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Transdermal delivery of *Rauvolfia serpentina* root extract for anxiety management

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Abstract
Rauvolfia serpentina is a well-established herbal medicine for anxiety management for several years in Bangladesh. In attempted work, six patch formulations of *Rauvolfia serpentina* root extract were formulated using Ethyl Cellulose (EC), Hydroxypropyl Methylcellulose (HPMC), Polyvinylpyrrolidone (PVP) and Polyvinyl acetate (PVA) in different ratios. Physicochemical characteristics and constant release profile was tested for all six formulations of four polymers. *In-vivo* tests such, marble burying behavior (MBBT), locomotor activity (LAT), and skin irritation tests performed by applying the patches on rats for assays the anxiety management activity. Physicochemical characteristics such, weight uniformity, thickness, folding endurance, moister content, surface pH, swell ability, percent elongation of all the six formulations were 508.7 mg, 1.53 mm, 115.1, 4.91%, 6.06, 49.21% and 20% on an average of different formulations respectively with constant release of extract from each formulations. In MBBT, PF-I showed (p<0.05) and PF-II – PF-IV showed (p<0.01) significance, on the other hand in LAT, PF-II – PF-IV showed (p<0.001) significance with zero skin irritation. Throughout the experiment, we developed herbal transdermal patches of *Rauvolfia serpentina* root extract using different polymeric combinations, which reduced anxiety on rat models. As concluded, with further research herbal transdermal patch can be emerging as new and much convenient way of management anxiety.

Keywords: Transdermal drug delivery**,** anxiety**,** physicochemical properties**,** polymers**,** skin

Introduction
Anxiety, it can be define as the dispositional tendency to experience the fear [1, 2] or can be characterized by excessive and uncontrollable worry of something probable or unknown [3, 4]. It is the most common class of psychiatric disorders [5] associated with pronounced functional impairments and reduced quality of life across the lifespan [6]. Severe anxiety condition may treat with long-term medication management process and a steady controlled release of drug into the blood stream is necessary for management-based treatment [7]. According to the criteria, a better alternative route for a sustained steady release of drug can be the transdermal route. Transdermal patches been useful in developing new applications for existing therapeutics, which is a convenient dosage form for the route, can be defined as self-contained, topically administered medications in the form of patches, by which the drug delivered through the skin at a controlled rate to the systemic circulation [8, 9]. Despites of some drawbacks, transdermal patches have some major advantages such, by pass, the first pass effect ergo better increased bioavailability, maintaining constant plasma level, longer duration of action of a single dose ergo reduction of multiple dosing, and there are some evidences showed the reduction of side effect [10, 17].
Rauvolfia serpentina (L.) Benth., a medicinal plant from the Apocynaceae family occasionally found in the wild around Bangladesh, famously used for hundreds of years for the treatment of insomnia, hypochondria, insanity and hypertension [18, 20]. It has alkaloids, saponins, tannins, steroids, flavonoids, and phenols [21]. Chemically it contains alkaloids constituents like serpentine, serpentinine, ajmaline, ajmalicine, reserpine and so on [22, 23]. Among those, reserpine is an anxiolytic compound mostly found into the root of *R. serpentina* is about 0.0382%-0.1442% which was proved to manage anxiety in the past many years [24, 25]. The compound can cross the blood brain barrier efficiently and can effect on the serotonin reuptake mechanism [26, 27]. In this experiment, matrix type patch was formulated by using six combinations of four polymers with methanolic extract of *Rauvolfia serpentina* root.

The goal was to establish an herbal anxiety management patch, which may ensure fewer side effects, zero skin irritation, and a constant release of the *R. serpentina* extract into blood stream. For that, *in-vitro* tests were performed to ensure the physicochemical characteristics, polymeric integrity, release constancy and *in-vivo* tests were performed to determine the effectiveness of the patch as anxiety management medication on rat model.

Materials and Methods

Materials

The root of *Rauvolfia serpentina* or locally known as Sharpagandha collected from Narayanganj, Bangladesh in November 2015. The collected samples were then identified by Sarder Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium Dhaka, Bangladesh and a voucher specimen (DACB: 43255) has been deposited in the Herbarium for further reference. Then the fully dried root was finely powdered and stored into an airtight container. Pharmaceutics Laboratory of Stamford University Bangladesh provided diazepam powder. Hydroxypropyl Methylcellulose, Polyvinylpyrrolidone, Polyvinyl Acetate, Glycerol, Ethanol and Methanol brought from Qingdao Sun Chemical Corporation Ltd, Loba Chemie Pvt Ltd, Sigma-Aldrich Corporation and Merck KGaA respectively. All the chemical reagents were pharmaceutical graded and highly purified.

