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Review

Evaluation of Anti-venom effect of *Tiryaq-e-arba* in rabbit models

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ABSTRACT

Background: *Tiryaq-e-arba* is a polyherbal Unani antidote/antivenom formulation used in the management of poisoning due to snake bite, scorpion bite as well as in cold poisons since time immemorial. Objectives: *Tiryaq-e-arba* was not evaluated scientifically before this study carried out, therefore it was studied for antivenom activity by testing on plasma fibrinogen level in Russell's Viper envenomation in rabbits. Material &Methods: The anti-venom activity of the test drug was studied by observing its effect on plasma fibrinogen level in Russell's Viper envenomation in rabbits by the method of Netelson. Results: The plasma fibrinogen level was found to be 171±665.04 mg/100 ml of blood, 36.18±1.12 mg/100 ml of blood, 33.14±0.52 mg/100 ml of blood and 17.9±1.65 mg/100 ml of blood at 0, 1, 3 and 6 hours respectively in control animals while in the test animal it was found to be 157.13±3.44 mg/100 ml of blood, 41.13±2.69 mg/100 ml of blood, 62.09±1.65 mg/100 ml of blood and 54.39±0.73 mg/100 ml of blood respectively. The test showed that though the plasma fibrinogen level in the test lower at 0 hour but it was greater in the control animals at 1, 3 and 6 hours. The increase in plasma fibrinogen level in the test animals at 3 and 6 hours was statistically significant (P<0.001). Conclusions: The finding of the present study was that *Tiryaq-e-arba* possesses antivenom activity which scientifically support the Unani claim that it is *Dafe-Sumoom-al-Hevan* (Antivenom or Antidote) and the use of this preparation in corresponding diseases.

Keywords Anti venom activity, Antidote, *Tiryaq*-e-Arba, Unani medicine, Plasma fibrinogen level

INTRODUCTION

Tiryaq is roughly translated as antidote or antivenom which is not only effective in diverse types of poisoning but also have other actions and uses. One of the famous *Tiryaq* is *Tiryaq*-e-arba. *Tiryaq-e-arba* is polyherbal pharmaceutical preparation consisting of four herbal ingredients due to which it is known as *Tiryaq-e-arba* as *arba* means four in Arabic. A unique feature of *Tibbe* Unani (Unani Medicine) is the possession of *Tiryaq*at roughly translated as antidotes which are not only effective in diverse types of poisoning but also have other important actions and uses. One of the most reputed *Tiryaq* is *Tiryaq-e-arba* (Ahmad M.H, 1913). *Tirayq-e-Arba* is a polyherbal Unani antidote formulation used in the management of poisoning due to snake bite, scorpion bite as well as in cold poisons since time immemorial. It is famous for its antidote action. It is reported to be brain tonic and cardiac tonic (Kabeeruddin, M, 1921; Jeelani, G, 1985), antidote (Attar A.N, 1329; Ghani, M.N, 1928), help in expulsion of dead foetus (Ghani, M.N, 1928; Shareef, M. 1873), liver corrective (Ghani, M.N, 1928; Antaki, D. 1930) and splenic corrective (Antaki, D. 1930; Rahman, S.Z, 1980). Classical text described its uses in biting of poisonous animals (Hasan M, 1893; Ali A.M, y.n.m), cold diseases (Hussain M.H. 1901; Ali A.M, y.n.m), cold poisons and cold oedema (Ghani, M.N, 1928; Hussain M.H. 1901), snake bite, scorpion bite, insect bite, spider bite (Ghani, M.N, 1928; Rahman, S.Z, 1980; Hasan M, 1893). As one of the aforeseen effect of animal bite is coldness and *Tiryaq-e-arba* temperament is hot in III[rd] and dry in IV[th] degree (Ghani, M.N, 1928; Shareef, M. 1873; Hussain M.H. 1901). So, it can be considered as best medicine to combat poisoning and coldness produced by it. As modern science also says that the efficacy of antivenom is its ability to bind venom components or toxins, while the effectiveness of antivenom is its ability to prevent or reverse the effects of envenoming in humans.

