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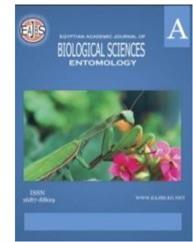

Synthesis and Characterization of a Novel PAA Carrier for Apitoxin of *Apis mellifera* L.

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ABSTRACT

The current study aimed to characterize a novel bee venom –loaded polyacrylic acid carrier intended for cancer therapy. Honey bee venom was collected by the electric shock method. Poly acrylic acid and venom-loaded polyacrylic acid were prepared using potassium peroxodisulfate as an initiator. Nanoformulation was achieved by emulsion-solvent diffusion. Characterization was achieved by FT-IR, ^{1}H-NMR, TEM, DFED, and UV spectrophotometry. TEM showed that the resultant spherical nanoparticles were either dispersed or in a nodular form and their diameter was 17-210 nm. DFED showed that the resultant nanoparticles did not show crystalline edges. FTIR and 1H-NMR concluded that there was no marked interaction between drug and selected polymer except in the physical encapsulation process. UV spectrophotometry indicated a 95% uniformity of drug content in the resultant nanoparticles. The study concluded that the PAA carrier may be a promising suitable carrier for bee venom.

INTRODUCTION

Bee venom is a unique weapon in the animal kingdom. It is an efficient and complex mixture of substances designed to protect bees against a broad diversity of predators from other arthropods to vertebrates. It contains several biologically active peptides, including melittin, apamin, adolapin, mast cell degranulating peptide, and enzymes (phospholipase A2, and hyaluronidase) as well as non-peptide components, such as histamine, dopamine, and norepinephrine (Habermann, 1972; Raghuraman, and Chattopadhyay, 2007). Bee venom and its constituents, such as melittin, phospholipase A2, and apamin, have shown direct anti-cancer activity on several tumor cells including renal, lung, liver, prostate, bladder, melanoma, osteosarcoma, and mammary cancer cells, as well as leukemia cells *in vivo* and *in vitro* (Orsolic 2012). Furthermore, recent studies reported several effects of bee venom such as induction of apoptosis and necrosis and effects on proliferation, cytotoxicity, and growth inhibition of different types of cancer cells (Liu *et al.,* 2002; Han *et al.,* 2007; Orsolic, 2012).

An ideal drug carrier aims to overcome the deficiencies of treatment with free drugs that frequently leads to failed therapies and poor therapeutic responses (Merisko-Liversidge *et al.,* 2003).

Many anticancer therapeutics are cytotoxic and, therefore, have to be released to the affected site only, to protect normal cells from possible side effects. The attachment of anti-

cancer agents to nano polymers could be a promising approach towards reducing the overall toxicity arising from poor delivery and targeting (Lee *et al.,* 2002).

Drug nanoparticles, due to their unique properties, have been studied for many delivery strategies. Their ability to permeate biological membranes, accessibility to remote tissues, and increased residence time in the body offer a more diverse portfolio of treatment options and improve drug efficacy through both local and systemic targeting (Ober and Gupta, 2011). Also, nanoparticles have been actively explored as delivery systems for small drug molecules as well as macromolecules such as nucleic acids, peptides, proteins, and hormones (Panyam and Labhasetwar, 2003; Brzoska *et al.*, 2004).

Nowadays hydrogels have become popular carriers for drug delivery applications due to their biocompatibility and resemblance to biological tissues (Lowman *et al.,* 2004 and Peppas *et al.,* 2004). Hydrogels are three-dimensional cross-linked polymeric structures that can absorb and retain a large amount of water while remaining insoluble in aqueous solutions (Hoffman, 2002).

Nano hydrogels are gaining tremendous importance in a wide variety of applications in medical, pharmaceutical, drug delivery and other fields in addition to being one of the upcoming classes of polymer-based controlled-release drug delivery systems. Besides exhibiting swelling-controlled drug release, hydrogels also show stimuli-responsive changes in their structural network and hence, the drug release (Gupta *et al.,* 2002; Vinogradov *et al.,* 2002). Conjugation of the therapeutic drug to the polymer improves the pharmacokinetic and pharmacodynamic properties of biopharmaceuticals through a variety of measures including, increased plasma half-life (which improves patient compliance because less frequent doses are required), enhanced blood circulation time of the drug, protection of the therapeutic drug from macromolecules as proteins and proteolytic enzymes, reduction in immunogenicity, enhanced stability of proteins, enhanced solubility of low molecular weight drugs, and the potential for targeted delivery (Roberts *et al.,* 2002; Duncan, 2003; Duncan, 2006; Liechty *et al.,* 2010; Vilar *et al.,* 2012).

Polyacrylic acid (PAA), according to The European Food Safety Authority as published by Toxipedia, (2015), and its precursor, acrylic acid, are considered to be safe compounds which have been extensively used in a wide variety of household and personal care products as diapers, nail polish, shampoo, moisturizers, and other products. Also, polyacrylic acid is an FDA- and EFSA-approved food additive and considered not to raise a concern for genotoxicity and it was concluded that the use of these substances does not raise a safety concern when used.