Methods

Rauvolfia serpentina root extraction

Approximate 60 g of finely crashed root of *Rauvolfia serpentina* was soaked into 250 mL methanol properly for 120 h. at 25±5 ºC in a beaker. It stirred for 1 h daily with a glass rod, then filtered by using cotton three times for removing the bulky material and finally filtered by filter paper for removing the fine particles. The solvent was removed by using rotary evaporator and about 4.87 g (% Yield = 8.116%) methanolic extract of *Rauvolfia serpentina* (MERS) obtained. MERS patch were formulated using the MERS and further investigations had been done.

Formulation of patches

The matrix type transdermal patches were prepared by using solvent casting method[8]. The casting solutions prepared by mixing polymer according to the formula tabulated in Table 1 with appropriate solvent. In this experiment, Ethyl Cellulose (EC) was dissolved in chloroform, on the other hand Hydroxypropyl Methylcellulose (HPMC), Polyvinylpyrrolidone (PVP) and Polyvinyl acetate (PVA) was dissolved in ethanol. The mixer was mix by mechanical stirrer by adding proper amount of Glycerol as plasticizer. The MERS added for MERS patch preparation and for the standard group standard patch were formulated using Diazepam powder. For the placebo, patch preparation neither MERS nor Diazepam used into the formulations. The formulas tabulated in Table 1.

Table 1: Composition of polymer for one 8cm × 8cm square sheet

No.	Formulation Code	Casting Solvent	Polymer				Plasticizer (mL)
			HPMC (%)	EC (%)	PVP (%)	PVA (%)	
1	PF-I	Ethanol	100	-	-	-	5
2	PF-II	Ethanol & Chloroform	50	50	-	-	5
3	PF-III	Ethanol	50	-	50	-	5
4	PF-IV	Ethanol	50	-	-	50	5
5	PF-V	Ethanol & Chloroform	40	40	20	-	5
6	PF-VI	Ethanol & Chloroform	30	30	20	20	5

After completed proper mixing, the mixer solvent poured into an 8 cm × 8 cm square petri plate. For removal of unwanted bubble formation and proper distribution of the extract, the mixer sonicated for 30 min and the sonicated mixer allowed drying into a LC-Oven maintaining 25±2 ˚C temperature for 24 h. Finally, after drying the 8 cm × 8 cm square sheet shaped into 16 pieces, 2 cm × 2 cm each.

In-Vitro evaluation

Weight uniformity

Randomly selected five different patches from each formulation weighted individually. Every patch weighted three times for precision. Then the weight variation calculated of the patches [28].

Thickness

Thickness measured in four different places of the same patch by using slide calipers and the mean thickness was calculated. From each formulation, five patches were randomly picked for the test [29].

Folding endurance

It's the number of folds required to break the polymer patch. It was required to test the strength and flexibility of the polymer combinations of different formulations. Longer folding number ensure better polymer integrity. To estimate, the patch folded repeatedly at the same place until it broke. The number of folds before the break of the patch was the folding endurance number. Random five patches from each formulation were selected for estimation [30].

Moisture content

Prepared five patches from each formulation were weighted individually and then put those into a desiccator with silica jell into it for 24 h. After the interval, all the patches weighted again, and presence of extra moister was calculated. The moister content were calculated by the formula below

$$M\ (\%) = ((W_o - W_t)/W_t) \times 100$$

In here, M (%) was the percentage of Moister Content, W_o was initial weight and W_t was final weight at't' time.

Surface pH

Randomly selected five patches from each formulation placed into the test tube continued 1mL of double distilled water and allowed to swell for an hour. Surface pH measured by using a pH meter, introducing the electrode into the surface water [30].

Swell ability

Randomly five patches from each formulation picked and weighed separately. All the patches allowed soak in to five petri dishes contained 5mL of double distilled water. Increase in weight measured with a time interval, until weight became constant. The degree of swelling (S) can be determined by following equation,

$$S\ (\%) = ((W_c - W_i)/W_i) \times 100$$

In here, S (%) is the percent of swelling, W_c was the constant weight and W_i was the weight of the patch initially [30].

Percent elongation

Randomly five patches of every formulation picked for the test. Percentage elongation is determined by measuring the length just before the breaking point. The percentage elongation can be determined from the below mentioned formula,

$$\%\ \text{elongation} = ((L_f - L_i)/L_i) \times 100$$

In here, L_f was final length of the single patch and L_i was the initial length of the single patch [8].

