Journal of Applied Pharmaceutical Science Vol. 3 (08), pp. 139-143, August, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.3824
ISSN 2231-3354

Comparative *in vivo* evaluation of three types of honey on topical wound healing activity in rabbits

Nagiat T Hwisa, Prakash Katakam*, Babu Rao Chandu, Einas Ghafar Abadi and Ebtihal Mosbah Shefha
Department of Pharmacology and Biochemistry, Faculty of Pharmacy, University of Zawia, Al-Zawia, Libya.

ARTICLE INFO

Article history:
Received on: 31/03/2013
Revised on: 09/07/2013
Accepted on: 08/08/2013
Available online: 30/08/2013

Key words:
Honey, Sidr, Thyme, Spring, wound healing, topical.

ABSTRACT

The present investigation has focused on the comparative evaluation of topical wound healing activity in rabbits using three types of honeys such as Sidr (SDH), Thyme (TYH), Spring (SPH). The activity was compared with commercial wound healing formulations, Mebo (MBC) and Fusidin (FSC) creams. Among the different types of the honey SDH was found to possess higher healing rate of wounds induced either by thermal or chemical methods. Whereas, in thermal induced burns, both TYH and SPH have shown similar wound healing activity but better than that of standard drugs. The activity for thermal and chemical induced burns was found in the order of SDH > TYH/SPH > MBC > control and SDH > TYH > SPH > FSC > control respectively. Among the three tested honeys, the t-test data was compared to those of controls and it was found that there was a significant ($p<0.05$) reduction in the wound area. It was concluded that honey in general could be employed as natural topical wound healing agent comparable to commercial synthetic analogs tested in the study. Further the present investigation proves that SDH is possessing superior wound healing activity than that of TYH and SPH.

INTRODUCTION

Honey is the sweet, viscous fluid produced by honey bees (the genus *Apis*) using the nectar of flowers. Historically, honey has been used by humans to treat a variety of ailments, from gastric disturbances to ulcers, wounds and burns, through ingestion or topical application, but only recently have the antiseptic and antibacterial properties of honey chemically explained (Drgrotte, 2013). Antibacterial properties of honey are the result of the low water activity causing osmosis, chelation of free iron, its slow release of hydrogen peroxide, low pH, and the antibacterial activity of methylglyoxal. Honey appears to be effective in killing drug-resistant biofilms which are implicated in chronic rhinosinusitis (Bansal *et al*., 2005; Lusby *et al*., 2002). It also possesses antiinflammatory activity by inhibiting prostaglandins (Al-Waili and Boni, 2003) and by thrombin-induced oxidative burst in phagocytes (Ahmad *et al*., 2009). The most remarkable discovery was the antibacterial activity of honey that has been mentioned in numerous studies (Al-Waili and Haq, 2004). Natural honey exhibits bactericidal activity against many enteropathogenic organisms, including those of the *Salmonella* and *Shigella* species, and enteropathogenic *E. coli* and methicillin-resistant *S. aureus* strains (Jeffrey and Echazarreta, 1996; Alvarez-Suarez, 2010; Emsen, 2007).

On the other hand, honey is well known for its advantages within the wound environment from recent clinical studies (Visavadia *et al*., 2008). It maintains a moist wound environment that promotes healing and its high viscosity helps to provide a protective barrier to prevent the infection. In addition, the mild acidity and low-level hydrogen peroxide release both aid tissue repair and contribute to the antibacterial activity of honey (Lusby *et al*., 2005). Honey has been reported to have immunomodulatory activities of monocytic cells to repair the wounded tissue by releasing anti-inflammatory cytokines and growth factors (Henriques *et al*., 2006; Tonks *et al*., 2003). Its effectiveness in rapidly clearing up the infection and promoting healing is not surprising in light of the large number of research findings on its antibacterial activity. The precise composition of honey varies according to the plant origin on which the bee feeds. (Rakha *et al*., 2008; Al-Mamary *et al*., 2002). Sidr honey is most revered honey and expensive honey in the world. It is obtained from the flowers of the ancient Sidr tree (*Ziziphus spina-christi*). Sidr tree grows in coastal, desert, and semi-desert areas and found extensively in the eastern part of Yemen. (Sidr Honey[a, b], 2013).