Hydrogels based on PAA can absorb a substantial amount of water which makes them potential candidates for drug delivery systems (Thakur *et al.,* 2011). These hydrogels have also been reported with adjustable swelling kinetics with applications for insulin release, (Vernon *et al.,* 1999) to improve osteoblast adhesion (Bearinger *et al.,* 1998) and showing special properties such as super-absorbent hydrogels (Aoki *et al.,* 1994; Dhara *et al.,* 1997). PAA and its lightly cross-linked commercial forms like Cabopol and Polycarbophi have been the most extensively used polymer for mucoadhesive drug delivery, and also appear to be biocompatible (Park and Robinson, 1987; Kellaway and Warren, 1996; Aiache *et al.,* 1997; Bourlais *et al.,* 1998; Hwang *et al.,* 1998; Abd El-Rehim *et al.,* 2007). Al-Noor *et al.,* (2015) reported the successful application of PAA as a carrier for captopril, an anti-hypertensive drug.

Recent studies have shown, using nanotechnology, that bee venom and its components can be delivered specifically *in vivo* in nanoparticles to kill different cancers without any cytotoxicity for normal tissues. This study aimed to characterize a bee venom carrier based on polyacrylic acid for future applications against cancer.

MATERIALS AND METHODS

Bee Venom Extraction:

Honey bee venom from forager honey bees (*Apis mellifera* L.), descendant from naturally mated queens, was collected by the electric shock method according to Benton *et al.,* (1963) with the modifications adopted by Sanad, R., and Mohanny, K., (2013). The resulting bee venom was scrapped up for collection, dissolved in distilled water and centrifuged at 12,000 *g* for 10 min to remove insoluble materials then, it was freeze-dried according to the method of Lariviere and Melzack, (1996) for later use.

Preparing the Polymer and Venom-loaded Polymer:

Polyacrylic acid (PAA) polymer was prepared according to the method of Braun, (2005) by preheating distilled water to 80 °C in a 50-ml round-bottom flask. Purified acrylic acid polymerization is initiated by a solution of potassium peroxodisulfate after 10-15 min. After polymerization, the solution was added to 0.1 N HCI, whereupon the polymer precipitated. The polymer was filtered, broken up, extracted in a Soxhiet apparatus with petroleum ether for 5 hours, and finally dried to constant weight in vacuum at 50 °C.

The venom loaded polymer was prepared in the same fashion as the nano-polymer was prepared, the only modification was introducing a drop-wise saturated solution of bee venom at the same time the acrylic acid and potassium peroxodisulfate were introduced.

Nano-formulation and Purification:

The resulting polymer was ground thoroughly, sonicated, centrifuged to remove insoluble materials then mixed with apitoxin in a solvent of acetone and ethanol. Afterward, this solution was poured into an aqueous PVA solution, to produce *o/w* emulsion. The nanospheres obtained were centrifuged and resuspended in distilled water to remove the solvent and free drug in the upper aqueous phase. Finally, the nanospheres were powdered by freeze-drying (Song *et al.,* 1997; Guarrero *et al.,* 1998; Takeuchi *et al.,* 2001 and Ober and Gupta, 2011). Further purification was performed by dialysis for 1 week to remove surfactant and unreacted molecules. The dialyzed particle dispersion was condensed by evaporation of water and dried to get hydrogel nanoparticles (Pelton and Chibante, 1986).

Characterization, Biocompatibility and Drug-Polymer Interaction Studies:

A full inspection was raised through the combination of field transmission infrared spectroscopy (FT-IR), proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), and transmission electron microscopy (TEM) to elaborate both compositional and morphological features of the novel modified venom-loaded polymer (VLP).

Determination of Surface Chemistry:

Transmission electron microscopy (TEM), to determine morphology, topography, and size of the resultant particles, allows fracturing of the particles and consequently an observation of their interior.

Darkfield electron diffraction (DFED), to determine the crystalline edges of the sample and determine its form as amorphous or crystalline, this will have an influence on the *in vitro* and *in vivo* release characteristics of the drug.

Determination of Drug Interaction and Biocompatibility:

Fourier transform IR (FTIR), to determine chemical analysis and functional groups of surfaces modified nanoparticles revealing interactions at the nanoparticle surface. It is most frequently used to qualitatively assess protein integrity in the solid-state without destruction of the formulation (Wolf *et al.,* 2003).

Proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, to determine the functional group and chemical composition. It is a non-destructive spectroscopic technique used for characterizing surface modification with the ability to render information on the chemical nature as well as the molecular or collective mobility of individual components

(Mayer *et al.,* 2002).

Determination of Drug Content:

UV spectrophotometry was performed at 246 nm to determine drug content and uniformity according to the method of Punitha *et al.,* (2013).

RESULTS AND DISCUSSION

Determination of Surface Chemistry:

Transmission electron microscopy (TEM; Figs. 1-4) analysis of VLPAA showed that the resultant nanoparticles were present in either a dispersed form or in an aggregated nodular form, this can be related to different levels of polymer concentration after the polymerization process, and can also be attributed to the swelling state of the PAA hydrogel. The morphology of the resultant particles was spherical and ranged in diameter between 17-210 nm, this makes the resultant particles well in the nano scale. This size range is in accordance with the study conducted by Omidirad *et al.* (2013) on the doxorubicin-loaded poly AA-coated magnetite nanoparticles, where the particles were 6-15 nm.

Dark field electron diffraction (DFED; Fig. 5) showed no well-defined crystalline edges were evident in VLPAA sample, resembling parent bare polymer while the bee venom showed well known crystalline edges indicating its poly-crystalline nature. This can indicate that the application of the VLPAA particles will not result in any cellular deformations due to the absence of crystalline nature in these particles. These findings are in complete agreement with the study of Liu *et al.* (2015) on pH-responsive PAA as a drug carrier.