Constant release study

Modified dissolution apparatus was used for the release test of the patches [31]. Each MERS patch placed on a round 41.2 mm diameter glass disk, covered by a 104 µm stainless steel mash, and placed into each vessel of dissolution apparatus. The dissolution media was 900 mL 7.4 pH phosphate buffer with a maintained temperature of 32±2 ºC and the paddle speed was 50 rpm. The samples pulled from the vessel for 5 h with a 30 min interval. Each pulled sample was measured by UV-Spectroscopy at 268 nm λ_{max} against blank [32, 33].

***In Vivo* evaluation**

Animal

Albino Swiss male rat were used that collected from International Centre for Diarrhoeal Disease Research, Bangladesh (icddr, b). All the rats were 9 weeks old and weight was between 140 g to 150 g. Rat house environment maintained carefully with 12:12 h light-dark circle and temperature maintained within 22 ºC-25 ºC. Fresh food and drinkable water supplied in daily basis. Each cage (length 42 × width 46 × height 15 cm) contained not more than but 5 rats with sawdust bedding. Bedding changed after every 5-7 days. Before every test, patches were applied for 24 h, during the time animal groups were separately housed under the same maintained condition. Each group contained 5 rats. All the experimental rules and protocol approved by the Institutional Animal Ethical Committee (SUB/IAEC/16.02) of Stamford University Bangladesh.

Patch preparation and dose

All the three types of patches (placebo patch, standard patch, and MERS patch) formulated using four different polymers (HPMC, EC, PVP and PVA). Placebo one did not contain any drug or sample in it. For the standard patch Diazepam powder was used and 24h dose was according to1.6 mg/kg/2 cm^2/h flux [34]. For the MERS drug, dose was determined by several trials and best significance dose was selected. MERS dose for 24 h was calculated according to 2 mg/kg/2 cm^2/h drug flux. Both standard and MERS patches formulated with a 24 h dose and stored into an airtight container wrapped with aluminum foil paper till use. Groups tabulated according to the formulations in Table 2.

Table 2: Groups of rats for each formula

Groups		Placebo	Diazepam	Root Extract
A	Formula Code	PF-I	PF-I	PF-I
B		PF-II	PF-II	PF-II
C		PF-III	PF-III	PF-III
D		PF-IV	PF-IV	PF-IV
F		PF-V	PF-V	PF-V
G		PF-VI	PF-VI	PF-VI

Animal preparation

The back of each rat shaved just one day before the experiment by using an electronic clipper. The patches applied on exposed skin of the rat. Before apply the exposed skin swabbed by cotton soaked with ethanol. 5-7 days old bedding was unchanged before every test for better result. Medical tap was used to hold stable the patches on rats.

Skin sensitivity test

For the study, 10 rats picked weight between 140-150 g. They already shaved a day before the test. Each day a new patch applied for 7 days on every rat. Everyday rats observed for any skin abnormalities. Reaction gradation showed in Table 3 [35].

Table 3: Skin reaction gradation

Score	Erythema scale
a	No reaction
b	Slight, patchy erythema
c	Slight but confluent or moderate but patchy erythema
d	Moderate erythema
e	Severe erythema with or without edema

Behavioral test

All the tests performed under low light and in a quiet condition.

Marble burying test

To conduct the experiment all the patches applied on the shaved skin of the rats before 24 h. Six different formulas were used for formulate all the placebo, standard and MERS patches. All the rats grouped into six groups with five rats in each group. Formula PF-I, PF-II, PF-III, PF-IV, PF-V and PF-VI were applied on group A, B, C, D, E and F respectively. Placebo patches applied on the rat groups for 24 h and performed the tests on them. According to the protocol of marble burying test, approximately 5cm deep sawdust were lightly tapped and over it 25 white glass marbles were evenly spaced into a 12×12×12 inch glass box. The duration of the test is 30 min. after that only two-third or fully buried marbles counted. At the same time, the locomotor activity recorded by a video tracking system. The system helped to count how many times a rat crossed a 6×6 inch box predetermined into the computer. Than standard patches applied on the rat groups for 24 h and tests performed. Finally after one-week of interval MERS patches applied on the rat groups and tests performed. Every test performed after removing the patch from rat. After observation of each rat, they all put back into their home box with fresh new bedding [36].