* *Corresponding Author*
Prakash Katakam,
Faculty of Pharmacy, University of Zawia, Al-Zawia, Libya.
Phone: +218-926855775
Email: pkatakam9@gmail.com

Thyme honey also known as Greek thyme honey is light coloured and, infused with layers of flavour from the abundant wild flowers carpeting the mountains in spring and summer. The presence of phenol is known for its antibacterial activity (Greek Thyme Honey, 2013). Wide medicinal applications are associated with spring honey. It strengthens the memory and resists aging, diabetes and diarrhoea (Spring Honey, 2013).

From the literature survey we have found that there is no research carried out on comparative evaluation of wound healing activity of honey from different types and sources. Therefore the present investigation is focused on the comparative evaluation of topical wound healing activity using three types of honey.

MATERIALS AND METHODS

Fusidin cream (FSC) (sodium fusidate 2% w/w ointment, Leo Pharmaceutical Products, Denmark. Batch No. EG3794), Mebo cream (MBC) (0.25% w/w β-sitosterol, Gulf Pharmaceutical Industries, UAE. Batch No. 509), Sidr honey (SDH), Thyme honey (TYH), Spring honey (SPH) were procured from local market. Conc HCl was of analytical grade from Merck, Germany.

Experimental protocol

A prior permission from the institutional animal ethics committee was obtained by the researchers to conduct the present investigation on White New Zealand rabbits. The animals were housed under standard laboratory conditions, maintained under 12/12 h light/dark cycle and fed with commercial foods such as crushed wheat and corn along with distilled water *ad libitum*. Each group consisting of five rabbits weighing about 1.5 to 3.0 kg was selected randomly for the study. The healthy rabbits of either sex were divided into five groups consisting of five animals in each group. Group 1 received FSC or MBC as a standard group. Group 2 received SDH, group 3 received SPH and group 4 received TYH. Group 5 animals were maintained untreated as a control. The wound healing activity was tested as per the modified procedures of literature (Mekonnen *et al*., 2013; Abu-Zinadah, 2009).

Induction of wound

Primary irritation to the skin was measured by a patch-test technique on the intact skin of the rabbits in accordance with the guidelines of the Consumer Product Safety Commission, Title 16, Chapter II, Part 1500 (Bonnette *et al.*, 2001; Derelanko and Hollinger, 2001, Yogeshwar and Vandana, 2010). Topical wound was induced in all groups by chemical and thermal means. The hair on the dorsal skin of rabbits was shaved mechanically about 7×7 cm area using an electric razor and marked with Indian ink. The animals were left aside for 24 h to observe any recurrence of skin damage caused by the shaving. The thermally induced wound was activated by thermal injury where direct heat was applied using a hot stainless steel metal rod of 6 mm diameter maintained at 80 ^{o}C. Both the chemical and thermal burns were induced simultaneously on either side of the dorsal skin of the rabbits (**Fig. 1A**). The chemically induced wound was affected by spreading few drops of conc. HCl 35% v/v upon the shaved skin of rabbits as described by Arwa (2011) and Abu-Zinadah (2009) and is shown in **Fig. 1B**.

Treatment of wounds

The animals of group 5 (control) were left without any treatment. The wound of the group 1 animals (standard) was topically treated by spreading a thin layer of FSC (500 mg) for chemically treated animals and MBC (500 mg) for thermally treated animals twice a day. The animals of groups 2, 3 and 4 were treated with 500 mg each of SDH, SPH and TYH respectively. The Fig. 4-7 show the procedure of treatment of the wounds. The diameter of the wound area was measured at each of the time intervals of 0, 7, 14 and 21 days. The animals were allowed for another 10 days without any treatment to see whether the wound was cured. The study was performed over a total period of 38 days.

Statistical analysis

The areas of the wound were calculated from the diameters measured and the data were compared using MS office EXCEL software. All values were expressed as mean ±S.D. The statistical significance of the difference between groups was assessed by Student's t-test where the values of $p<0.05$ were considered as significant.

RESULTS AND DISCUSSION

The wound healing effect of three types of honey (Cider, Spring and Thyme) was observed in three groups of animals and compared with that of control (untreated) and standard (FSC and MBC) groups. **Fig. 2** shows thermally induced burns after treatment with the three types of honey. The progress of treatment after 38 days was shown in **Figs. 3** and **4** and the results were incorporated in **Tables I** and **II**. The results showed that there is a considerable wound healing activity among the treated groups over control. The wound healing activity of the treated groups was measured in terms of decrease in the area of the wounds caused by the burns.