Determination of Drug Interaction and Biocompatibility:

Fourier transform IR (FTIR; Fig. 6) comparison between bare poly acrylic acid (PAA), pure bee venom (BV) and venom-loaded poly acrylic acid (VLPAA) showed that VLPAA resembled its parent bare PAA, and showed no traces of the venom peaks especially in the fingerprint region with special nomination to the peak existing in the region 800-1000 cm^{-1} which corresponds to the aromatic characters present in BV components as melittin.

Proton nuclear magnetic resonance (^{1}H-NMR; Figure 7) spectroscopy showed that the majority of the peaks resembled bare PAA as the peaks appeared with a chemical shift equivalent to 1.42, 2.25, 4.18 and 4.67 ppm with the disappearance of the characteristic peaks of BV components. However, the presence of multiple peaks with aromatic nature around a chemical shift of 5 ppm may be attributed to the partial release of the venom from its PAA carrier in the surrounding solution.

1-Consequently, the results of ^{1}H-NMR and FT-IR concluded that there was no marked interaction between drug and selected polymer except in the physical encapsulation process without the formation of any chemical bonds between the BV and PAA, and without any alteration to the structural integrity of the BV.

In terms of biocompatibility, the graphs obtained indicate as a result that the drug is compatible with the excipients used.

2-Determination of Drug Content:

UV spectrophotometry (Table 1) of VLPAA showed that BV-content uniformity was achieved and was in the range of 95%. In other words, the BV particles were distributed evenly and in a uniform fashion in the polymer matrix.

The results obtained by FT-IR and UV spectroscopies are in agreement with the study conducted by Nassar (2013) on the potential of BV to control Sitophilus granarius.

3-By combining the observations obtained from the used characterization techniques, it can be expected that the resultant VLPAA particles were in the nano scale with nearly equal content of encapsulated BV without any alteration to the chemical or structural integrity of

the BV or PAA. The crystalline properties of VLPAA showed smooth edging, thus avoiding any cellular lacerations when applied.

Conclusion: These results indicate that the PAA carrier shows promising results when conjugated with bee venom.

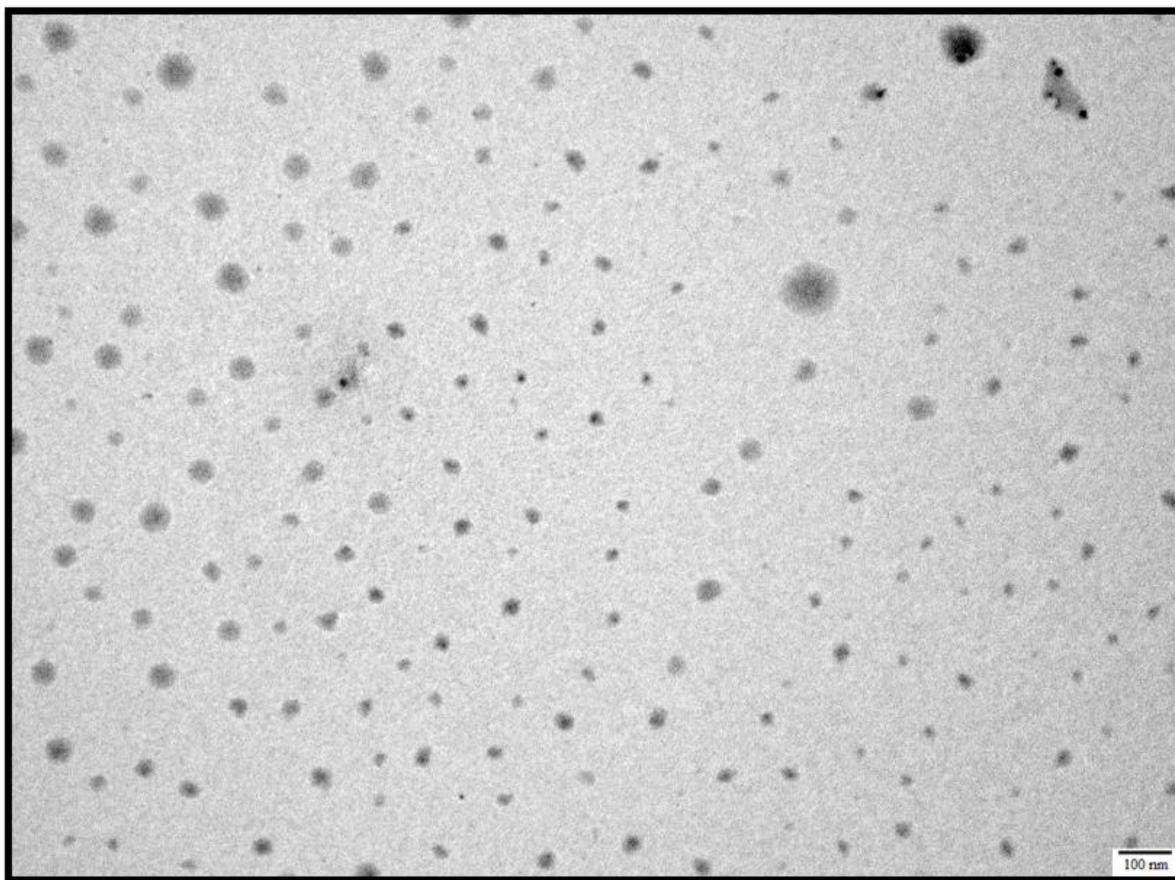


Fig.1: Magnified TEM micrographs showing dispersed nano-VLPAA.