Statistical analysis

Both *in-vitro* and *in-vivo* data were expressed as mean ± SEM (n=5). The number of buried marbles and locomotor activity during the marble burying test was analyzed by one-way analysis of variance (ANOVA) dunnett's test. All the data were analyzed for significant level $p<0.05$, $p<0.01$ and $p<0.001$.

Results

All the patches were stored into an airtight container wrapped by foil paper in room temperature and no physicochemical changes like appearance, color, or flexibility occurred during the storage. All the patches showed weight variation from 425.36 (PF-I) to 640.22 (PF-VI) mg with an acceptable standard deviation value. Combination of different polymers showed variation in the weight of different patches. The thickness of the patches varied from 1.02 (PF-III) to 2.18 (PF-VI) mm. For better performance, the patch should have acceptable flexibility. It should have the ability to withstand with normal skin bent and fold of skin. It can be determined by folding endurance and percent elongation of patches. All the patches showed 100 (PF-V) to 150 (PF-IV) folding endurance and 8% (PF-III) to 29% (PF-V) percent elongation. Patches showed swell ability from 25.61% to 77.73%. In here, PF-IV showed low swell ability in contrast PF-V highest swell ability. Surface pH varied from 5.35 (PF-V) to 6.74 (PF-III), which indicated that there would be no occurrence of skin irritation after application. All the parameters represented in Table 4.

Table 4: Characteristics of Transdermal Patches (Weight Uniformity, Thickness, Folding Endurance, Moister Content, Surface pH, Swell Ability, Percent Elongation)

Formula Code	Weight Uniformity (mg)	Thickness (mm)	Folding Endurance	Moister Content (%)	Surface pH	Swell Ability (%)	Percent Elongation (%)
PF-I	425.36±0.185	1.39±0.072	103.4±0.554	0.672±0.066	5.67±0.034	47.88±0.758	10±0.236
PF-II	449.6±0.705	1.86±0.054	127±0.591	1.2±0.0711	5.67±0.064	61.17±0.979	26±0.915
PF-III	445.94±0.459	1.02±0.037	105.4±0.144	3.17±0.213	6.74±0.205	42.51±0.976	8±0.356
PF-IV	540.64±0.351	1.18±0.036	150.6±0.660	1.04±0.266	6.67±0.101	25.61±0.825	23±0.549
PF-V	550.52±0.976	1.57±0.0351	100.6±0.6	4.71±0.221	5.358±0.179	77.73±0.048	29±0.870
PF-VI	640.22±0.555	2.18±0.021	103.6±0.007	18.69±0.321	6.29±0.268	40.37±0.236	24±0.870

Constant release rate observed among the formulations for five hours. MERS used as release substance into the polymer formulations. PF-II (71.56%), PF-V (72.22%), and PF-VI (70.24%) showed almost close released rate, which was steady than others. On contrary, PF-IV (97.10%) showed almost full release of MERS within 5 h, which bit faster release then other formulations. PF-I (79.49%) showed something in the middle (Figure 1).

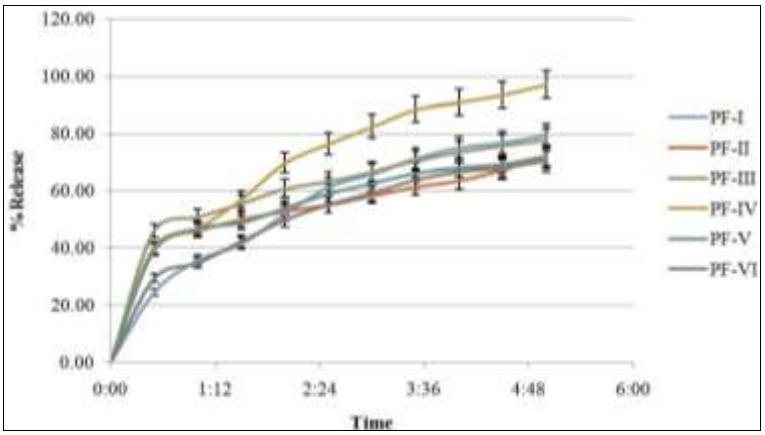

Fig 1: Percent Release of Six Formulas (PF-I, PF-II, PF-III, PF-IV, PF-V and PF-VI)

For the skin irritation test, 7 patches applied in 7 days on the shaved rats' skin. The results of the skin irritation tabulated in the Table 5.