In the present investigation, thermal injury was chosen as an alternative to mechanical injury of the skin of rabbits (Borhan, 2012). In the thermally induced burns, after the treatment period of 4 weeks, the wound in group 1 (standard, MBC) animals were found to be 3.38 cm, whereas as in group 2 (SDH treated) the areas of wound were reduced to 2.36 cm. In group 3 (SPH treated) and group 4 (TYH treated) the area of wound was found to be same with 2.92 mm^2. The group 5 (untreated control) showed a decrease in the wound area with 3.41 mm^2 owing to the natural phenomenon of healing process. The results are shown in **Table I** and **Fig. 3**. The wound healing activity in thermal induced burns was found to be in the order of SDH > TYH/SPH > MBC > control. The results indicated that the three types of honey used in the study were found to be having a significant wound healing activity than that of standard used. Among the different types of the honey SDH was found to possess higher wound healing rate as against thermally induced wound. In chemical induced wounds, after the treatment period of four weeks, the areas of wounds were found as 4.25, 3.33, 4.01 and 3.43 mm^2 in group 1 (standard, FSC), group 2 (SDH treated), group 3 (SPH treated) and group 4 (TYH treated) respectively. The group 5 (untreated control) showed a decrease in the wound with 4.73 mm^2 of area owing to the natural phenomenon of healing process. The results are summarized in **Table II** and **Fig. 4**. The wound healing activity in chemical induced burns was found to be in the order of SDH > TYH > SPH > FSC > control. The results indicated that the three types of honey used in the study were found to possess significant wound healing activity compared to that of the standard. Interestingly, all the types of honey were found to have better wound healing activity than that of commercial products indicating the superior potential of natural products over synthetic ones. After completion of 38 days it was observed that the wound was cured among all rabbits. The statistical treatment of data was done using student's t-test and the values were shown in **Tables I** and **II**. The data containing t-test were compared to those of standard and control ($p<0.05$) indicated that there was no significant difference exists between these values. However, among the data of three tested honeys compared with those of the controls, it was found that there was a statistically significant reduction in the wound area of experimental models. The possible mechanism of the wound healing activity of honey could be due to the inhibition of prostaglandins (Al-Waili and Boni, 2003) and by thrombin-induced oxidative burst in phagocytes (Ahmad *et al*., 2009). In addition, the mild acidity and low-level hydrogen peroxide release, both aided tissue repair and could have contributed to the antibacterial activity of honey resulting in the healing of the wound (Lusby *et al*., 2005; Visavadia *et al*., 2008). The wound healing activity of honey could also be due to modulation of the activity of monocytic cells to repair the wounded tissue by releasing anti-inflammatory cytokines and growth factors (Tonks *et al*., 2003; Henriques *et al*., 2006).

Among the different types of the honey SDH was found to possess higher healing rate of wounds induced by thermal and chemical methods. However, in thermal induced burns, both TYH and SPH have shown similar wound healing activity but better than that of standard drugs.

Table. 1: Effect of different types of honey on wound healing activity of thermal burns.

Treatment	Average area of wound (mm^2)[a] (n=3)					Student's t-test values[b]	Result
	First day	1 week	2 weeks	3 weeks	4 weeks		
Control	4.81 (±0.07)	4.83 (±0.08)	4.54 (±0.06)	3.81 (±0.11)	3.41 (±0.10)	-	-
Standard	4.86 (±0.08)	4.79 (±0.06)	4.49 (±0.11)	3.95 (±0.09)	3.38 (±0.12)	0.359	NS
SDH	4.78 (±0.06)	4.64 (±0.03)	3.79 (±0.08)	2.92 (±0.04)	2.36 (±0.05)	0.022	S
TYH	4.75 (±0.11)	4.68 (±0.08)	4.24 (±0.09)	3.42 (±0.06)	2.91 (±0.09)	0.012	S
SPH	4.74 (±0.09)	4.89 (±0.07)	4.31 (±0.12)	3.45 (±0.10)	2.93 (±0.06)	0.045	S

[a] Values are mean ± S.E.M. (area reduction). [b] $p < 0.05$. S=significant, NS=not significant.

Table . 2: Effect of different types of honey on wound healing activity of chemical burns.

