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African Journal of
Pharmacy and Pharmacology

8 July, 2019
ISSN 1996-0816
DOI: 10.5897/AJPP
www.academicjournals.org



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Full Length Research Paper

Evaluation of anthelmintic activity of ethanolic extracts of *Carica papaya* leaves using *Paramphistomum cervi* and *Haemonchus contortus*

Md. Rabiul Islam^{1*}, Syeda Fatema Tuz Zahra³, S. M. Ibrahim Sumon¹, Shahnaj Parvin³, Kamrul Hasan², Moudud Ahmed¹, Md. Abu Talha Siddique¹ and Tazmel Haque⁴

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Received 19 February, 2019; Accepted 27 May, 2019

The aim of this present study is to evaluate the anthelmintic activity of leaves extracts of *Carica papaya* using *Paramphistomum cervi* and *Haemonchus contortus* as the test worms. A range of concentrations (100, 50 and 25%) of ethanolic extracts of *C. papaya* were tested to assay the procedure. This is mainly applied for the determination of time of death (D) and time of paralysis (P) of the tested worms. After the analysis, it was shown that for the *H. contortus* at 100% concentration, the paralysis occurred within the shortest time (P=24.5 min) and death came at the lowest possible time (D=56 min). The time of death and paralysis increased at 50% (D=64 min and P=28 min) and 25% concentration (D=74 min and P=34 min), respectively compared to the Piperazine citrate (P= 24 min and D= 54) at concentration of 10 mg/ml. Here, distilled water is as a control solution. The results of this study revealed that the ethanolic extracts of the leaves of the *C. papaya* expressed a demonstrated paralysis significantly, and also responsible for the death of *P. cervi* and *H. contortus* especially at the higher concentration (100%) compared to the standard reference of Piperazine citrate. Therefore, from the results it is declared that the ethanolic extracts of the leaves of *C. papaya* showed a great anthelmintic activity against *P. cervi* and *H. contortus* worms. Hence, the present research work signifies that the leaf of *C. papaya* has a major anthelmintic activity and also can be used as a potent drug for its low cost and availability.

Key words: *Paramphistomum cervi* and *Haemonchus contortus*, anthelmintic, *Carica papaya* leaves, Albendazole.

INTRODUCTION

The word “anthelmintic” is from the Greek word, “anti” which means “against” and “helminis” means “worm”

which means “to kill or wipe out worms or parasites”. Anthelmintics are drugs that either kill (vermicide) or

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expel (vermifuge infesting helminthes. Parasites have been of concern to the medicinal field for centuries.

Helminthiasis or helminth infection is a parasitic disease of humans and other animals in which a part of the body is infected with parasitic worms (*Paramphistomum cervi* and *Haemonchus contortus*). They often live in the gastrointestinal (GIT) of their hosts, but may also stay in other organs. Scientific studies have shown that many plants that are used in the human ethnomedicinal practice showed huge pharmacological activities and can be helpful in ethno - veterinary practice. In human body, GIT is the abode of many helminths, but some also live in tissues, or their larvae migrate into tissues. They harm the host by depriving them of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins (Tripathi, 2003).

Anthelmintics drugs can either expel (vermifuge) or kill (vermicide) the infesting helminths. Vaccinations play a role also in the lungworms. On the other hand, some others problems have come up with the use of the anthelmintics. Resistance also occurred in the helminths (Dhar et al., 1965) to a variety of anthelmintic drugs and classes, and chemical residue and toxicity problems also occurred (Kanthal et al., 2012). Studies have shown that the plant kingdom is widely known to be rich sources of botanical antibacterial, anthelmintics, and insecticides activities (Hounzangbe-Adote et al., 2005). Also, a lot number of medicinal plants have been applied to treat some parasitic infections in animals and man (Steppek et al., 2006).

Carica papaya Linn (caricaceae) is a large, stemmed, singled, herbaceous perennial tree that is cultivated worldwide; it contains huge biologically active compounds, for example: chymopapain and papain. The concentration of these compounds can vary in the latex, fruit, roots and leaves. Additionally, the quantity of the phytochemicals compounds also differs from female and male trees. For example, phenolic compounds are normally found higher in male trees than female trees.

MATERIALS AND METHODS

Test material

Ethanollic extract of C. papaya leaves

Test pathogen (Helminth): *H. contortus* and *P. cervi*

Leaves collection: The leaves of *C. papaya* (family Caricaceae) were collected from Savar (Dist., Dhaka, Bangladesh) in January 2019.