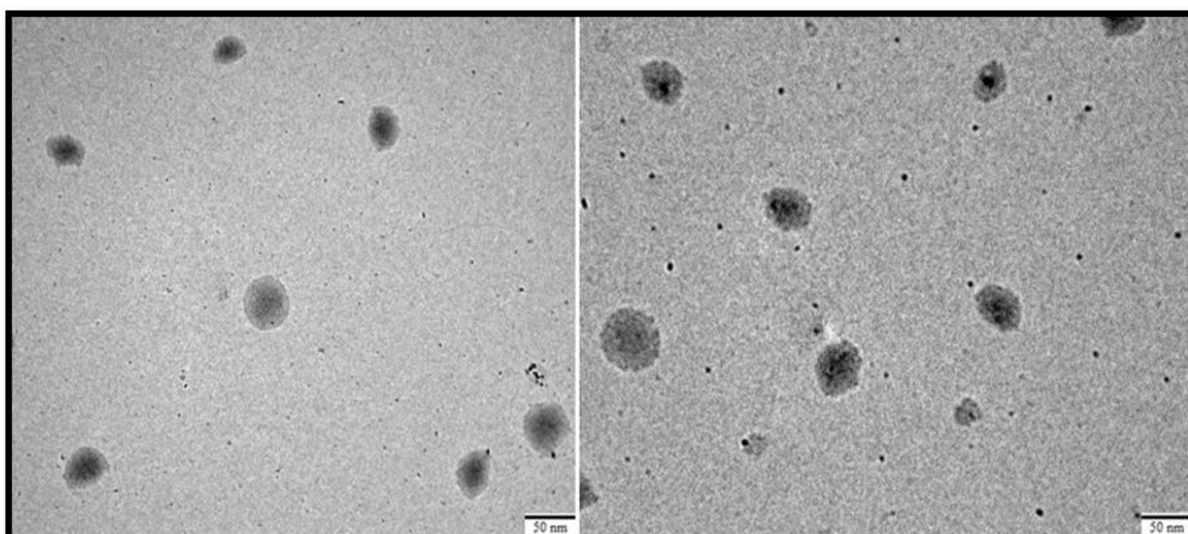


Fig.2: Magnified TEM micrographs showing dispersed nano-VLPAA.

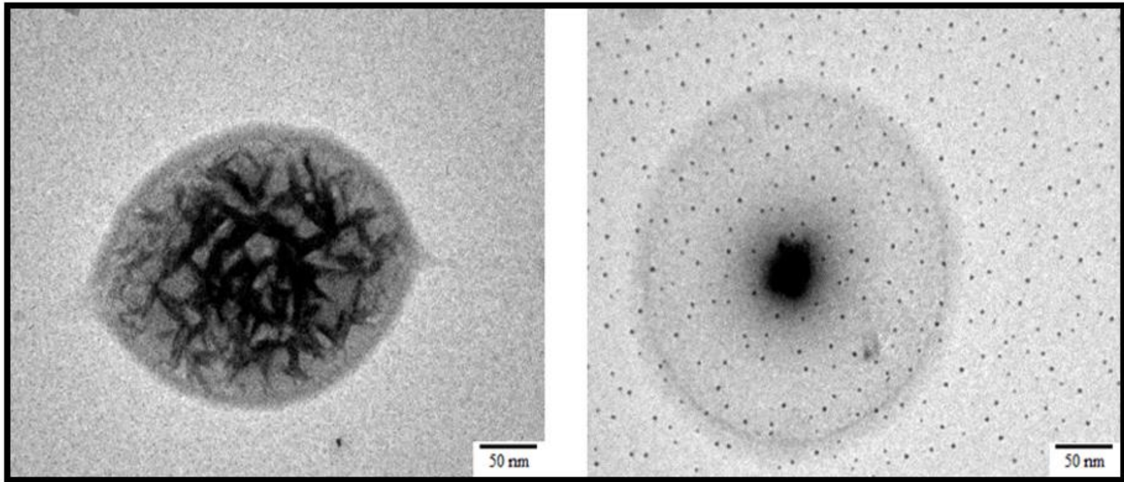


Fig.3: Magnified TEM micrographs showing nano-PAA spheres with encapsulated BV.