Treatment	Average area of wound (mm^2)[a] (n=3)					Student's t-test values[b]	Result
	First day	1 week	2 weeks	3 weeks	4 weeks		
Control	5.67 (±0.09)	5.65 (±0.05)	5.45 (±0.10)	4.89 (±0.12)	4.73 (±0.07)	-	-
Standard	5.65(±0.07)	5.6 (±0.04)	5.36 (±0.11)	4.85 (±0.10)	4.25 (±0.09)	0.096	NS
SDH	5.61 (±0.10)	5.53 (±0.12)	5.09 (±0.09)	3.93 (±0.05)	3.33 (±0.11)	0.045	S
TYH	5.66 (±0.05)	5.53 (±0.08)	5.06 (±0.11)	4.09 (±0.07)	3.43 (±0.12)	0.046	S
SPH	5.64 (±0.12)	5.58 (±0.06)	5.24 (±0.07)	4.58 (±0.09)	4.01 (±0.10)	0.048	S

[a] Values are mean ± S.E.M. (area reduction). [b] $p < 0.05$. S=significant, NS=not significant

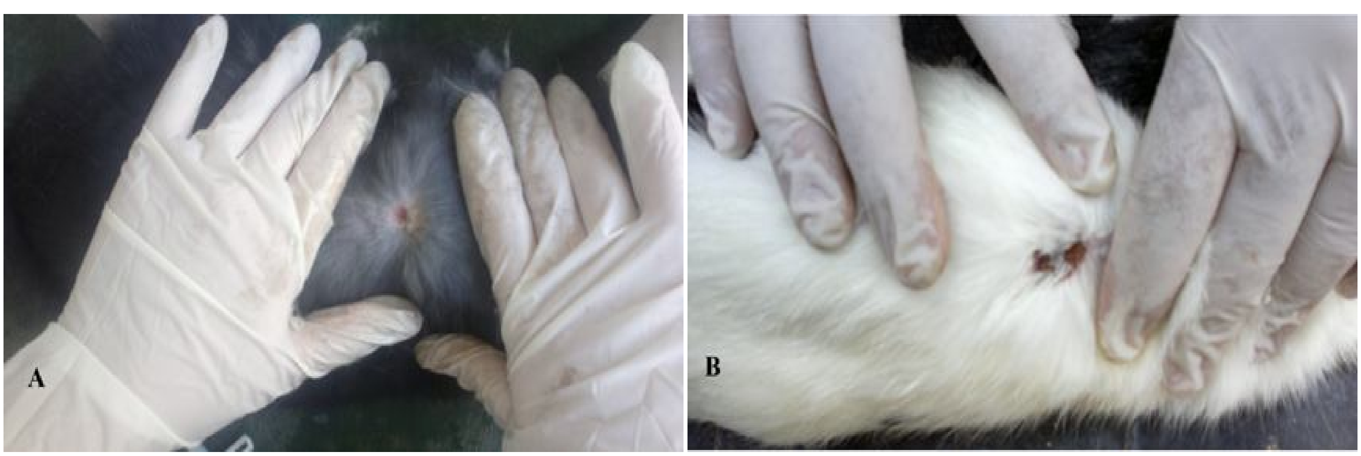

Fig. 1: Untreated (A) thermal and (B) chemical induced burns.

Fig. 2: Thermal burns after 38 days treatment with (A) Sidr honey, (B) Thyme honey and (C) Spring honey.