Table 5: Seven days skin reaction

Days	Polymer Combinations					
	PF-I	PF-II	PF-III	PF-IV	PF-V	PF-VI
1	a	a	a	a	a	a
2	a	a	a	a	a	a
3	a	a	a	a	a	a
4	a	a	a	a	a	a
5	a	a	a	a	a	a
6	a	a	a	a	a	a
7	a	a	a	a	a	a

In this table, *a* - No reaction, *b* - Slight, patchy erythema, *c*- Slight but confluent or moderate but patchy erythema, *d*- Moderate erythema, *e*- Severe erythema with or without edema.

Results indicated there was no skin irritation during the application. All the polymer combinations showed reduction in marble burry test and reduction in locomotor activity test. PF-II, PF-III, PF-IV, PF-V and PF-VI showed marble burry reduction from 1.6±1.864 to 2.4±2.776 as compare to the control with acceptable significance ($p<0.01$) and standard groups of all the formulations showed reduction compared to control groups. On the other hand, PF-I did not showed that much reduction but still showed significance ($p<0.05$) compared to control (Figure 2).

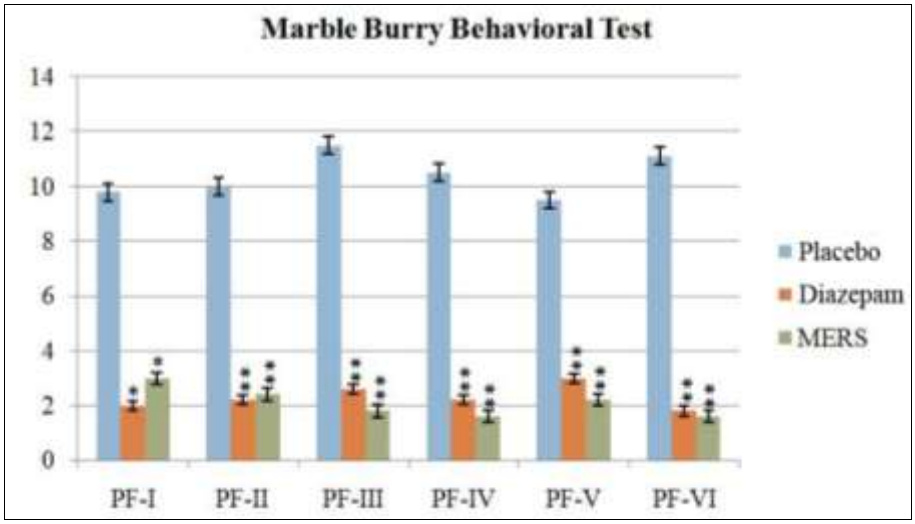

Fig 2: Marble Burry Behavioral Test

In case of locomotor activity test, PF-II, PF-III, PF-IV, PF-V and PF-VI showed good enough reduction with a high significance (p<0.001) against control but PF-I didn't show any significance at all (Figure 3).

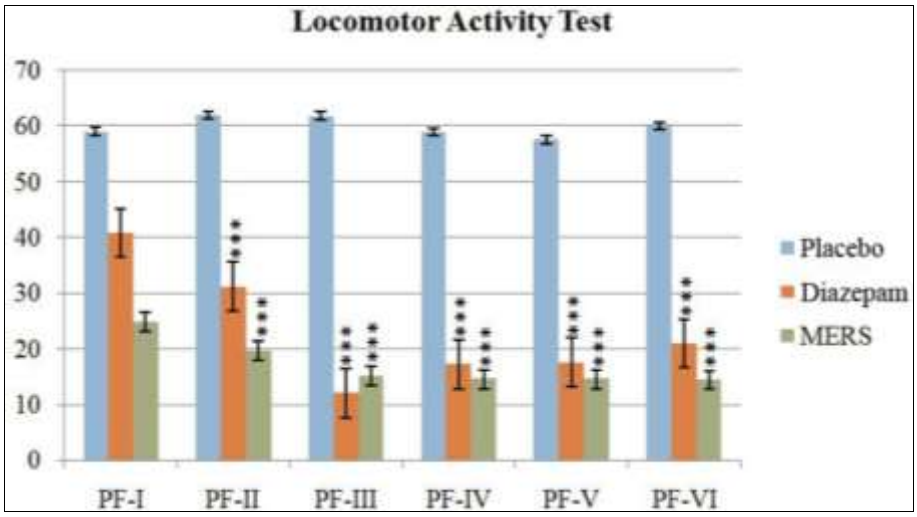

Fig 3: Loco motor Activity Test

All the results tabulated in Table 6.