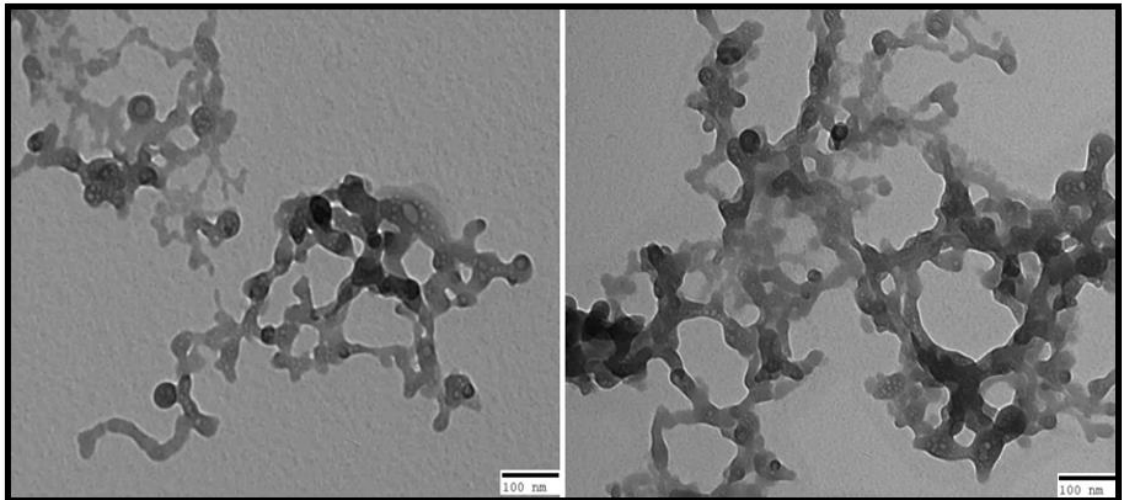


Fig.4: Magnified TEM micrographs showing nodular aggregations of nano-VLPAA.

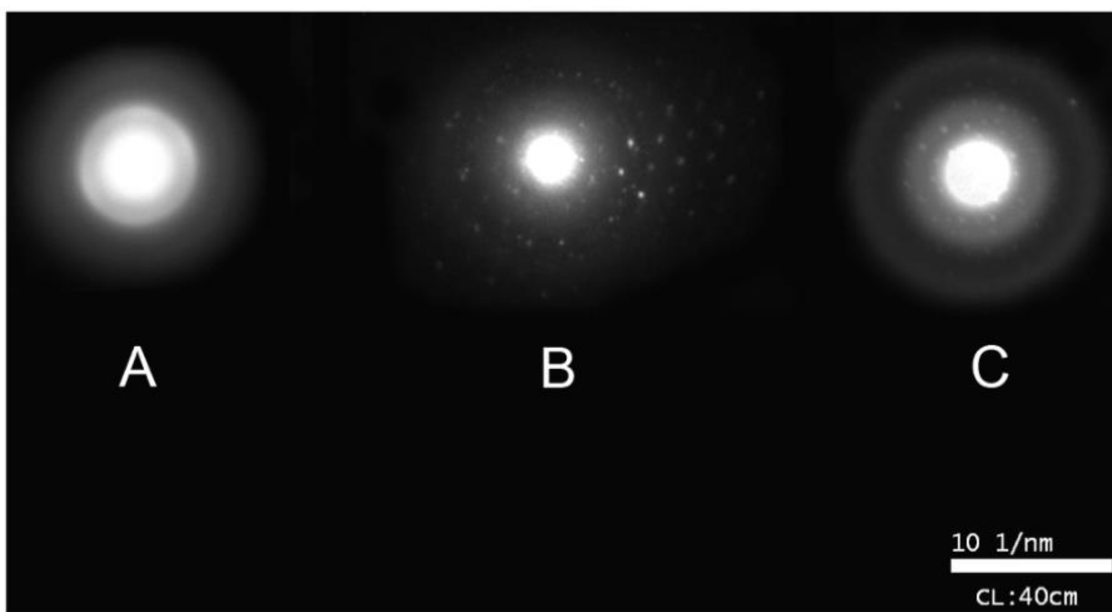
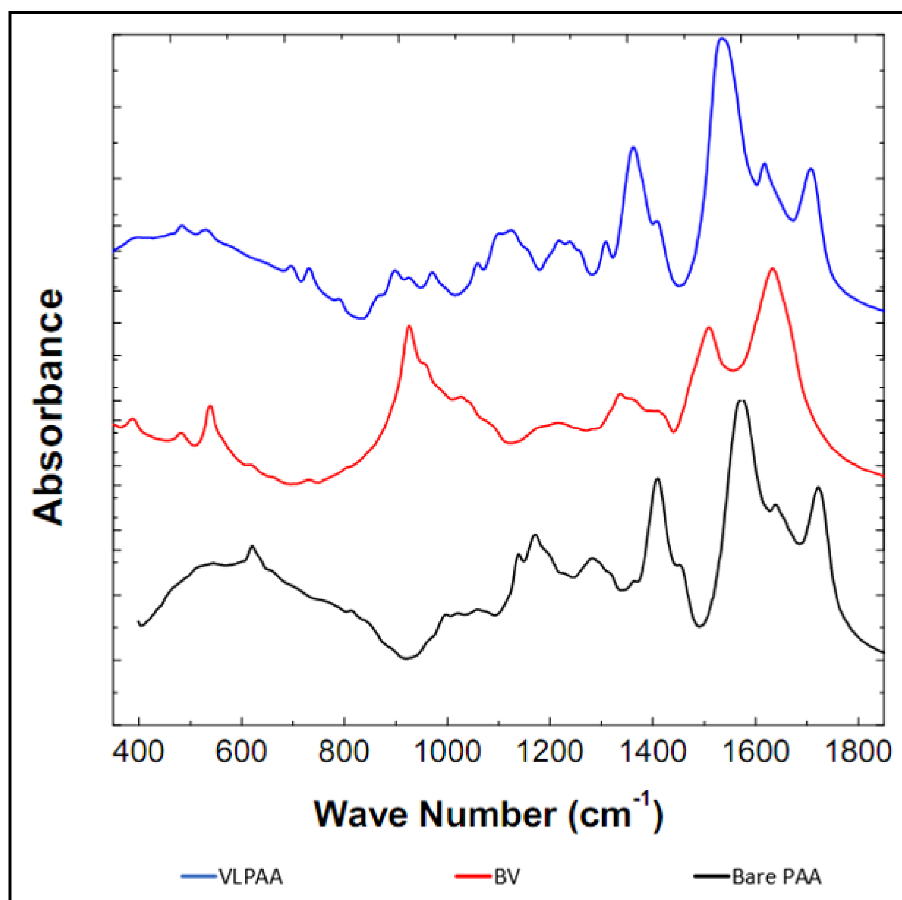


Fig.5: Dark field electron micrographs showing the crystalline properties of (A) bare polymer, (B) pure BV, and (C) VLPAA.