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MATERIAL AND METHODS

Ingredients of *Tiryaq-e-Arba*

Table I. The Ingredient details of *Tiryaq-e-arba* (Jeelani, G, 1985)

S. no	Drug name	Botanical name	Part used	Ratio
1.	*Habb-ul-Ghar*	*Laurus nobilis* Linn.	Fruit	1 part
2.	*Juntiana Roomi*	*Gentiana Lutea* Linn.	Root	1 part
3.	*Mur Makki*	*Commiphora myrrha* Ness Engl.	Gum	1 part
4.	*Zarawand Mudharj*	*Aristolochia rotunda* Linn.	Root	1 part
5.	*Shehad*	Honey	Secretion	3 part

Method of preparation
All the ingredients from 1-4 are taken in equal quantity and honey three times to others. Ingredients were obtained from Dawakhana Tibbiya College, A.M.U., Aligarh, except for *Habb-ul-Ghar* (*Laurus nobilis* Linn), which was obtained from Riyadh, Kingdom of Saudi Arabia. The authenticity of the drugs was confirmed botanically in the department of *Ilmul Advia*, AMU, Aligarh. The drugs were first crushed and then powdered separately and sifted from 60 mesh sieve to obtain a fine powder. The powder of 4 drugs was taken in equal proportion mixed thoroughly. Further prepare *qiwam* by mixing them in the suspension of honey into 1:3 ratio. Discontinue the heat as the *qiwam* was prepared and added the above powders and mixed thoroughly. Allow it to cool at room temperature and all the powdered ingredients in equal weight mixed in the *qiwam* as in *Majoon*. Then it was stored in tightly closed containers. It was prepared in departmental laboratory, AMU. (Ministry of Health and Family Welfare, 2010)

Animal dose preparation
The test drug combination (TDC) or *Tiryaq-e-arba* was suspended in distilled water for oral administration to the animals. The animal dose of the test preparation expected to be equivalent to the human dose was calculated by multiplying its Unani clinical dose described in Unani texts with appropriate conversion factors (Dhawan, B.N, 1982). Thus, the dose determined for rat was 75 mg/100 gm, body weight.

Antivenom activity study by testing Plasma Fibrinogen Level in Russell's Envenomation
The effect of test drug i.e. *Tiryaq-e-arba* was studied for Antivenom activity. The freeze-dried Russell's Viper venom was obtained from CSIR, Centre for Biochemicals, V.P. Chest Institute Building, Delhi University Campus, New Delhi. It was reconstituted (10 mg/10ml) in normal saline immediately before use. Male rabbits weighing 1.2-1.7 kg were divided into 2 groups of 3 animals each. The animals in Group I were administered orally with the test drug in the dose of 350 mg/kg daily for 5 days. Animals in Group II were administrated with the same volume of distilled water and served as control. On the 5th day, 2 hours after *Tiryaq-e-arba* administration, the animals were injected 2 mg/kg of the venom intra- peritoneally.

The dose of 2 mg/kg of the venom was selected as at this dose falls plasma fibrinogen level quite low, but the animal do not die within 24 hours. (Seth, S.D.S. *et.al*., 1972) The blood was taken out from the ear veins of rabbits at 0,1,3 and 6 hour later in both the groups. The blood was collected in double oxalated test tubes. The plasma fibrinogen level was estimated by the colorimetric method of Natelson. (Netelson, S, 1971)

Methods of Estimation
The method for fibrinogen estimation comprised the conversion of fibrinogen to fibrin with calcium salts and the determination of its concentration by determination of the optical density. (Jenkins, G.L. *et.al*., 1957)

Reagents
1. Calcium chloride (2.5%): 2.5 gm of crystalline dehydrate was made up to 100 ml With water
2. Biuret's reagent: The reagent was prepared by adding rapidly 25 ml of 12.5% NaOH to 5 ml of 1% $CuSO_4$.
3. NaOH (12.5%):50 gm. NaOH was dissolved in water and made upto 100 ml allowed to stand overnight. The supernatant was diluted 4 times. It was kept in well stoppered polytheline container.
4. $CuSO_4$ (1%):1 gm $CuSO_4.5H_2O$ was made upto 100 ml. of water.
5. Protein Standard (1.6%): A standardized, pooled protein sample was dissolved to obtain a solution of 1.6% i.e. protein value of standard = 6.4% -1 ml of the protein standard was diluted with 4 ml. of normal saline. Commercial standard like versatol (Warner-Chillot) was used.