Test pathogen collection

The live parasite (*H. contortus* and *P. cervi*) was collected from freshly killed cattle at the local abattoirs. After cleaning, parasites

were stored in 0.9% phosphate-buffered saline (PBS) of pH 7.4 prepared with 8.01 g NaCl, 0.20 g KCl, 1.78 g Na₂HPO₄ and 0.27 g KH₂PO₄ in 1 L of distilled water at 37±1°C.

Preparation of sample

To prepare the suspension of extract at concentrations of 25, 50 and 100 mg/ml; 0.25, 0.5 and 1 g of extracts were taken and triturated with 0.2% v/v of Tween 80 as a suspending agent. Final volume was made to 10 ml for respective concentration with PBS. For the preparation of standard albendazole at concentrations of 15 mg/ml; 150 mg of albendazole powder were taken and triturated with 0.2% v/v of Tween 80 as a suspending agent and final volume was made to 10 ml for respective concentration with PBS.

Study design

The anthelmintic activity of ethanolic leaves extract of *C. papaya* was examined on the live parasites (*H. contortus* and *P. cervi*). The anthelmintic assay procedure was carried out by Mali and Manoj method with some minor modifications (Manoj et al., 2009; Mali, 2007). The Indian adult earthworms (*H. contortus* and *P. cervi*) of 5 cm in length and 0.3 cm in width were used here. Three different concentrations of 100, 50 and 25% in distilled water (each of 50 ml preparation) were prepared. Also, the standard solution (Albendazole) was poured in the different Petri dishes. The two earthworms were then washed in the normal saline solution. The time period of paralysis was then recorded whilst no movement was seen unless shaken strongly. After that, the death time period was recorded after making sure that the parasite did not move when shaken forcefully, dipped in the warm water (50°C), or set to the external stimuli. The anthelmintic activity was presented as the time required for the paralysis and the death of parasites in contrast to control.

RESULTS AND DISCUSSION

The suspensions of ethanolic extracts of *C. papaya* were made in Normal Saline using the Tween80 to get 25, 50 and 100% concentration of the solution. The similar concentration of the solution of the reference standard (albendazole drug) was made in normal saline as well. 2 ml of each solution of various ethanolic extracts and standard drug solution were then diluted to 10 ml with normal saline separately and poured in different Petri dishes.

The Petri dishes were then divided into ten separate groups. Group 1 consists of normal saline and group 2 consists of the standard drug (albendazole). Groups 3-10 consist of 8 extracts. Each group contains 25, 50 and 100% concentrations. After that, 2 equal sizes of adult earthworms were released in each concentration. Times were then recorded also at the time of releasing these earthworms in the solution.

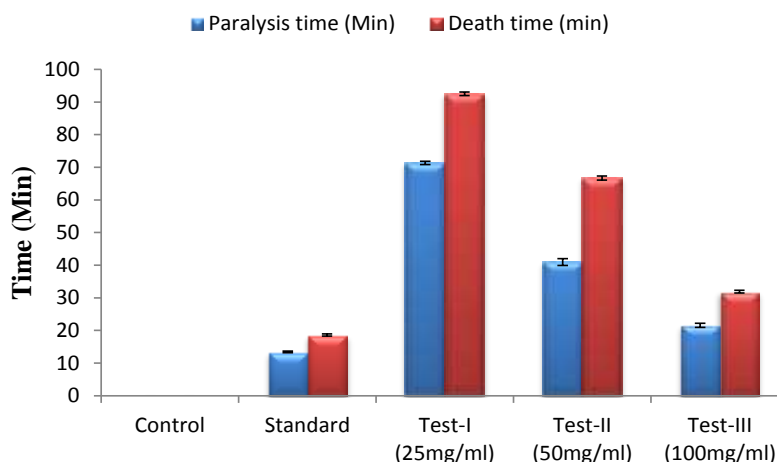
The time that is taken in minutes for death and paralysis of these earthworms were then recorded. The anthelmintic activity of the *C. papaya* was evaluated on the adult earthworm *P. cervi* and *H. contortus* due to the

Table 1. Anthelmintic activity ethanolic extract of *C. papaya* leaves against *H. contortus*.