Fig,6: FT-IR spectrographs for VLPAA, BV, and bare PAA.

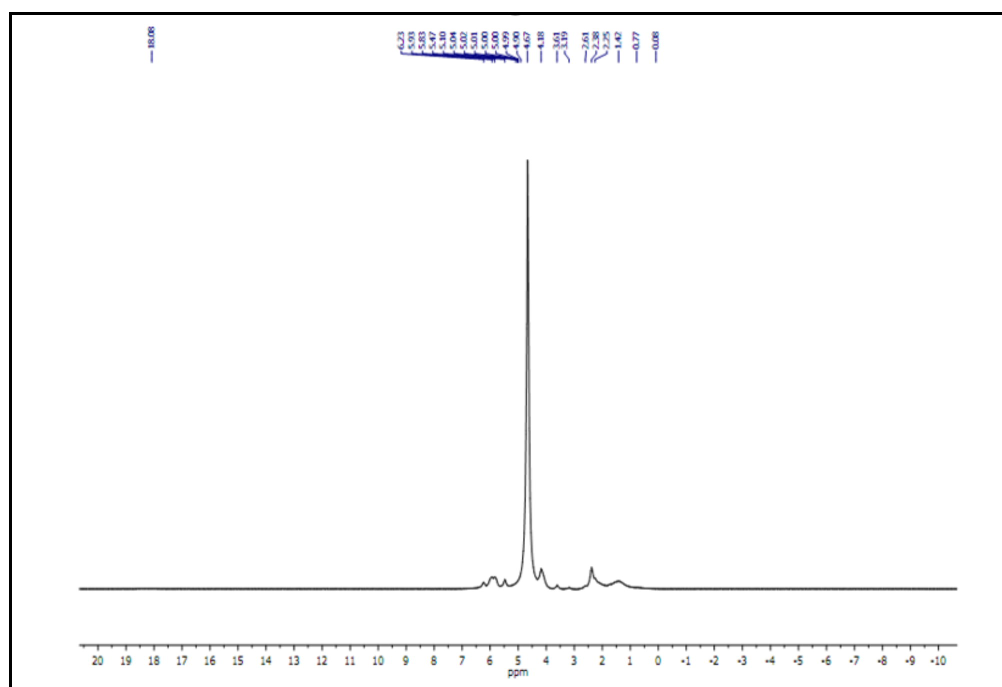


Fig.7: ^{1}H-NMR spectrograph of VLPAA.

Table 1: BV content (%) in VLPAA samples measured under UV spectrophotometer at 246 nm.

Reading	BV Content (%)
1	96.4 ± 0.55
2	93.7 ± 0.36
3	94.2 ± 0.28
4	98.6 ± 0.11
5	95.4 ± 0.37

REFERENCES

Abd El-Rehim, H. A.; Hegazy, E. A.; Khalil, F. H. and Hamed, N. A. (2007). Radiation preparation of drug carriers based polyacrylic acid (PAAc) using poly (vinyl pyrrolidone) (PVP) as a template polymer. Nuclear Instruments and Methods in Physics Research, 254: 105–112.

Aiache, J. M.; Meski, S. E. and Beyssac, E. (1997). The formulation of drug for ocular administration, J. Biomater. Appl., 11: 329–348.

AL-Noor, T. H.; Ali, F. M. and Ali S. M. (2015). Synthesis of drug carrier polyacrylic acid with spacer group. International Journal of Technical Research and Applications, 3(3): 300-306.

Aoki, T.; Kawashima, M.; Katono, H.; Sanui, K.; Ogata, N.; Okano, T. and Sakurai, Y. (1994). Temperature-Responsive Interpenetrating Polymer Networks Constructed with Poly (acrylic acid) and Poly (N, N-dimethylacrylamide). Macromol. 27(4): 947-952.

Bearinger, J. P.; Castner, D. G. and Healy, K. E. (1998). Biomolecular modification of p(AAm-co-EG/AA) IPNs supports osteoblast adhesion and phenotypic expression. J. Biomater. Sci., Polym. Ed., 9(8): 629-652.

Benton, A.W.; Morse, R. A. and Stewart, J. B. (1963). Venom collection from honey bees. *Science*, 142: 228-230.

Bourlais, C. L.; Acar, L.; Zia, H.; Sado, P. A.; Needham, T. and Leverge, R. (1998). Ophthalmic drug delivery systems- Recent advances, Prog. Retinal Eye Res., 17: 33–58.

Braun, D.; Cherdron, H.; Rehahn, M.; Ritter, H. and Voit, B. (2005). Polymer synthesis: theory and practice. 4th ed., Springer, Heidelberg, Germany., P. 170.