Table 6: Anti-anxiety effect of MERS using different polymer in marble burry and loco motor activity test

Polymer Combination Code	Marble Burry Test	Loco motor Activity Test
PF I	3±2.88*	24.8±28.432
PF II	2.4±2.776**	19.6±20.144***
PF III	1.8±1.952**	15.2±15.408***
PF IV	1.6±1.864**	14.6±15.784***
PF V	2.2±2.328**	14.6±15.984***
PF VI	1.6±1.864**	14.4±12.976***

*p<0.05, **p<0.01 and ***p<0.001compared with the control group (Dunnett's test)

Discussion

Because of the bypass of first pass effect and immediate action of transdermal patch, this can be a better alternative of oral dosage form. For the CNS disorders like anxiety or depression or even for hypertension a constant drug management may needed. Transdermal patch can be very promising for these kinds of conditions. In case of matrix type transdermal patch, by several polymer combinations drug release can be control. Medicinal plants can be used for better reliability with less/zero side effects than the synthetic drugs. Medicinal plants can be used for better reliability with less/zero side effects than the synthetic drugs. MERS used in the preparation of the patches.

Six (PF-I, PF-II, PF-III, PF-IV, PF-V and PF-VI) formulations were prepared using four polymers (HPMC, EC, PVP and PVA) by several trials. PF-I was 100% HPMC, where PF-II, PF-III and PF-IV was a 50:50% combination of HPMC:EC, HPMC:PVP and HPMC:PVA respectively. PF-V was a 40:40:20% combination of HPMC:EC:PVP polymer. Finally, PF-VI was a four polymer combination where HPMC:EC:PVP:PVA present at 30:30:20:20% respectively. Percentage of each polymer

in the formulations corrected several times to ensure well formation of the membrane. According to the observation, Increase in the percentage of PVP destabilized the membrane formation. It either became very soft or did not form at all into membrane. Every formulation contained plasticizer, because without plasticizer the membrane became brittle and hard. For maintaining acceptable flexibility with MERS and diazepam 5 mL of glycerol as plasticizer added in each formulation. A soft and weak polymer combination showed low percent elongation and folding endurance, on the other hand a hard and strong polymer combination showed low folding endurance and high percent elongation, finally a soft and strong polymer combination showed high folding endurance and high percent elongation. Accordingly, PF-V and PF-VI was soft and strong membrane which can withstand the normal bents and folds of skin. Study of swell ability depends on the hydrophilicity of the polymers and polymer combination with the presence of plasticizer. The combination of HPMC, EC and PVP showed maximum swell ability, in where into the distilled water polymers lost the plasticizer and eventually increased penetration of water and eventually increased wettability. Release constant study showed gradual and steady release by all the formulas. Formulations contained EC polymer showed almost the same release percentage of MERS. Combination of HPMC: PVA showed faster release and only HPMC showed variations in several trials of MERS release.

The protocol of the marble burying tests dictates, Control group can be expected to bury most of the marbles during a 30 min test. Benzodiazepine, which is Diazepam, would markedly reduce burying indeed with the movement of mice. On the other hand, *Rauvolfia serpentina* was proved as anti-anxiety drug [20, 37]. Each group of rats were received MERS via different formulation of transdermal patch and showed significant avoidance of marble burying with reduction of movement as compared to control group [36]. The spontaneous locomotor activity was recorded by video tracking system. *Rauvolfia serpentina* contains 50 alkaloids including therapeutically important reserpine, deserpidine, rescinnamine and yohimbine [18]. Among these reserpine is responsible for the anxiolytic effect on petients [25]. Reserpine is highly lipophilic, so it can cross the skin from the matrix membrane rapidly and has the ability to cross the blood brain barrier so that it can achieve the brain level and inhibit the serotonin reuptake within minimum time [26, 27]. Because of the bypass of first pass effect via transdermal route, MERS can affect rapidly and because of the constant continuous release from the patch with zero skin irritation, it can effectively manage anxiety for longer period. At the end, if extended release considered better for management based treatment then PF-VI showed better result among all formulations.

Conclusion

Transdermal patch formulated by the MERS in this research showed acceptable result for anxiety management. In the formulations, all six formulations of four polymers passed through all the physicochemical tests, also showed accepted significance in *in-vivo* tests, and reduced anxiety on rats with zero skin irritation. Over the past few years, there are several anxiety management methods and therapies developed and it has always been the search for new much convenient ways. Therefore, in conclusion if considered the efficacy, safety and tolerability of herbal medicine and fast, safe bioavailability of transdermal patch, this can be a well-accepted alternative for anxiety management.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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