Group	Paralysis time (Min)	Death time (min)
Control	-	-
Standard	13.41±0.24*	18.59±0.35*
Test-I (25 mg/ml)	71.35±0.53*	92.53±0.51*
Test-II (50 mg/ml)	40.98±1.06*	66.71±0.63*
Test-III (100 mg/ml)	21.59±0.61*	31.88±0.41*

Values are expressed as mean ± standard error of mean (n = 6)

* indicates P<0.05 when compared with control.

**Figure 1.** Anthelmintic activity ethanolic extract of *C. papaya* leaves against *H. contortus*.

resemblance of its physiological and anatomical characteristics with human beings. Paralysis occurred while the worm did not exist in normal saline. Therefore, death was recorded when the worms lost their mobility followed by the fading of body color. As seen in Table 1, the ethanolic extracts of *C. papaya* exhibits the anthelmintic activity in the very dose dependent manner in taking the shortest time for the paralysis (P) and death (D) with highest concentration (100%). Hence, the ethanolic extracts of *C. papaya* exhibited different percentage of anthelmintic activities at different concentrations. It shows the shortest time of paralysis (P=21.59 min) and death (D= 31.88 min) in 100% concentration against *H. contortus*, while the time increased for 50% of concentration (P= 40.98 min and D= 66.71 min) and for 25% of concentration (P= 71.35 min and D=92.53 min) respectively. The standard solution (albendazole) took the very short time (P= 13.41 min and D= 18.59). Anthelmintic activity of ethanolic extract of *C. papaya* leaves against *P. cervi* is also similar to the effect against *H. contortus*. For 100% solution, the effect is highest {(P=12.11 min) and death (D= 17.11 min)} and for 50% solution the effect is comparatively slow {(P= 17.14

min) and death (D= 25.26 min)}. At the 25% solution the effect is very low {(P= 27.45 min) and death (D= 38.64 min)} as compared to the standard solution {(P= 7.79 min) and death (D= 13.8 min)}. The effect of albendazole on the worm is due to the flaccid paralysis in expulsion of the worm by peristalsis. Thus, ethanolic extract of *C. papaya* showed a significant anthelmintic activity as compared to the standard reference (albendazole) and control (distilled water).

Anthelmintic activity against *H. contortus*

The data revealed that the ethanolic extract of *C. papaya* leaves showed dose dependent anthelmintic activity. The effect was maximum at a concentration of 100 mg/ml, whereas the other test concentrations showed marked degree of anthelmintic activity (Table 1 and Figure 1).

Anthelmintic activity against *P. cervi*

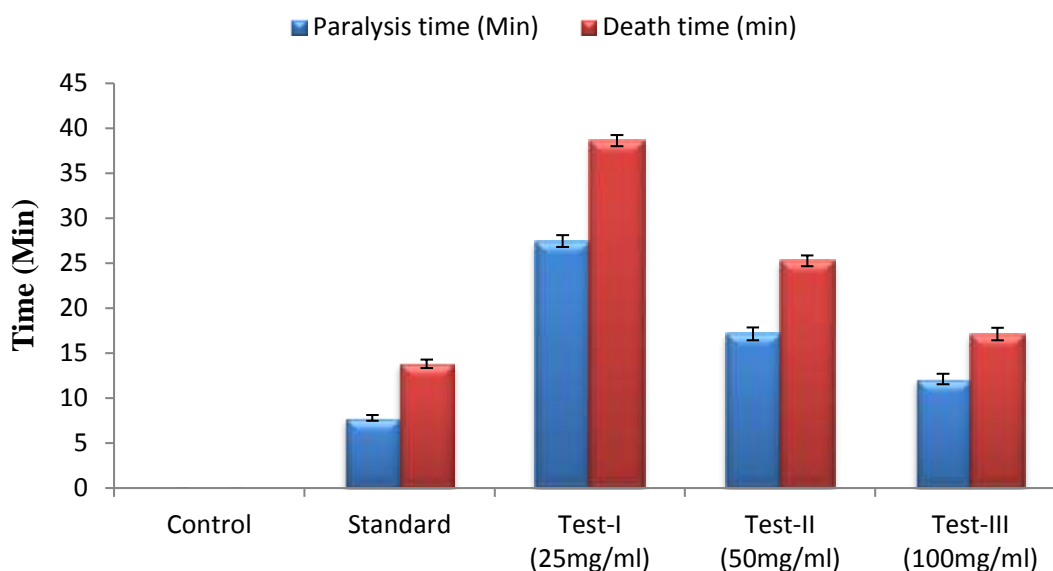
The data revealed that the ethanolic extract of *C. papaya* leaves showed dose dependent anthelmintic activity

Table 2. Anthelmintic activity ethanolic extract of *C. papaya* leaves against *H. contortus*.