Brzoska, M.; Langer, K.; Coester, C.; Loitsch, S.; Wagner, T. and Mallinckrodt, C. V. (2004). Incorporation of biodegradable nanoparticles into human airway epithelium cells - *in vitro* study of the suitability as a vehicle for drug or gene delivery in pulmonary diseases. Biochem. Biophy. Res. Com., 318: 562–570.

Dhara, D.; Nisha, C. K. and Chatterji, P. R. (1997). Super absorbent hydrogels: interpenetrating networks of poly (acrylamide-co-acrylic acid) and poly (vinyl alcohol): swelling behavior and structural parameters. JMSF Pure Appl. Chem. A36 (2): 197-210.

Duncan, R. (2003).The dawning era of polymer therapeutics. *Nat. Rev. Drug Disc.*, 2(5): 347–60

Duncan, R. (2006). Polymer conjugates as anticancer nanomedicines. Nat. Rev. Cancer, 6(9): 688–701.

Guarrero, Q.; Allemann, E.; Fessi, H. and Doelker, E. (1998). Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. Drug Dev. Ind. Pharm., 24: 1113–1128.

Gupta, P.; Vermani, K. and Garg, S. (2002). Hydrogels: from controlled release to pH-responsive drug delivery. Drug Disc. Today, 7: 569–579.

Habermann, E. (1972). Bee and wasp venoms. Science, 177(46): 314-22.

Han, S.; Lee, K.; Yeo, J.; Kweon, H.; Woo, S.; Lee, M.; Baek, H.; Kim, S. and Park, K. (2007). Effect of honey bee venom on microglial cells nitric oxide and tumor necrosis factor-alpha production stimulated by LPS. J. Ethnopharmacol., 111 (1): 176–181.

Hoffman, A. S. (2002). Hydrogels for biomedical applications. Adv. Drug Del. Rev. 54(1): 3-12.
Hwang, S. J.; Park, H. and Park, K. (1998). Gastric retentive drug delivery systems, Crit. Rev. Ther. Drug Carrier Syst., 15: 243–284.
Kellaway, I. W. and Warren, S. J. (1996). Mucoadhesive hydrogels for buccal delivery, Drugs Pharm. Sci., 74: 221–239.
Lariviere, W. R. and Melzack, R. (1996). The bee venom test: a new tonic-pain test. Pain, 66(2–3):271–277.
Lee, N. J.; Koo, J.; Ju, S.; Moon, S. B.; Cho, W. J. and Jeong, I. (2002). Synthesis and biological activity of phthalimide-based polymer containing 5-fluorouracil. Polym. Int., 51: 569–76.
Liechty, W. B.; Kryscio, D. R.; Slaughter, B. V. and Peppas N. A. (2010). Polymers for drug delivery systems, Annu. Rev. Chem. Biomol. Eng. 1: 149-173.
Liu, X.; Chen, D.; Xie, L.; and Zhang, R. (2002). Effect of honey bee venom on proliferation of K1735M2 mouse melanoma cells in-vitro and growth of murine B16 melanomas in-vivo. J. Pharmacy Pharmacol, 54(8): 1083–1089.
Lowman, A. M.; Dziubla, T. D.; Bures, P. and Peppas, N. A. (2004). Structural and dynamic response of neutral and intelligent networks in biomedical environments. In: Peppas, N. A. and Sefton, M. V., editors. Molecular and cellular foundations of biomaterials. New York, Academic Press, Pp. 75–130.
Mayer, C.; Hoffmann, D. and Wohlgemuth, M. (2002). Structural analysis of nanocapsules by nuclear magnetic resonance. Int. J. Pharm., 242: 37–46.
Merisko-Liversidge, E.; Liversidge, G. G. and Cooper, E. R. (2003). Nanosizing: a formulation approach for poorly water-soluble compounds. Eur. J. Pharm. Sci., 18:113–120.
Nassar, M. I. (2013). The potential of natural venom of *Apis mellifera* for the control of grains weevil adults (*Sitophilus granaries* – Coleoptera - Curculionidae). Int. J. Entomol. Res., 1(1): 25-31.
Ober, C. A. and Gupta, R. B. (2011). Nanoparticle technology for drug delivery, Ide@s Concyteg, 6(72): 714-726.
Orsolic, N. (2012). Bee venom in cancer therapy. *Cancer Met. Rev*. 31: 173-194.
Panyam, J. and Labhasetwar, V. (2003). Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv. Drug Deliv. Rev., 55: 329–347.
Park, H. and Robinson, J. R. (1987). Mechanisms of mucoadhesion of poly (acrylic acid) hydrogels. Pharm. Res., 4(6): 457-64.
Pelton R. H. and Chibante P. (1986). Preparation of aqueous latices with N-isopropylacrylamide. Colloids Surf., 20: 247-56.
Peppas, N. A.; Wood, K. M. and Blanchette, J. O. (2004). Hydrogels for oral delivery of therapeutic proteins. Expert Opin. Biol. Ther., 4: 881–887.
Punitha, S.; Sabitha, G.; Kalal, V. and Rajasekar, S. (2013). Formulation and evaluation of Atorvastatin calcium sustained release matrix tablets. Int. J. Pharm. Res. Pharm. Sci., 4(1): 82-87.
Raghuraman, H. and Chattopadhyay, A. (2007). Melittin: a membrane-active peptide with diverse functions. Bioscience Reports, 27(4–5): 189S–223S.
Roberts, M. J.; Bentley, M. D. and Harris, J. M. (2002). Chemistry for peptide and protein PEGylation. Adv. Drug Deliv. Rev., 54(4): 459–76.
Sanad, R. and Mohanny, K. (2013). The efficacy of a new modified apparatus for collecting bee venom in relation to some biological aspects of honey bee colonies. J. Am. Sci., 9(10): 177-182.
Song, C. X.; Labhasetwar, V.; Murphy, H.; Qu, X.; Humphrey, W. R.; Shebuski, R. J. and Levy, R. J. (1997). Formulation and characterization of biodegradable nanoparticles

for intravascular local drug delivery. J. Control. Release, 43:197–212.