Procedure
To a test tube (13 X 100) 0.2 ml of plasma was added from oxalated blood, 5 ml. of normal saline and 0.5 ml of 2.5% calcium chloride were added, mixed centrifuged. The supernatant was decanted carefully to avoid the inclusion of the clot. 5.0 ml. of normal saline was added, mixed, centrifuged and decanted. The washing with normal saline was repeated once more. 2.5 ml of Biuret's reagent was added. Then it was placed in a water bath at 370C for about 20 minutes or until the fibrinogen was dissolved. Then it was centrifuged. The optical density of the supernatant was read at 540 mu in the spectrophotometer against the Biuret's reagent as the blank. For the standard 0.05 ml of protein standard (1.6%) was mixed with 2.5 ml of Biuret's reagent and incubated as for the test sample. (Rastogi RP, 1991)

Fibrinogen concentration was calculated by the following formula:
Absorbance unknown/Absorbance standard X 400 = Fibrinogen mg/100 ml.

RESULTS

The antivenom activity of the test drug was studied by observing its effect on Plasma Fibrinogen level in Russell's Viper Envenomation in rabbits by the method of Netelson (1971). The plasma fibrinogen level was found to be 171±665.04 mg/100 ml of blood, 36.18±1.12 mg/100 ml of blood, 33.14±0.52 mg/100 ml of blood and 17.9±1.65 mg/100 ml of blood at 0,1,3 and 6 hours respectively in control animals while in the test animal it was found to be 157.13±3.44 mg/100 ml of blood, 41.13±2.69 mg/100 ml of blood, 62.09±1.65 mg/100 ml of blood and 54.39±0.73 mg/100 ml of blood respectively as shown in table II. Further graphical presentation of effect of *Tiryaq-e-arba* on plasma levels after Russell's Viper envenomation in rabbits is shown in Figure I.

Table II. Effect of *Tiryaq-e-arba* on Plasma fibrinogen levels (mg/100 ml. of blood) after Russell's viper envenomation in rabbits

Groups	Plasma Fibrinogen (mg/100 ml)			
	0 Hr.	1 Hr.	3 Hr.	6 Hr.
Control	171.65±04	36.18±1.12	33.14±052	17.91±65
Tiryaq-e-Arba	157.13±3.44	41.13±2.69	62.09±1.65*	54.39±0.73*

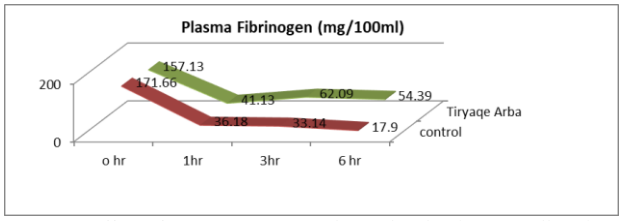

Figure I. Effect of *Tiryaq-e-arba* on Plasma levels after Russell's Viper Envenomation in rabbits

DISCUSSION

Animal envenoming including snake, scorpion and other poisons are responsible for more than 20,000 deaths annually, making it one of the world's most neglected tropical diseases according to the WHO. Venom-induced consumption coagulopathy (VICC) is a major effect of snake envenoming. Antivenom is the main treatment for VICC, but, although it appears to neutralize procoagulant toxins, clotting factor resynthesis and full recovery of clotting function takes 24–48 h. The prothrombin activators activate the clotting pathway and cause complete consumption of fibrinogen, factor V, and FVIII, resulting in unrecordable times for clotting tests (International Normalised Ratio [INR] and activated partial thromboplastin time), as well as very high D-dimer levels. (Isbister GK *et al.*, 2013)