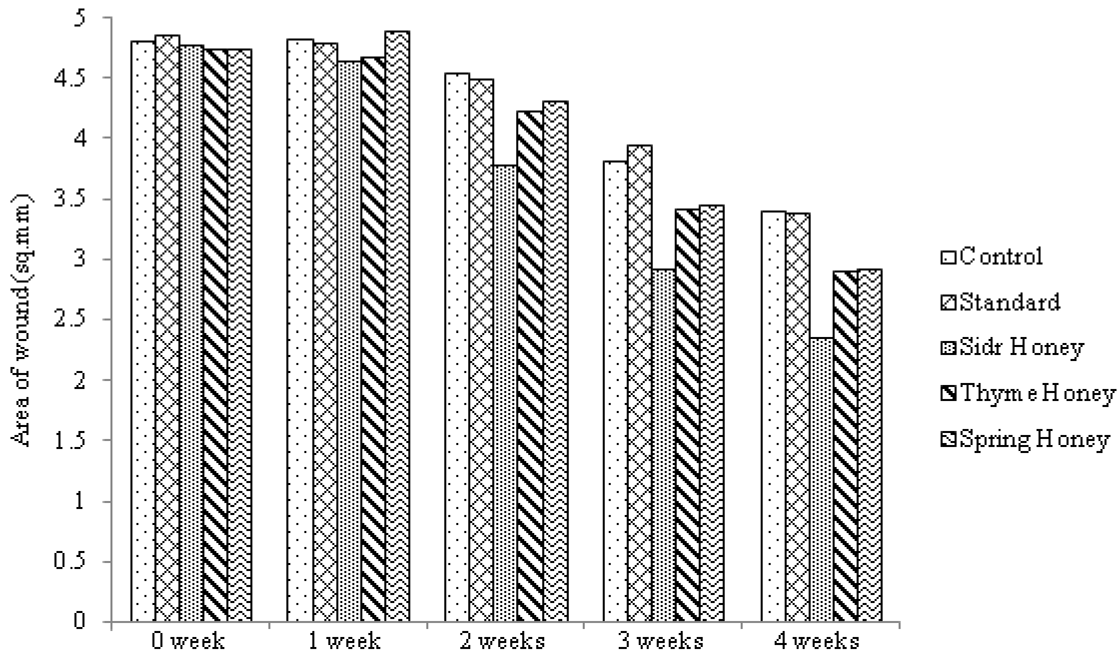

Fig. 3: Wound healing effect of different types of honey on thermal burns.

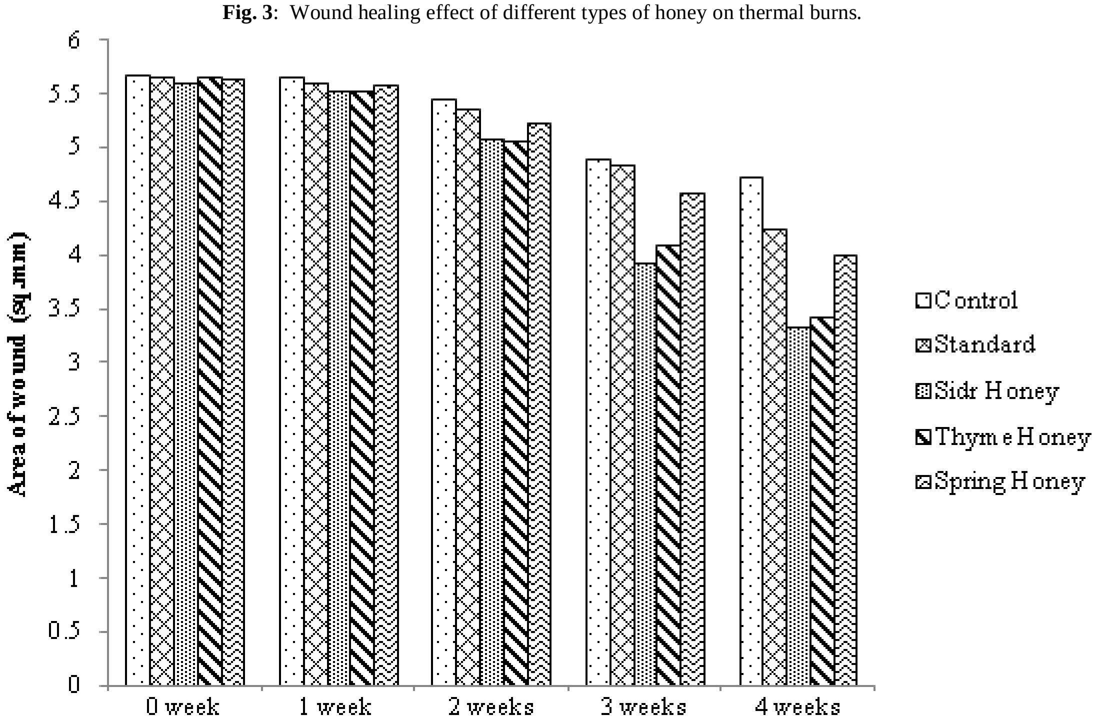

Fig. 4: Wound healing effect of different types of honey on HCl induced burns.

CONCLUSIONS

It was concluded that honey in general could be employed as topical wound healing agent and was also proved that SDH, SPH and TYH honeys could be used as natural wound healing agents besides commercial synthetic analogs tested in the study. Further the present investigation established that SDH was possessing better wound healing activity than that of TYH and SPH.

ACKNOWLEDGEMENT

The authors are thankful to the Government Medical Research Center, Zawia, Libya and Research Faculty of Pharmacy, University of Zawia, Libya for providing the necessary research facilities.

REFERENCES

Abu-Zinadah OA. Using nigella sativa oil to treat and heal chemical induced wound of rabbit skin. JKAU: Sci. 2009; 2(2): 335-346.

Ahmad A., Alam KR., Mesaik MA. Anti-inflammatory effect of natural honey on bovine thrombin-induced oxidative burst in phagocytes. Phytother Res. 2009; 23: 801-808.