Takeuchi, H.; Yamamoto, H. and Kawashima, Y. (2001). Mucoadhesive nanoparticulate systems for peptide drug delivery, Adv. Drug Deliv. Rev. 47: 39–54.

Thakur, A.; Wanchoo, R. K. and Singh, P. (2011). Hydrogels of Poly (acrylamide-co-acrylic acid): In-vitro Study on Release of Gentamicin Sulfate. Chem. Biochem. Eng. Q., 25(4): 471–482.

Toxipedia (2015). *Polyacrylic acid.* Retrieved May, 2015, from http://www.toxipedia.org/display/toxipedia/Polyacrylic+Acid

Vernon, B.; Kim, S. W. and Bae, Y. H. (1999). Insulin release from islets of Langerhans entrapped in a poly (N-isopropylacrylamide-co-acrylic acid) polymer gel. J. Biomater. Sci., Polym. Ed., 10 (2): 183-198.

Vilar, G.; Tulla-Puche, J. and Albericio, F. (2012). Polymers and drug delivery systems. Current Drug Delivery, 9(4): 367-394.

Vinogradov, S. V.; Bronch, T. K. and Kabanov, A. V. (2002). Nanosized cationic hydrogels for drug delivery preparation, properties and interactions with cells. Adv. Drug Delivery Rev., 54:135–47.

Wolf, M.; Wirth, M.; Pittner, F. and Gabor, F. (2003). Stabilisation and determination of the biological activity of asparaginase in poly(lactide-co-glycolide) nanospheres. Int. J. Pharm., 256:141–152.

ARABIC SUMMARY

تركيب وتوصيف حامل مستحدث من الحمض الاكريلي المتعدد لسم النحل المستخلص من نحل العسل

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٢- مركز التدريب والأبحاث لناقلات الامراض – جامعة عين شمس

هدف الدراسة: هدفت الدراسة الحالية إلى توصيف مركب مستحدث من حمض الاكريليك المتعدد مُحمل بسم نحل العسل بهدف تطبيقه كعلاج محتمل للسرطان.

المواد والطرق البحثية المُستخدمة والنتائج:

١-استخلاص سم النحل وتهيئته لدراسة السمية: تم استخلاصه من شغالات نحل العسل بطريقة الصعق الكهربي ثم كشطه صورة مسحوق واذابته في مياه مقطرة وحفظه بطريقة التجفيف بالتجميد ثم تحضير عدة تركيزات لتطبيقها علي خلايا السرطان الكبدية.

٢-البوليمر الخاوي والمحمل بسم نحل العسل: تم تحضيرهم باستخدام باديء التفاعل (ثنائي كبريتيد البوتاسيوم).

٣-التحضير في مستوي النانو وتنقية العينات: عن طريق انتاج مستحلب زيتي/مائي.

٤-الدراسات الوصفية، التوافقية والتفاعلية للبوليمر:

نقل المجال الطيفي بالأشعة تحت الحمراء وطيف الرنين النووي المغناطيسي بالبروتون اظهروا ان جزئيات النانو بوليمر تشابه تلك للبوليمر الخاوي بدون اظهار أي قمم طيفية مميزة لسم النحل وبخاصة في المناطق البصمية المميزة للصفات الاروماتية التي يظهرها سم نحل العسل بمكوناته المختلفة واهمها المليتين. وهذا يؤكد اقتصار دور البوليمر على الارتباط الفيزيائي فقط بجزيئات سم النحل اثناء تحميله علي جزيئات النانو بوليمر بدون تكوين أي روابط كيميائية من شأنها تغيير التركيب المميز لسم النحل.

نتائج المجهر الاليكتروني الناقل أوضحت ان جزيئات البوليمر المُحمل بسم نحل العسل كانت إما متفرقة في أشكال دائرية أو مجمعة في اشكال عنقودية وقد تراوحت اقطار هذه الجزيئات بين ١٧-٢١٠ نم.

دراسة الحيود الاليكتروني بالمجال المظلم لم توضح وجود أي حيود تبلورية في جزيئات البوليمر المحمل بسم النحل، مظهرة بذلك تشابه مع البوليمر الخاوي وتعارض مع سم النحل المنقي الذي اظهر حدود تبلورية جلية، مؤكدا بذلك الطبيعة المتبلورة لمكونات سم النحل.

الدراسة المطيافية في مجال الاشعة فوق البنفسجية أكدت ان كمية سم نحل العسل كانت متساوية بالجزيئات المختلفة من النانو بوليمر وقد كانت في نطاق ٩٥٪.

١- الاستنتاجات:

يمكن اعتبار البوليمر المستحدث من حمض الاكريليك المتعدد حامل مناسب لسم النحل.