The diagnosis and monitoring of VICC requires coagulation studies and clotting times. Internationally, the most commonly used test is the 20-minute whole blood clotting test (WBCT20). However, the reliability of the WBCT20 as a diagnostic test has come into question for the diagnosis of Russell's viper envenoming. The duration of the WBCT ranges from 10 minutes in some studies to 30 minutes in others. (Yong Jun Jeon *et al.*, 2019)

There are a number of proposed mechanisms by which the binding of antivenom to venom results in prevention or reversal of envenoming. Antivenom can potentially block the active site of a toxin or bind to a toxin to prevent it interacting with its substrate (steric hindrance) to neutralize the toxin. Antivenom-venom complex formation in the central compartment may prevent the distribution of toxins to the target tissues (e.g., nervous system) or cause the redistribution of toxins from their target tissues back to the vascular compartment. Antivenom can increase the elimination of toxins from the circulation and body. In the case of VICC, the toxins act in the central compartment, so antivenom must either bind to the toxins in the blood, and therefore prevent the action of the toxins, or increase the elimination of toxins.

Fresh frozen plasma appeared to speed the recovery of coagulopathy and should be considered in bleeding patients. Thus it is important to consider the proteolytic events of the proteinases on plasma proteins. In addition, most snake venom proteases affect coagulation by hydrolyzing the fibrinogen and inducing/prolonging clot formation. Several venom proteases with fibrinogenolytic activity have been reported and they preferentially hydrolyse Aα and Bβ subunits of fibrinogen. Similarly, T. malabaricus venom proteases hydrolyze Aα and Bβ subunits of human fibrinogen. Snake venom also contains prothrombin activators which cause a variable deficiency in factor V, VII and fibrinogen. (Kalana Maduwage and Geoffrey K. Isbiste, 2014)

In this study, the test animals were administered with 350 mg/kg of *Tiryaq-e-arba* by oral route once a day for 5 days while the control animals were administered with the vehicle i.e. distilled water in the same volume and manner. Two hours after the last dose all the animals were injected with 2 mg/kg of Russell's viper venom intra-peritoneally. Blood was collected at 0,1,3 and 6 hours later for the estimation of plasma fibrinogen by the colorimetric method of Netelson (1971). The test showed lower values at 0 hour than in the control animals but it was greater than in the control animals at 1,3 and 6 hours. The increases in plasma fibrinogen level in the test animals at 3 and 6 hours was statistically significant ($P< 0.001$) (Afaq SH, 1994). The significant results of increases in plasma fibrinogen level with increasing intervals revealed that *Tiryaq-e-arba* possesses significant antivenom effect.

CONCLUSION

Antivenom is the recommended standard treatment for snake envenoming. Antivenoms consist of polyclonal antibodies to the toxins in snake venoms. They may be whole immunoglobulins (IgG) or fractionated IgG, either F(ab′)2 or Fab. *Tirayq-e-Arba* is a famous antidote or antivenom of Unani Medicine which is used in the management of poisoning due to snake bite, scorpion bite etc. as well as by cold poison since time immemorial. But it was not evaluated scientifically before this study carried out, therefore it was studied for antivenom activity. The findings revealed that *Tiryaq-e-arba* possesses significant antivenom effect and scientifically support the Unani claim that it is *Dafe-Sumoom-al-Hevan* (Antivenom) and the use of this preparation in corresponding diseases. It can also be used as a unique emergency medicine where it is not possible to give modern emergency services immediately i.e., it can save one's life. It may be used as an alternative to chemical antidotes and relieve the after effects of animal bite poisoning without side effects. Despite antivenom being efficacious and binding to the multiple toxins in the venom, there are a number of reasons that it may not be effective. For antivenom to be effective against such irreversible effects, it must be administered early, so it can bind with toxins before they distribute to their target sites and cause irreversible toxicity.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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