Al-Mamary M., Al-Meeri A., Al-Habori M. Antioxidant activities and total phenolics of different types of honey. Nutr Res. 2002; 22: 1041-1047.

Alvarez-Suarez JM., Tulipani S., Romandini S., Bertoli E., Battino M. Contribution of honey in nutrition and human health: a review. Mediterr J Nutr Metab. 2010; 3: 15-23.

Al-Waili NS., Boni NS. Natural honey lowers plasma prostaglandin concentrations in normal individuals. J Med Food. 2003; 6: 129-133.

Al-Waili NS., Haq A. Effect of honey on antibody production against thymus-dependent and thymus-independent antigens in primary and secondary immune responses. J Med Food. 2004; 7: 491-494.

Arwa HMA. Evaluation activity of zinc sulphate on wounds healing induced by chemical burning in normal rabbits. Journal of Basrah Researches. 2011; 37(4A): 100-104.

Bansal V., Medhi B., Pandhi P. Honey: A remedy rediscovered and its therapeutic utility. Khatmandu Univ Med J. 2005; 3: 305-309.

Bonnette KL., Rodabaugh DD., Wilson CW (2001). Dermal irritation and sensitization. In: Derelanko MJ., Hollinger MA (2001). Handbook of Toxicology (pp. 127-201). London: 2nd ed. CRC Press.

Borhan M., Ali R., Daryoush M., Saeid S. comparison of in vivo wound healing activity of verbascum thapsus flower extract with zinc oxide on experimental wound model in rabbits. AEJTS. 2012; 4(1): 24-30.

Drgrotte. http://www.drgrotte.com/honey-medicine.shtml (retrieved on 28-02-2013).

Emsen IM. A different and safe method of split thickness skin graft fixation: Medical honey application. Burns. 2007; 33: 782-787.

Greek thyme honey. The health benefits of greek thyme honey. http://sufidreamer.hubpages.com/hub/Greek-Foods-The-Health-Benefits-of-Greek-Thyme-Honey (retrieved on 28-03-2013).

Henriques A., Jackson S., Cooper R., Burton N. Free radical production and quenching in honeys with wound healing potential. J Antimicrob Chemother. 2006; 58: 773-777.

Jeffrey AE., Echazarreta CM. Medical uses of honey. Rev Biomed. 1996; 7: 43-49.

Johnston JE., Sepe HA., Miano CL., Brannan RG., Alderton AL. Honey inhibits lipid oxidation in ready-to-eat ground beef patties. Meat Sci. 2005; 70: 627-631.

Lusby PE., Coombes A., Wilkinson JM. Honey: a potent agent for wound healing? J Wound Ostomy Continence Nurs. 2002; 29(6): 295-300.

Lusby PE., Coombes AL., Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Res. 2005; 36: 464-467.

Mekonnen A., Sidamo T., Asres K., Engidawork E. In vivo wound healing activity and phytochemical screening of the crude extract and various fractions of Kalanchoe petitiana A. Rich (Crassulaceae) leaves in mice. J Ethnopharmacol. 2013; 145(2): 638-646.

Rakha MK., Nabil ZI., Hussein AA. Cardioactive and vasoactive effects of natural wild honey against cardiac malperformance induced by hyperadrenergic activity. J Med Food. 2008; 11: 91-98.

Sidr honey. http://sidrhoney.tripod.com/id12.html (Retried on 30th January, 2013).

Sidr honey. The health benefits of honey Sidr. http://quran.ketaballah.net/vb/showthread.php?t=945 (retrieved on 28-02-2013).

Spring honey. Benefit of Spring Honey. http://www.basaernews.com/news11453.htm. (retrieved on 28-02-2013).

Tonks AJ., Cooper RA., Jones KP., Blair S., Parton J., Tonks A. Honey stimulates inflammatory cytokine production from monocytes. Cytokine. 2003; 21: 242-247.

Visavadia BG., Honeysett J., Danford MH. Manuka honey dressing: An effective treatment for chronic wound. Br J Oral Maxillofac Surg. 2008; 6(1): 55-56.

Yogeshwar GB., Vandana BP. Formulation of meloxicam gel for topical application: In vitro and in vivo evaluation. Acta Pharm, 2010; 60; 153–163.

How to cite this article:

Nagiat T Hwisa, Prakash Katakam, Babu Rao Chandu, Einas Ghafar Abadi and Ebtihal Mosbah Shefha., Comparative *in vivo* evaluation of three types of honey on topical wound healing activity in rabbits. J App Pharm Sci, 2013; 3 (08): 139-143.