Group	Paralysis time (Min)	Death time (min)
Control	0	0
Standard	7.79±0.31*	13.8±0.46*
Test-I (25 mg/ml)	27.45±0.65*	38.64±0.61*
Test-II (50 mg/ml)	17.14±0.71*	25.26±0.62*
Test-III (100 mg/ml)	12.11±0.58*	17.11±0.69*

Values are expressed as mean ± standard error of mean (n = 6).

* indicates P<0.05 when compared with control.

**Figure 2.** Anthelmintic activity ethanolic extract of *C. papaya* leaves against *H. contortus*.

against *P. cervi*. The effect was highest at a concentration of 100 mg/ml, whereas the other test concentrations showed marked degree of anthelmintic activity (Table 2 and Figures 1 and 2).

Conclusion

From the end result, it is said that the leaves of *C. papaya* give a clear idea about major anthelmintic activity compared with standard anthelmintic drug (Albendazole). This drug should be explored further for phytochemical profile to recognize the active constituents responsible for the anthelmintic activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are really thankful to head of Pharmacy Department of University of Asia Pacific, Dhaka, Bangladesh, for providing the superb facility to carry out this research work. The authors are also grateful to Dr, Anisur Rahman, Associate Professor, Khulna university of Pharmacy discipline, for his great guidance as an expert to do this work.

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Full Length Research Paper

Rational and experiential decision-making preferences of pharmacy students in Karachi, Pakistan

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Received 28 February, 2019; Accepted 17 June, 2019

This study was conducted to examine the rational (systematic and rule-based) and experiential (fast and intuitive) thinking preferences of pharmacy students. This may assist in formulating learning and teaching tactics for decision-making leading to safe and optimized patient care services. Here, a validated psychometric tool, that is, the Rational Experiential Inventory (REI-40) survey was used. It was distributed to students studying in fourth and final years of pharmacy degree program in private as well as public sector universities of Karachi city. A composite rational score was obtained by adding responses from the rational ability (r-ability) and rational engagement (r-engagement) items, while a composite experiential score was obtained by adding the experiential ability (e-ability) and experiential engagement (e-engagement) items. Results revealed that the mean rational scores obtained were 3.269 ± 0.28 for rationality, 3.259 ± 0.29 for r-ability and 3.289 ± 0.28 for r-engagement. Mean experiential scores were 3.143 ± 0.21 overall, 3.247 ± 0.18 for e-ability and, 3.039 ± 0.23 for e-engagement. Influence of gender, year of study and, type of institute on students' responses was evaluated by applying independent t-test. The present study thus highlighted that most of the pharmacy students favored rational over experiential decision-making styles.

Key words: Decision making, pharmacy students, rational engagement, experiential engagement

INTRODUCTION

The World Health Organization (WHO) stresses on the need of pharmacists in patient care especially in developing countries. The role of a pharmacist in

healthcare has been established worldwide. This role has transcended from a conventional drug dispensing to direct patient care. Pharmacists are now involved in

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direct patient care alone or as a member of the healthcare team to provide pharmaceutical care and clinical pharmacy services to the patients in the achievement of treatment outcomes. Besides, the drug information service is also provided by pharmacists. Kaboli and colleagues highlighted that pharmacists have an integral role in clinical care that involve carrying out medication reconciliation services, improved patient safety as well as adverse drug events (ADE) monitoring (Al-Tajir and Kelly, 2005; Kaboli et al., 2006). Thus, there is a plethora of studies that highlight the role of pharmacists in healthcare that requires sound clinical decision making (Phansalkar et al., 2009; Williams et al., 2016).

Evidence indicates that human reasoning operation constantly utilizes two cognitive approaches for processing of information. Experiential processing is characterized by fast, heuristic, associative, intuitive, recognition primed and automatic. The other mode known as rational processing gained by formal and cultural tuition utilizes conscious, slow, explicit, deliberate, rule-based, analytic and controlled (Stanovich and West, 2000). In the Heuristic-Systematic Information Processing Model, an approach used by a decision maker can either be simple heuristic decision approach or a systematic approach. The making of decision depends upon the extent of involvement of the person with decision (Chaiken, 1980). Factors that include dispositional, that is, individual and, situational, that is, environmental effects the association between experiential and rational reasoning approach. Participation in a simultaneous cognitive job, time pressure, time of performing tasks and mood tend to impair counteractive actions of the rational approach. The rational mode of operating positively correlates with intelligence, need for cognition and exposure to statistical training (Kahneman, 2003).

