67 ± 01.45**	250.22 ± 01.37**

Results are presented as mean ± SEM. (n = 6), ANOVA followed by Dunnett's test.* ($P < 0.05$), **($P<0.01$) when compared with diabetic control group. Where, NC: Normal control group received 0.9% w/v saline (1ml/ kg, p.o.), STD: Glipizide (5 mg/kg, p.o), HT: Herbal tablet (89 mg/kg, p.o.), TEST: Transdermal patch of *M. charantia* (2 cm2; 10 mg/patch), ## - ($P<0.01$) when compared with normal control group

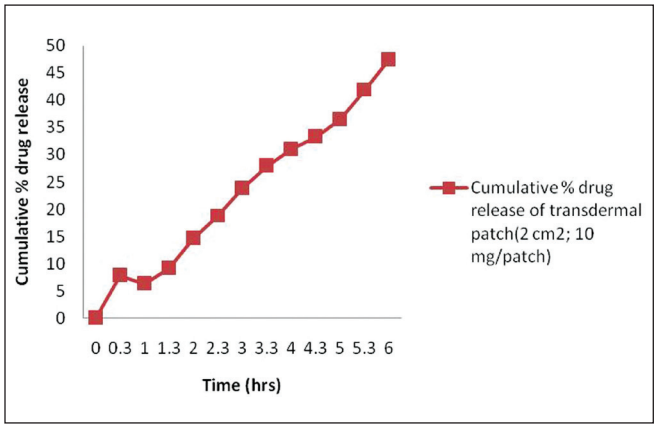

Figure 2: Cumulative % drug release from transdermal patches of *M.Charantia*

Table 4: Effect of transdermal patches on lipid profile in diabetic rats

Groups	Total cholesterol (mg/dl)	HDL-C (mg/dl)	Triglyceride (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
NC	81.6 ± 01.4	39.29± 01.87	59.41±02.19	11.88± 00.43	30.43± 02.55
DC	140.9 ± 02.72##	27.54 ± 02.00##	150.01± 02.07##	30 ± 00.41##	83.43 ± 02.57##
STD	85.3 ± 01.53**	36.38 ± 02.12**	61±01.00**	12.2 ± 00.20**	36.76 ± 03.35**
AT	122.16±01.76**	33.35±0 1.29	107.81± 01.64**	21.56±00.32**	67.24 ± 01.97**
TEST	124.1 ± 01.53**	31.71 ± 01.09	114.02±01.96**	22.8 ± 00.39**	69.58 ± 01.93**

**- (P<0.01) when compared with diabetic control group, ## - (P<0.01) when compared with normal control group

transdermal patches of *M. charantia* in diabetic rats was higher at 6 hr. Its preparations are reported to increase glucose utilization by liver and increase cellular uptake of glucose, promote, insulin release and potentiate its effect, which is probably responsible for the anti-diabetic effect.

The other species of this genus, *Momordica cymbalaria* have been reported to have anti-hyperglycemic activity.[10]

A variety of orally active hypoglycemic agents are frequently used to help to manage the glucose intolerance of NIDDM patients. But the effectiveness of these drugs is limited and suffers from a variety of side effects including hypoglycemia.[11]Many patients develop failure to oral anti-hyperglycemic agents. All these factors together reduce compliance. On the other hand, plant extracts evaluated in this study are commonly used vegetable in India and juice of fruits of this plant is commonly employed as a household remedy for diabetes.

CONCLUSION

In conclusion, this experimentation is one of the few attempts to utilize herbal drugs through transdermal drug delivery system. Above study demonstrates that transdermal patches of *Momordica charantia* exhibited better *in vivo* performance in rats and further study on higher animals and on human beings are required.

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