Clinical decision-making may not accommodate either or models well, because motivation is considered as fundamentally significant determinant of an individual's mode of processing. The more appropriate frame work for medical decision making Cognitive-Experiential Self Theory (CEST) involves equally an experiential and rational system control incessantly in an incorporated communication (Epstein, 2003). The model operates without emphasizing on importance of a judgment or decision maker's level of motivation.

Research studies have highlighted that individuals decision making style tends to switch from one mode to another and depends upon presentation of information and type of population under consideration for example, undergraduates or professional decision making style may be different owing to responsibilities, circumstances, resources, and common individual distinctiveness (Epstein, 2003; Phansalkar et al., 2009; Calder et al., 2011). This study aimed to examine the pharmacy student rational, that is, systematic and rule-based, and

experiential, that is, fast and intuitive, thinking preferences, that may provide an approach in formulating learning and teaching methods for decision making leading to safe and optimized patient care services.

MATERIALS AND METHODS

A cross-sectional study was conducted in October 2015 among undergraduate students studying in fourth and final years of pharmacy degree program in private and public-sector universities in Karachi.

Venue and duration of the study

The venue of the study was the pharmacy teaching institutes located in Karachi city. According to the literature, Karachi has three public-sector and six private sector universities. The total yearly intake of these institutes is estimated to be over 1200 students.

Participants

The participants were the undergraduate students studying in their fourth and final year pharmacy degree program. Students studying in other degree programs were excluded. Those who did not consent to participate were also excluded from the study.

Sample size and sampling procedure

Sample size was calculated through an online software and probability sampling technique was adopted. A total of 434 pharmacy students participated in the study.

Research instrument

The study was conducted using a previously validated survey tool known as the Rational Experiential Inventory (REI-40) survey.

Data analysis

The data was analysed through SPSS version 20 (IBM Corp, Armonk, NY). The continuous data were presented as mean (X) \pm standard deviation (SD). An independent t-test was adopted to examine differences between groups.

Ethics review and statement of consent

This study was exempted from review by the ethical committee. The participants were briefed about the study prior to handing the questionnaire. The participation was voluntary, and the questionnaire was handed to those who consented to participate.

RESULTS

Out of 500 survey questionnaires, only 434 were returned giving a response rate of 86.8%. Most students were

Table 1. Characteristics of study population.

Characteristics	Number (Percentages)
Gender	
Male	77(17.74%)
Female	357(82.25%)
Academic year	
Fourth year	238(54.83%)
Final year	196(45.16%)
Institute	
Private	196(45.16%)
Public sector	238(54.83%)

Table 2. Comparison of rational-experiential inventory means of participants.

Demographics	Rational	R-Ability	R-Engage	Experiential	E-Ability	E-Engage
	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)
Gender						
Male(77)	3.257(0.295)	3.242(0.358)	3.272(0.232)	3.148(0.201)	3.244(0.212)	3.053(0.191)
Female(357)	3.238(0.274)	3.192(0.296)	3.284(0.252)	3.14(0.170)	3.243(0.160)	3.037(0.181)
Academic year						
Fourth year(238)	3.248(0.218)	3.207(0.170)	3.290(0.267)	3.123(0.165)	3.184(0.158)	3.063(0.172)
Final year(196)	3.313(0.361)	3.353(0.378)	3.273(0.345)	3.163(0.283)	3.316(0.213)	3.010(0.354)
Institute						
Private(196)	3.313(0.361)	3.353(0.378)	3.273(0.345)	3.163(0.283)	3.316(0.213)	3.010(0.354)
Public sector(238)	3.248(0.218)	3.207(0.170)	3.290(0.267)	3.123(0.165)	3.184(0.158)	3.063(0.172)

female (357, 82.25%) and belonged to public-sector pharmacy teaching institute (238, 54.83%). The majority of students (238, 54.83%) studied in fourth year (Tables 1 and 2). The mean rational score was 3.269±0.28 for rationality, 3.259±0.29 for r-ability and, 3.289±0.28 for r-engagement. Mean experiential score was 3.143±0.21 overall, 3.247±0.18 for e-ability and, 3.039±0.23 for e-engagement. Influence of gender, year of study and type of institute on responses was evaluated by applying independent t-test. No significant differences in decision-making styles were found among students based on gender except an 'experiential engagement statement', (p=0.002). Significant association was found between 'type of institute' and 'rational ability', (p<0.0001). R-Engagement included; 'thinking is not my idea of an enjoyable activity', p=0.002, 'learning new ways to think would be very appealing to me', (p < 0.0001), 'I enjoy thinking in abstract terms', (p < 0.0001) and, 'I don't like to have to do a lot of thinking', (p < 0.0001). The influence

on e-ability and engagement included, 'I don't have a very good sense of intuition', (p=0.036), 'I don't like situations in which I have to rely on intuition', (p=0.001), 'I don't think it is a good idea to rely on one's intuition for important decisions', (p=0.001), and 'I think it is foolish to make important decisions based on feelings', (p=0.028).

Significant association was found between 'year of study', and 'rational scores', that is, 'I have a logical mind and I usually have clear, explainable reasons for my decisions', (p < 0.0001), 'thinking is not my idea of an enjoyable activity', (p=0.002), 'learning new ways to think would be very appealing to me', (p < 0.0001), and 'I enjoy thinking in abstract terms and I don't like to have to do a lot of thinking', (p < 0.0001). Association was found significant between 'year of study' and 'experiential scores', that is, 'I don't have a very good sense of intuition', (p=0.036), 'I don't like situations in which I have to rely on intuition', and 'I don't think it is a good idea to rely on one's intuition for important decisions', (p=0.001).

DISCUSSION

In Pakistan, the conventional pharmacy practice is still prevalent. Pharmacist's role is in transformation to address the demands of healthcare system. For the attainment of therapeutic benefits and achievement of clinical outcomes, understanding the pharmacy student's decision-making preferences would guide in developing educational strategies that could promote sound decision making for the safe and proper use of medicines by the patient.

This study was based on dual-processing theory and CEST proposing that decision making involves two independent modes that process and operate simultaneously and sequentially (Epstein et al., 1996; Epstein, 2003). In our study, prospective pharmacy students used both rational and experiential decision-making styles, which was similar to the study conducted on third year pharmacy student in the United States (McLaughlin et al., 2014). This novel study assessed REI in pharmacy students of Pakistan. The outcomes demonstrated that pharmacist preferred fact, order and logic over intuition. The results are consistent with studies using the Myers-Briggs Type Indicator (Hardigan and Cohen, 1999; Shuck and Phillips, 1999; McLaughlin et al., 2014).

A significant association was found between professional year and rational scores in our study. It reflected that Pharm. D program courses directed students to use systemic and analytical analysis for the evaluation of patient medication regimen appropriateness that encompassed several factors and considerations that were involved to ensure overall health status of the patient. As pharmacy students demonstrated rational thinking preferences. This highlighted the need to teach evidence based medicine strategies to support systematic and analytic decision making (Brown et al., 1995; McLaughlin et al., 2014; Williams et al., 2016).

In support of making clinical decisions in healthcare field, a balance between rational and experiential thought processes are required in time efficient way. Even though cognitive psychology has comprehensively explored issues underlying the decision-making process, the extent to which pharmacists rely on rational and experiential modes of information processing is not recognized well. The present study outlined pharmacy student decision-making style reflecting knowledge and skill obtained during classroom teaching of various pharmacy practice courses with special emphasis on clinical problem-solving skills to students in clinical practice. These decision-making skills helped simplify complex drug-therapy management and therapeutic dilemmas.

Our study had some limitations. The study highlighted the decision-making preferences of fourth and final year pharmacy students. To understand rational and

experiential decision making among pharmacists, the research should be carried out in workplace settings. It is also suggested that research must be carried out covering all the batches in the year to experience impact of the study. Therefore, future studies may be carried out to determine the decision-making preferences of pharmacists along a continuum of experience as likelihood of their dependence on the rational decision making may subside over time.

Conclusion

Most of the student pharmacists in this study favored rational over experiential decision-making styles. In support of making clinical decisions in healthcare field, a balance between rational and experiential thought processes are required in time efficient way as pharmacists face a wide range of situations that require effective and appropriate decision making.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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